Beneficial effects of Beta-carotene injections prior to treatment with PGF$_{2\alpha}$ on the fertility of postpartum dairy cows


1Ondokuz Mayis University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, 55139, Kurupelit, Samsun, TURKEY.
2Kafkas University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, 36300, Pasaccayri, Kars, TURKEY.
3Dicle University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, 21280, Diyarbakir, TURKEY.
4Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, 15030, Burdur, TURKEY.
5Harran University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, 63200, Urfa, TURKEY.
6Clinic for Horses, Department of Veterinary Medicine, Freie Universität Berlin, GERMANY.
7Ankara University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, 06110, Diskapi, Ankara, TURKEY.

*Corresponding author: serhan.ay@gmail.com or serhan.ay@omu.edu.tr

SUMMARY

This study examined the effects of Beta-carotene (βC) injections before estrus synchronization with PGF$_{2\alpha}$ on fertility parameters in cows. A total of 124 postpartum (pp) cows were used. In GI (n=25), βC (Carofertin®, 0.4 mg/kgBW, i.m.) was injected on d 15 and 45 pp. In GII (n=25), βC was administered on d 15 pp. In GIII (n=25), βC was administered on d 45 pp. In GIV (n=25), βC was injected on d 35 and 45 pp. GV (n=24) was set aside as a control group. All animals received PGF$_{2\alpha}$ (Iliren®, 0.0015 mg/kgBW, i.m.) on d 50 and 61 pp. Blood samples were collected on d 15, 48 and three days after each βC injection. The overall pregnancy rate was determined to be higher in GI (100%) and GIV (88.0%) than GV (70.8%) (P<0.01). The number of services per conception was the lowest in GI (1.4) and GII (1.4), while days of first service were lower in GII (90.3 d) and GIV (90.8 d) (P<0.05). The insemination index was found to be lowest in GI (1.4). These results suggest that two administrations of βC in the early and late pp period before administering PGF$_{2\alpha}$ can improve fertility. It was concluded that when serum βC concentrations remain high for a long time (30d) as a result of βC injections, fertility can be improved.

Keywords: Beta-carotene, fertility parameters, postpartum cows.

RÉSUMÉ

Effets d’administrations de bêta-carotène avant le traitement avec des PGF$_{2\alpha}$ sur la fertilité des vaches laitières au cours du post-partum

Cette étude a examiné les effets d’administrations de bêta-carotène (βC) réalisées avant un traitement de synchronisation de l’ovulation avec des PGF$_{2\alpha}$ sur les paramètres de la fertilité chez la vache en post-partum (pp). L’étude a concerné au total 124 vaches en post-partum. Les vaches du groupe I (n = 25) ont reçu une administration de βC (Carofertin®, 0.4 mg/kgBW, im) et 15 et 45 jours après la parturition. Les vaches du groupe II (n = 25) et du groupe III (n = 25) ont reçu une seule administration de βC à la même dose au stade 15 jour pp. Les vaches du groupe IV ont reçu une administration de βC 35 et 45 jours pp. Les vaches du groupe V (n=24) ont constitué le groupe contrôle. Tous les animaux ont reçu une administration de PGF$_{2\alpha}$ (Iliren®, 0,0015 mg/kgBW, im) 50 et 61 jours après la parturition. Les prélèvements de sang ont été réalisés au cours des 15ème et 48ème jours pp et trois jours après chaque administration de βC. Les taux de gestation des vaches du groupe I (100 %) et du groupe IV (88,0 %) ont été supérieurs à celui du groupe contrôle (GV, 70,8 %, P < 0,01). Le nombre d’inséminations par conceptions a été le plus faible pour les vaches des groupes I et II (1,4), tandis que le nombre de jours avant le premier service a été le plus faible pour les vaches des groupes III (90,3 d) et IV (90,8 d, P < 0,05). Nos résultats suggèrent que deux administrations de βC, réalisées au début et à la fin de la période pp avant un traitement aux PGF$_{2\alpha}$, pourraient améliorer la fertilité. Il a été conclu que le maintien de concentrations sériques élevées pendant les 30 jours suivant la parturition pourrait améliorer de la fertilité associée au traitement.

Mots clés : Bêta-carotène, paramètres de fertilité, vaches, post-partum.

Introduction

Beta-carotene (βC) is the primary precursor for vitamin A but it also acts independently from vitamin A. It is mainly intake from forages. After being metabolized in the intestine, it is absorbed as retinol [35] and transported with high density lipoproteins and stored in liver and adipose tissue. The corpus luteum (CL) contains high βC levels [3]. Beta-carotene serum concentrations vary depending on breed, lactation [34], peripartal stage [23] and nutrition [35]. Beta-carotene concentrations in plasma, CL and follicular fluid also vary according to the stage of the estrous cycle or pregnancy [18].

A positive relationship between supplemental βC and reproductive function has been demonstrated [20]. Low βC concentrations in blood serum have been associated with prolonged estrus, silent estrus [19] and signs of estrus that are less pronounced [24]. It has been reported that the addition of βC to daily rations during pre-and post-partum periods significantly increases conception rates while reducing the pregnancy index (PI; number of services per pregnancy) [22]. Many authors reported an increase in infertility as a result of deficient of βC in cows [5, 31].

It has been reported that delayed formation of the CL and low progesterone (P$_{4}$) production after estrus are observed in
cows with low serum concentrations of βC [5]. A positive correlation has been reported between βC concentrations in the CL, CL diameter and plasma P4 concentrations during different stages of estrus and the second trimester of pregnancy [6, 18]. KAWASHIMA et al. [27] demonstrated that changes in plasma βC concentrations during the peripartum period differ between ovolitary and anovulatory cows during the first follicular wave postpartum. This indicates that low βC concentrations in plasma during the prepartum period may be one of the factors related to the complex mechanism of first postpartum anovulation in dairy cows.

Beta-carotene is also a potent antioxidant [10] and giving it has an important role in immunity. During the first 24 hours after parturition, plasma βC concentrations decrease in cows, but increase in newborns fed colostrum, and a positive correlation between the plasma concentration in cows and colostrum has been reported [26].

Calving, the involution period and the start of lactation result in metabolic stress associated with an increase in the production of free radicals and reactive oxygen species [16]. Administration of antioxidants and an adequate supply of nutrients are the most appropriate strategies for eliminating or minimizing metabolic stress and its detrimental effect [12]. It is well known that during this period, especially during the first week of the pp period, the βC concentrations in plasma dramatically decrease in cows [33]. Therefore this study was designed to determine the effects of postpartum βC injections on blood serum βC concentrations, fertility parameters and reproductive disorders in cows before synchronization with PGF2α.

**Material and Methods**

**ANIMALS AND TREATMENTS**

The study consisted of 124 postpartum (pp) Holstein-Friesian cows between 4 to 5 years of age without any pp problems, such as retained fetal membranes and acute metritis. The study was performed on a commercial dairy farm and cows were fed with a total mixed ration twice daily that included corn and grass silage, hay, canola and a balanced grain ration. Animals were divided randomly into five groups.

First, βC was administered to all groups through an injection. The βC dose was determined in accordance with previous data [13] and the manufacture’s recommendation. The total dose was divided into two doses and injected in different areas of the rump. In group I (GI; n=25), βC (Carofertin®, 0.4 mg/kgBW i.m., Alvetra® Werft AG) was injected twice on d 15 and 45 pp. In group II (GII; n=25), βC was administered only once on d 15 pp. In group III (GIII; n=25), βC was also injected only once on d 45 pp. In group IV (GIV; n=25), βC was administered two times on d 35 and 45 pp. In group V (GV; n=24), no βC injection was given and it was kept as a control group. All of the animals in the study received PGF2α (Triprost trometamol, Iliren® 0.0015 mg/kgBW; i.m., Intervet) on d 50 and 61 pp after the βC injection (Figure 1).

**BLOOD SAMPLES AND BETA-CAROTENE ASSAYS**

Blood samples were collected just before βC injections and on d 15 pp, i.e. at the start of the study, and three d after each βC injection in all groups, i.e., on d 18 pp in GI; on d 48 pp in GII and on d 38 pp in GIV. Blood samples were also collected on d 48 pp in all groups. In GV, it was only collected on d 15 and 48 pp (Figure 1).

Ten milliliters of blood samples for determination of serum concentration of βC was obtained from jugular vein by use of vacutainer vials. Blood samples were centrifuged immediately after collection for 15 min at 2000 x g at room temperature; serum was decanted and stored at -20°C until assayed.

Beta-carotene concentrations were determined by spectrophotometric analysis and measured as described previously [39] after extraction using a carotene photometer (Schimadzu UV-mini 1240). Extraction efficiency was > 95%. The inter-assay coefficients of variation averaged 9.7%.

**FERTILITY PARAMETERS**

The following parameters were evaluated in this study: The reproductive disorder rate (endometritis and cystic ovaries; %), first service pregnancy rates (FSPR; pregnancy rates after first insemination; %), overall pregnancy rates (OPR; pregnancy rates after a maximum of three consequent insemina-
Beta-carotene concentrations were found to increase substantially ($P < 0.001$) on d 15 and 48 in all groups injected with βC (GI, GII, GIII and GIV). βC serum concentrations were increased in GII ($P < 0.01$) on d 48, but remained lower than the values obtained in the other groups. The greatest increase in βC concentrations was found on d 48 pp compared to d 15 pp in GIV, to which βC injections were administered on d 35 and 45 pp ($P < 0.001$). No increase was found in the control group ($P > 0.05$) (Table I).

There were no differences in βC concentrations in any of the groups on d 15 pp ($P > 0.05$) when the βC concentrations were compared. However, a significant increase was found in the groups on d 48 pp, when βC concentrations were found to be significantly higher in groups injected with βC (GI, GII, GIII and GIV) than in GV ($P < 0.001$). The βC concentrations were found to be higher on d 18 than on d 15 pp in GI and GII ($P < 0.001$). There was a significant difference between the values obtained on d 15 and 38 pp in GIV ($P < 0.001$). On the other hand, there was no difference between on d 38 and 48 pp ($P > 0.05$). However, no statistically significant difference was found between GIII and GIV ($P > 0.05$) on d 48 pp (Table I and Figure 2).

After the injections, OPR was determined to be significantly higher in GI (100%; $P < 0.01$) and GIV (88.0; $P < 0.05$) than it was in other groups (GII, GIII and GV; 80%, 84% and 70.8%, respectively). On the other hand, the INI of GI was determined to be significantly lower than GIII and GV ($P < 0.05$). The D-FS were found significantly lower in GII, GIII and GIV than in GV ($P < 0.05$) (Table II). The D-OP values of GI and GII tended to be lower than those of GIII and GV ($P < 0.07$).

The number of cases of endometritis in the study was found to be significantly ($P < 0.05$) lower in GIV (0.0%) than in the control group (16.66%) while it was lower in other study groups (8.0%; GI, GII and GIII) compared to GIV (Table III). All cases of endometritis were detected after d 61.

### Discussion

It has been demonstrated that βC is normally transported to the ovary and CL with high density lipoproteins in the cow...
The lower plasma βC concentrations during the prepartum period might explain why the double injection of PGF2α is considered to increase fertility. The uptake of βC by the CL and the endometrium in various species has been demonstrated by previous studies [14, 36]. Therefore, as an antioxidant, βC in the endometrium provides a more optimal uterine environment for implantation and embryo development [43]. This finding might explain the high OP results after βC injections. The data obtained from this study suggest that βC injections prior to PGF2α injections increase OP. Moreover, the report from ARECHIGA et al. [1] might explain why the double injection of βC is more effective in terms of improving OP than the single injection in our study. According to this report, there is a need for long term supplementation in heat stressed cows in order to provide adequate βC concentration in uterine tissue.

DRILLICH et al. [14] reported that days open in cows with spontaneous estrus and induced estrus (PGF2α) yielded 110.9 ± 34.5 and 113.5 ± 35.5 d (P < 0.05), respectively. While D-FS was found to be similar to GV (112.9 ± 10.8 d) in this study, it was found to be lower in all injected groups. It was also determined to be significantly lower in GI, GIII and GIV than in the GV. Although, D-OP was found between 100.2 and 114.9 d in all groups, which is similar to our present research, no significant difference was found among the groups.

KAWASHIMA et al. [27] reported that both the energy status and plasma βC concentrations during the peripartum period may affect ovulation in the first follicular pp wave in dairy cows. Plasma concentrations of βC decrease throughout the dry period and reach their nadir in about the first week pp. The lower plasma βC concentrations during the prepartum period directly affect the occurrence of anovulation [27, 42].

Table II: Fertility parameters evaluated in the present study.

<table>
<thead>
<tr>
<th>Parameters (%)</th>
<th>GI (n=25)</th>
<th>GII (n=25)</th>
<th>GIII (n=25)</th>
<th>GIV (n=25)</th>
<th>GV (n=24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INI(No)</td>
<td>1.4a</td>
<td>1.4a</td>
<td>1.6a</td>
<td>1.6a</td>
<td>1.5a</td>
<td>NS</td>
</tr>
<tr>
<td>PI (No)</td>
<td>1.4a</td>
<td>1.8a,b</td>
<td>2.0b</td>
<td>1.9a,b</td>
<td>2.0b</td>
<td>a,b: &lt; 0.05</td>
</tr>
<tr>
<td>D-FS (X±SD; d)</td>
<td>95.9±6.2a,b</td>
<td>98.2±7.4a</td>
<td>90.3±7.2a,b</td>
<td>90.8±5.5a,b</td>
<td>112.9±10.8c</td>
<td>b,c: &lt; 0.05</td>
</tr>
<tr>
<td>OP (%)</td>
<td>100a,c</td>
<td>80.0b</td>
<td>84.0b</td>
<td>88.0a</td>
<td>70.8b,d</td>
<td>a,b: &lt; 0.05; c,d: &lt; 0.01</td>
</tr>
<tr>
<td>FSPR (%)</td>
<td>60.0a</td>
<td>56.0a</td>
<td>44.0a</td>
<td>40.0a</td>
<td>45.8a</td>
<td>NS</td>
</tr>
<tr>
<td>D-OP (X±SD; d)</td>
<td>100.2±7.6a</td>
<td>101.6±6.3a</td>
<td>114.9±13.6a</td>
<td>111.2±7.7a</td>
<td>114.4±10.4a</td>
<td>NS</td>
</tr>
<tr>
<td>OPR (%)</td>
<td>100.2±10.4a</td>
<td>101.6±6.3a</td>
<td>114.9±13.6a</td>
<td>111.2±7.7a</td>
<td>114.4±10.4a</td>
<td>NS</td>
</tr>
<tr>
<td>INI(No)</td>
<td>1.4a</td>
<td>1.8a,b</td>
<td>2.0b</td>
<td>1.9a,b</td>
<td>2.0b</td>
<td>a,b: &lt; 0.05</td>
</tr>
</tbody>
</table>

Table III: Rate of reproductive disorders in groups.

<table>
<thead>
<tr>
<th>Parameters (%)</th>
<th>GI (n=25)</th>
<th>GII (n=25)</th>
<th>GIII (n=25)</th>
<th>GIV (n=25)</th>
<th>GV (n=24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometritis</td>
<td>8.0a</td>
<td>8.0a</td>
<td>8.0a</td>
<td>0.0a,b</td>
<td>16.66a,c</td>
<td>b,c: &lt; 0.05</td>
</tr>
<tr>
<td>Cystic ovaries</td>
<td>0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
<td>4.0a</td>
<td>8.3a</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values with different superscripts (a,b,c) in the same row are significantly different (P < 0.05; P < 0.01). NS: Non-Significant.
since it takes about six weeks for antral follicles to reach the preovulatory stage [42] while the dominant follicle of the first or second follicular wave reaches preovulatory size at two to three weeks pp. These physiological explanations demonstrate why the PGF$_2\alpha$ application after the $\beta$C injections on d 15 and 45 pp, which were administered to positively influence ovarian activity, increased fertility parameters. In our opinion, $\beta$C injections applied during this period enhance the positive effect of some other hormones and various factors that are important to the genital tract by increasing serum $\beta$C concentrations. Thus $\beta$C injections make the sexual cycle’s synchronization with PGF$_2\alpha$ more successful.

The supplementation of $\beta$C reduced the incidence of retentio secundinarum and endometritis in cows [30]. ASLAN et al. [5] also demonstrated that $\beta$C injections combined with GnRH injections during the postpartum period reduced periperal disorders and improved fertility in cows. The data showed that the endometritis rate after the $\beta$C injection was found to be higher in the GV (16.66%) than in injected groups. It might be supposed that the incidence of endometritis in GV was higher considering that the number of animals in each group is equal though the incidence of endometritis in the GV was only significantly higher compared to GIV. The relationship between immunity and $\beta$C is well known. Beta-carotene involves the leukocyte membrane [8] and stimulates lymphocyte proliferation and phagocytosis. As a result, the activated natural defense mechanism leads to a decreased incidence of mastitis [9, 33] and retentio secundinarum [33]. KAEWLAMUN et al. [25] have shown that the percentage of neutrophils in endometral swab from the cervix and uterus at d 28 pp is lower in the group that received $\beta$C than it is in the control group. These data may explain the lower incidence of endometritis in the groups that received $\beta$C in our study.

Beta-carotene is found in the bovine CL in extremely high concentrations and is responsible for the characteristic bright yellow color of the CL [36]. Using the Raman spectroscopy method, ARIKAN et al. [3] have shown that $\beta$C is spread over the entire volume of the luteal cells with higher concentrations occurring at distinct sites, including the surface. It has been reported that high LH and $\beta$C concentrations in luteal cells may be involved in luteolysis [2]. It is also known that the $\beta$C deficiency plays an important role in the formation of ovarian cysts [7]. INABA et al. [21] reported that cows with ovarian cysts had significantly lower plasma $\beta$C concentrations than cows without ovarian cysts. It has also been reported that cows which receive $\beta$C supplements cows have a much lower incidence of ovarian cysts than cows without supplements [32]. As a result of the early pp $\beta$C injections, ovarian cysts were not found due to an increase in the concentrations of serum $\beta$C. However, ovarian cysts were found in only one case in GIV (4.0%), and two cases in the GV (8.3%). Ovarian cyst results in this study correspond to the findings of earlier research, which are consistent with follicular wave and anovulation. Several studies have failed to find beneficial effects on fertility from $\beta$C supplementation [4, 15], but both studies are related to follicular wave [27, 42] and our findings suggest that the injection time of $\beta$C is important.

It has also been reported that more than 50% of the cysts regressed spontaneously in the early pp period [27, 42], and the majority of ovarian cysts (70%) developed within 45 d after calving [44]. The rate of 4.0% suggests that ovarian cysts in GIV might be a consequence of injections of $\beta$C during the late pp period. In this study, the fact that groups which were injected with $\beta$C in the early pp period did not develop ovarian cysts supports the authors referred to above.

In summary, the data obtained from this study clearly show the positive effect of $\beta$C injections on fertility parameters. Even a single injection of $\beta$C during the pp period before PGF$_2\alpha$ treatment is sufficient to increase blood $\beta$C concentrations and improve fertility. On the other hand, a double injection of $\beta$C in both early (d15) and late (d45) pp period is much more effective at improving fertility, especially OPR, in cows. These positive effects can be related to various factors in the endometrium [12]. Therefore, further studies are necessary to investigate the relationship between endometrial factors and $\beta$C supplementation; its dose, supplementation route, and the time of administration.

In conclusion, this study demonstrated that $\beta$C injections have a significant positive effect on fertility parameters at d 15 and 45 pp before the PGF$_2\alpha$ injections. It was concluded that with this method of administration in GI results in a better OPR, PI and INI as well as significantly lower D-OP than in the other groups in this study.

Acknowledgement

The authors thanks Alvetra&Werfft AG (Wien, Austria) for their financial supports; and Akbel Milk and Milk Product Company (Eregli, Turkey) for making this study possible in their dairy farm.

References


