#### **RESEARCH ARTICLE**

## Effects of Peripartum Supplementation of $\beta$ -Carotene on Postpartum Fertility in Lactating Kankrej Cows

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#### Abstract

A study was undertaken to assess the effect of  $\beta$ -carotene supplementation on fertility in lactating Kankrej cows. Advanced pregnant cows (n=18) were randomly assigned into three groups. Experimental animals in Group I were fed on basal diet, while in Group-II and III were supplemented with 500 and 1000 mg of  $\beta$ -carotene/animal/day, respectively, for a period of 42 days, 21 days each before and after calving. All the cows were subjected to per-rectal and ultrasonographic observations at the 7<sup>th</sup>, 15<sup>th</sup>, 21<sup>st</sup> and 30<sup>th</sup> day postpartum. The gross uterine involution occurred at 28.50±1.50, 25.50±2.01 and 24±1.89 days in Group I, II and III, respectively. The diameter of the cervix was found to differ non-significantly between the groups on the 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> day in Group II and II. There was a highly significant reduction in the mean diameter of the gravid and non-gravid horn on the 7<sup>th</sup> day in Group II and III as compared to Group I, and also on the 21<sup>st</sup> and 15<sup>th</sup> day, respectively, within Group I and II. First service conception rate and overall conception rate were observed to be higher in Group III followed by Group II and I. It was concluded that  $\beta$ -carotene supplementation peripartum @ 1000 mg/animal/day for a period of 42 days accelerates uterine involution and improves postpartum fertility in dairy animals. **Key words:** Beta-carotene, Kankrej cattle, Postpartum fertility, Ultrasonography, Uterine involution

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#### INTRODUCTION

'itamin A does not exist as such in plants, but it is present as pro-vitamins in the form of certain carotenoids. One such carotenoid is Beta-carotene which is red in colour, but in dilute solutions it appears as orange to yellow. Green plant tissue contains 90%  $\beta$ -carotene and 10% gamma carotene and other forms. Beta-carotene has a specific role in reproduction and is involved in the formation of estradiol-17 $\beta$  in tertiary follicles and progesterone in corpora lutea, maturation and functional integrity of oviduct, uterus and placenta (Kumar et al., 2010). In cow reproduction, the transition period is crucial. Nutritional and non-nutritional factors have an impact on bovine reproduction. Diets deficient in β-carotene had been shown to affect the reproductive performance of animals due to decreased P4 output, delayed ovulation, low estrus intensity, high incidence of cystic ovarian degeneration, embryonic mortality and abortions (Jukola et al., 1996; Arthur et al., 2001; Gaikwad et al., 2007). Uterine involution is characterized by uterine contractions, physical shrinkage, necrosis, caruncle sloughing, and endometrial regeneration (Noakes et al., 2009). Hence, this study was planned to assess the effect of supplementation of  $\beta$ -carotene on postpartum fertility parameters in lactating Kankrej cows.

#### **MATERIALS AND METHODS**

The experiment was conducted at Livestock Research Station, Kamdhenu University, Sardarkrushinagar, Gujarat, India following approval vide VETCOLL/IAEC/2022/19/ PROTOCOL-09 by the Institutional Animal Ethics Committee <sup>1</sup>Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar-385506, India

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(IAEC) of the College under Kamdhenu University, Sardarkrushinagar, Gujarat.

#### **Experimental Design**

Apparently healthy advanced pregnant Kankrej cows (n=18) of 2<sup>nd</sup> to 7<sup>th</sup> parity were selected and randomly assigned to three experimental groups (6 cows per treatment). Group I was fed on basal diet (containing concentrate mixture, green maize fodder and groundnut straw) and experimental animals in Group-II and Group-III were allocated to basal diet supplemented peripartum with 500 and 1000 mg of  $\beta$ -carotene/animal/day (DSM Rovimix 8%  $\beta$ -carotene),

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# respectively, for a period of 42 days, 21 days prepartum and 21 days postpartum. $\beta$ -carotene was mixed with concentrate thoroughly and provided once daily throughout the experimental period. The basal diet was formulated to meet the nutrient requirements as per ICAR (2013).

#### **Assessment of Uterine Involution and Biometry:**

The uterine involution was considered complete when the uterus regained its nearly normal pre-gravid location, size and tone by per rectal examination (Noakes *et al.*, 2009). The transrectal ultrasonography was performed using a real-time B-mode ultrasound scanner (Titan, Sonosite Ltd; Hitchin, UK) equipped with a 5-10 MHz linear array transducer designed for intra-rectal placement on the 7<sup>th</sup>, 15<sup>th</sup>, 21<sup>st</sup> and 30<sup>th</sup> day postpartum to assess uterine involution as well as uterine and cervical diameters, and ovarian activity.

### Occurrence of Postpartum Estrus and Conception Rate:

First postpartum expressed estrus was observed and recorded by the number of days between calving and first behavioral estrus. The conception rates with respect to first service and overall conception up to three consecutive Artificial Inseminations were calculated for each of three groups.

#### **Statistical Analysis:**

The data on cervical and uterine diameter was analyzed using one-way ANOVA in SPSS 20.0 Software and significant differences among the treatment means were determined using DMRT at p<0.05 (Snedcor and Cochram, 1994).

#### **R**ESULTS AND **D**ISCUSSION

Mean days at gross uterine involution in different groups of postpartum Kankrej cows are given in Table 1. Genitalia were found to be situated within the pelvic cavity in 16.66 %, 33.34 % and 50. 00% of cows in group I, II, and III, respectively, on day 15 postpartum. The organs were found to have a further reduction in size and on day 21 postpartum with 50.00%, 50.00% and 100% of cows in Group I, II and III, respectively, had their genitalia located within the pelvic cavity. The remaining 2 (33.34%) and 1 (16.66%) cow from Group I and Group II, respectively, had their genitalia back within the pelvis by the 30<sup>th</sup> day postpartum.

**Table 1:** Days at gross uterine involution (Mean  $\pm$  SE) in different groups of Kankrej cows supplemented with  $\beta$ -carotene peripartum

Groups	Uterine involution (days) Per rectal		
Group-I (n=6)	28.5±1.50		
Group-II (n=6)	25.5±2.01		
Group-III (n=6)	24.0±1.89		

Postpartum uterine tonicity, elasticity and curling were observed increasing from 7<sup>th</sup> to 21<sup>st</sup> day in all animals, being more pronounced in beta-carotene supplemented groups. However, the parturition to uterine involution interval was not affected (p>0.05) by  $\beta$ -carotene feeding. These results support previous observations on dairy cows (Rakes *et al.*, 1985; Zubova *et al.*, 2021). Contrary to this result, Akordor *et al.* (1986) reported longer time for involution in Holstein cows.

The mean diameter of the cervix was found to differ non-significantly between the groups on the 7<sup>th</sup>, 15<sup>th</sup> and

**Table 2:** Ultrasonographic measurements of cervical, gravid and non-gravid uterine diameters (Mean $\pm$ SE) in different groups of postpartum Kankrej cows supplemented with  $\beta$ -carotene peripartum

		Treatment groups (n=6 each)			
Parameter	Postpartum days	Group I (Control, Basal diet)	Group II (500 mg β-carotene/day)	Group III (1000 mg β-carotene/day)	
Cervical diameter (cm)	7	4.63±0.18 <sup>c</sup>	4.30±0.17 <sup>c</sup>	4.07±0.26 <sup>b</sup>	
	15	4.15±0.16 <sup>bc</sup>	3.98±0.17 <sup>bc</sup>	3.66±0.24 <sup>ab</sup>	
	21	$3.97 \pm 0.15_{B}^{ab}$	3.58±0.15 <sub>AB</sub> <sup>ab</sup>	3.32±0.19 <sub>A</sub> <sup>a</sup>	
	30	3.57±0.15 <sup>a</sup>	3.17±0.17 <sup>a</sup>	2.97±0.19 <sup>a</sup>	
Gravid horn diameter (cm)	7	4.77±0.21 <sup>c</sup>	4.01±0.13 <sup>b</sup>	3.83±0.21 <sub>A</sub> <sup>b</sup>	
	15	4.04±0.11 <sub>B</sub> <sup>b</sup>	3.62±0.19 <sub>AB</sub> <sup>b</sup>	3.23±0.30 <sup>ab</sup>	
	21	3.37±0.2 <sup>a</sup>	3.16±0.11 <sup>a</sup>	2.85±0.26 <sup>a</sup>	
	30	2.98±0.13 <sup>a</sup>	2.77±0.13 <sup>a</sup>	2.48±0.20 <sup>a</sup>	
Non-gravid horn diameter (cm)	7	4.26±0.23 <sup>c</sup>	3.55±0.15 <sub>A</sub> <sup>c</sup>	3.01±0.15 <sub>A</sub> <sup>b</sup>	
	15	3.23±0.12 <sub>B</sub> <sup>b</sup>	2.99±0.15 <sub>AB</sub> <sup>b</sup>	2.63±0.15 <sub>A</sub> <sup>ab</sup>	
	21	2.81±0.12 <sup>ab</sup>	2.64±0.17 <sup>ab</sup>	2.49±0.15 <sup>a</sup>	
	30	2.44±0.16 <sup>a</sup>	2.32±0.14 <sup>a</sup>	2.21±0.13 <sup>a</sup>	

Means bearing different subscripts within the row (A-B) and superscripts within the column (a-c) differ significantly (p<0.05) and/or highly significantly (p<0.01) for a parameter.

Groups (n=6)	First postpartum expressed estrus (days)	Total no. of animals inseminated	1 <sup>st</sup> service conception rate (%)	Fertile Estrus (days)	Overall conception rate (%)
Group I	96.17±6.16	6	16.67 (1/6)	120±12.04 (2/6)	33.33 (2/6)
Group II	81.66±6.38	6	33.33 (2/6)	119.33±7.00 (3/6)	50.00 (3/6)
Group III	76.66±6.51	6	33.33 (2/6)	110.5±4.37 (4/6)	66.67 (4/6)

**Table 3:** First postpartum expressed estrus, conception rates (%) to first AI and overall of three AIs in different groups of Kankrej cows supplemented with  $\beta$ -carotene peripartum

 $30^{th}$  day and a significant (p<0.05) reduction in diameter was found on the  $21^{st}$  day in Group III as compared to Group I and II. Fonseca *et al.* (1983) and Wang *et al.* (1988) noted nearly similar observations in Holstein cows. Rakes *et al.* (1985) recorded smaller cervical diameter at  $21^{st}$  and  $28^{th}$  day postpartum in Holstein cows. Kaewlamun *et al.* (2011) shown that the percentage of neutrophils in an endometrial swab from the cervix and uterus at day 28 postpartum was lower in the group that received  $\beta$ -carotene than in the control group. Thus,  $\beta$ -carotene might have played a role in the faster reduction of cervix diameter in the present study.

The mean diameter of the gravid and non-gravid horns differed non-significantly between the groups on 21<sup>st</sup> and 30<sup>th</sup> days (Table 2). There was a highly significant (p<0.01) reduction in diameter on the 7<sup>th</sup> day in Group II and Group III as compared to Group I, while on the 15<sup>th</sup> day, significant (p<0.05) reduction was observed in Group III as compared to Group I in both uterine horns. Statistical analysis revealed that there was a highly significant (p<0.01) reduction on the 21<sup>st</sup> and 15<sup>th</sup> day in gravid and non-gravid horn within Group I and Group II. The mean diameters of gravid and non-gravid uterine horns were in agreement with previous reports (Rakes et al., 1985; Kaewlamun et al., 2011; Zubova et al., 2021). According to Colitti and Stefanon (2006) an adequate supply of  $\beta$ -carotene is the most appropriate strategy for eliminating or minimizing metabolic stress and its detrimental effect. Bindas et al. (1984) did not fine significant beneficial effect of supplementation with  $\beta$ -carotene on any of the reproductive performance traits.

The observations on first postpartum expressed estrus, conception rates (%) to first AI and overall conception rates (%) of 3 Als in different groups of Kankrej cows are presented in Table 3. There was no significant influence of  $\beta$ -carotene supplementation on first postpartum expressed estrus, though it was quite shorter in supplemented than nonsupplemented groups. These findings supported those obtained by Arechiga et al. (1998) in dairy cows, in which they also recorded less time for first postpartum estrus in  $\beta$ -carotene supplemented group. The findings of the 1<sup>st</sup> service conception rate as 16.67, 33.33 and 33.33% in group I, II and III, respectively, were in full agreement with the earlier reports in dairy cows (Bian et al., 2007; Ay et al., 2012; Oliveira et al., 2015), wherein an improvement in first service conception rate was noted in  $\beta$ -carotene supplemented cows. This might be due to enhanced uterine involution and favourable uterine environment for the establishment of a successful conception in  $\beta$ -carotene supplemented cows. Contrary to

our findings, De Ondarza *et al.* (2009) observed a lower 1<sup>st</sup> service conception rate, while Fonseca *et al.* (1983) reported a higher 1<sup>st</sup> service conception rate in HF cows. Overall conception rate was higher in the 1000 mg/day  $\beta$ -carotene supplemented group (66.67%) as compared to 500 mg/day  $\beta$ -carotene (Group II, 50.00%) and non-supplemented control (Group I, 33.33%). However, the days open or service period did not vary between three groups (Table 3).

#### CONCLUSIONS

Based on the results of the present study, it can be concluded that  $\beta$ -carotene can be supplemented peripartum in Kankrej cows ration at the rate of 1000 mg/animal/day for a period of 42 days (21 days prepartum and 21 days postpartum) for early uterine involution and improved first service and overall conception rates.

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