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Relationship between Plasma β-Carotene Concentration and Embryo Quality in Superovulated Japanese Black Cattle

Kazufumi GOTO, Osamu KAJISA, Kanta EZOE, Yoshihiko NAKANISHI, Kiyohiko OGAWA, Michihiro TASAKI*, Hitoshi OHTA*, Satoru INOHAE*, Shoichi TATEYAMA* and Takeshi KAWABATA*

(Laboratory of Animal Reproduction)

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Introduction

Vitamin A is essential for reproduction in cattle[8]. β-Carotene, a provitamin A, by itself may also function in reproduction in cattle[7,13-14].

The importance of β-carotene in bovine reproduction is equivocal. Lotthammer[13-14] reported that dairy cows fed little β-carotene showed an increased incidence of silent estrus, ovarian cysts, delayed ovulation, extended calving interval, lower conception rate, and an increased incidence of embryonic mortality compared with cattle supplemented with β-carotene. Inaba *et al.*[10] also reported that the plasma levels of β-carotene were significantly lower in cows with ovarian cysts than those in cows without ovarian cysts. In contrast, there are reports that the cattle fed diets containing varying levels of β-carotene do not significantly differ in the incidences of silent estrus[22], ovarian cysts[12,17], and postpartum anestrus[1,12]. Similarly, conception rate[1,3,22] and plasma progesterone concentration[1,3,12,22] were not influenced by β-carotene. Recently, Greenberg *et al.*[7] have reported that it is unlikely that β-carotene plays a major role in bovine fertility. However, β-carotene supplementation improved weight gain of heifers and increased the concentration of progesterone prepartum.

Lotthammer & Ahlswede[15] and Schams *et al.*[19] reported that serum β-carotene content was related to ovarian function in cattle. They showed an improvement in conception rate, intensity of estrus, and changes in luteinizing hormone patterns in the case when cattle received β-carotene supplementation of low carotene diets. Meyer *et al.*[18] reported that heifers of low carotene diets had a smaller corpus luteum than those of carotene-supplemented group. Lotthammer *et al.*[16] reported that blood and milk progesterone were lower for cows fed low-carotene rations.

As we described above, there are so many controversial reports about the effects of β-carotene on bovine reproduction and some of them have indicated that β-carotene may have effect on endocrine profile of cattle. We[4-6] have recently reported that plasma humoral profiles had a significant effect on embryo quality in superovulated Japanese Black cattle. There are few reports which have examined the effect of β-carotene on embryo quality of superovulated cattle. Therefore, we tried to clarify the relationship between plasma concentration of β-carotene and embryo quality in superovulated cattle.

*Kagoshima Prefectural Livestock Experimental Station, *Kokubu*-shi, Kagoshima 899-44 Japan

Materials and Methods

Hormones

Follicle stimulating hormone (FSH-P, Antrin) and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) analogues, estrumate and pronalgon F were from Denka Co., Japan, ICI Pharma, Canada and Upjohn Co., Japan, respectively. β-Carotene was from Merck, Germany.

Animals and superovulation treatment

Total 33 non-lactating Japanese Black cattle (6-14 years of age) were used for experiment. The cows were kept in paddocks as a group and fed corn silage and alfalfa hay *ad libitum*. The cows were superovulated by FSH-P treatment. Total doses of FSH-P ranged from 30-39 mg, given twice daily over 4 or 4.5 days. The dose was decreased each day of treatment. $PGF_2\alpha$ analogues (pronalgon F, 40 mg, 20+10+10 mg ; estrumate 0.75 mg) were administered on the third day of FSH-P treatment either in one dose or two doses (estrumate) or three doses (pronalgon F) given 6h apart. Animals were observed every 6-12 h for standing estrus. The animals exhibiting standing estrus were artificially inseminated with frozen semen from bulls of known fertility approximately at 0, 8, 24, and 32 h after standing estrus.

Embryo collection and blood sampling

The embryos were collected nonsurgically on the 11th or 12th day of treatment (Day 0=first treatment day), namely, 7 or 8 days after estrus, and classified as transferable or non-transferable (unfertilized, degenerated and retarded), based on the morphological evaluation.[11] The number of corpora lutea (CL) and unovulated follicles (FL) were estimated by a rectal palpation on the day of embryo collection. Blood samples were obtained twice per day from the first to the 5th day of treatment and once on the 8th and 11th or 12th days. The plasma was separated by centrifugation and stored at −40℃ until β-carotene assay. Only the samples from the first and the last collections for each animal were used for β-carotene assay.

Extraction of plasma β-carotene

Plasma β-carotene was extracted by the method of Ikeda *et al.*[9]. Briefly, 2 ml of plasma was added to the mixture of ethylalchol (2 ml) and petroleum ether (4 ml) and vortexed vigorously for 2 min, and stood for 10 min and the upper phase was used for the assay.

Assay of β-carotene

Optical densities of extracted samples were measured at 430 nm and the concentrations of β-carotene were calculated as described by Ikeda *et al.*[9].

Grouping of cattle before statistical analysis

Animals were divided into 2 groups, depending on the plasma concentration of β-carotene ($<$ 200 or $>$200 μg/dl) on the first treatment day and on the day of embryo collection. This classification was based on the report of Lotthammer[14] which indicated that bovine fertility was to be affected when plasma contained less than 200 μg/dl of β-carotene.

Statistical analysis

Data were tranformed into square root response and Student's t-test[20] was used for the statistical significance of differences between means.

Results

Table 1 shows the relationship between the plasma β-carotene concentration on the first treatment day and the numbers of CL, total (recovered) embryos (TE), normal (transferable) embryos (NE) and FL. The numbers of CL and TE tended to be larger in Group 2 (β-carotene concentration >200 μg/dl) than those in Group 1 (β-carotene concentration <200 μg/dl). The number of NE was significantly ($P<0.05$) larger in Group 2 than that in Group 1.

Table 2 shows the relationship between the plasma β-carotene concentration on the day of embryo collection and the numbers of CL, TE, NE and FL. The numbers of CL and TE tended to be larger in Group 2 than those in Group 1. The number of NE was significantly ($P<0.05$) larger in Group 2 than that in Group 1. The number of FL was significantly ($P<0.05$) smaller in Group 2 than that of Group 1.

Discussion

The objective of this study was to examine the relationship between plasma β-carotene concentration and embryo quality in superovulated Japanese Black cattle. The result indicated that plasma β-carotene concentration was related to embryo quality. Plasma β-carotene concentration

Table 1. Relationship between the plasma β-carotene levels on the first treatment day and the numbers of corpora lutea (CL), total (recovered) embryos (TE), normal (transferable) embryo (NE) and unovulated follicles (FL) in superovulated Japanese Black cattle

	β-carotene (μg/dl)	
	<200	>200
N	11	22
CL	11.9±1.8	18.6±2.5
TE	10.2±1.8	15.9±2.8
NE	3.6±1.7	7.8±1.7*
FL	1.6±0.4	1.0±0.3

Values are mean ± s.e.m.
*$P<0.05$

Table 2. Relationship between the plasma β-carotene levels on the day of embryo collection and the numbers of corpora lutea (CL), total (recovered) embryos (TE), normal (transferable) embryo (NE) and unovulated follicles (FL) in superovulated Japanese Black cattle

	β-carotene (μg/dl)	
	<200	>200
N	8	25
CL	12.0±2.5	17.7±2.3
TE	10.0±2.2	15.3±2.5
NE	2.6±1.5	7.6±1.6*
FL	2.0±0.6	1.0±0.3*

Values are mean ± s.e.m.
*$P<0.05$

of above 200 μg/dl was required for good superovulation response in cattle. This was agreed with the report of Lotthammer[14] that indicated bovine fertility was affected when plasma contained less than 200 μg/dl of β-carotene.

Suzuki & Hane[21] also reported a similar relationship between plasma β-carotene concentration on the day of embryo collection and the number of NE. In addition, the present result indicated that plasma β-carotene concentration not only on the day of embryo collection but also on the first treatment day was related to the quality of embryos. This finding suggests that the quality of embryos may be predicted before the cattle are superovulated.

Goto *et al.*[4-6] reported that the ovarian function, especially the function corpus luteum on the first treatment day is an important factor for reliable superovulation in cattle. Lotthammer & Ahlswede[15] and Schams *et al.*[19] reported that serum β-carotene concentration was related to ovarian function in cattle. Meyer *et al.*[18] reported that heifers of low carotene diet had a smaller corpus luteum than those of carotene-supplemented group. Lotthammer *et al.*[16] reported that blood and milk progesterones were lower for cows fed low carotene rations. These reports in conjunction with our present result suggest the importance of β-carotene on the normal function of ovary, especially on the function of corpus luteum, both in the normal and in the superovulated cattle.

The result of the present study suggests that plasma β-carotene concentration is related to embryo quality in superovulated cattle. Approximately over 200 μg/dl of plasma β-carotene is required for good superovulation response in cattle.

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Summary

The relationship between plasma β-carotene concentration and embryo quality was examined in 33 superovulated Japanese Black cattle. The superovulation was induced by follicle stimulating hormone (FSH) and prostaglandin (PG) analogue treatment. Both plasma β-carotene concentrations on the first treatment day and those on the day of embryo collection were apparently related to embryo quality. Approximately over 200 μg/dl of plasma β-carotene appears to be required for good superovulation response in cattle.