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Mem. Fac. Agr. Kagoshima Univ., **25**, 109–112, 1989

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Received for Publication July 28, 1988

Introduction

Major problems with superovulation in cattle include high variability in responses, treated cattle not expressing overt estrus, and cows ovulating over a wide interval of time[27].

The factors affecting superovulation response of donor cattle have been investigated[3,16,19,24,25]. The factors reported to be influence on superovulation response are the kind of hormone used[8,22], season[9,16], breed[5,21], age[4,15], nutrition[6,10], health status[7,16], level of milk production[2,23] and superovulation treatment-day in the estrus cycle[13].

Recently, several studies have indicated that the function of ovary on the first treatment day is an important factor for making a reliable superovulation in cattle[11,12,16,28].

Therefore, the present study was conducted to examine the effect of the ratios of estradiol-17 β (E_2) and progesterone (P_4), on the first treatment-day, on superovulation response of cattle.

Materials and Methods

Total 40 non-lactating Japanese Black cattle (6-14 years of age) were superovulated by follicle stimulating hormone (FSH-P, antrin, Denka Co., Japan) treatment. Total doses FSH-P ranged from 30-39mg were given twice daily over 4 or 4.5 days. The dose was decreased each day of treatment. Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) analogues (pronalgon F, 40mg, 20+10+10mg, Upjohn CO., Japan; estrumate, 0.75mg, ICI Pharma, Canada) was administered on the third day of FSH-P treatment either in one dose or two doses (estrumate) or in three doses (pronalgon F) given 6 h apart.

Animals exhibiting standing estrus were artificially inseminated with frozen semen from bulls of known fertility 3 to 4 times. Seven out of 40 cattle showed weak or persistent estrus (abnormal responses) were not bred.

The embryos were collected nonsurgically from 33 cattle 7 or 8th days after estrus, and classified as transferable or nontransferable, based on the morphological evaluation[18].

The number of corpora lutea (CL) was estimated by a rectal palpation on the day of embryo collection.

Blood samples were obtained two to four times per day from the first to the 5th days of treatment and once on the 8th and the day of embryo collection. The plasma was separated by centrifugation

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and stored at −40℃ until hormone assay. The methods of P_4 and E_2 assay were described elsewhere[11,12].

At the end of experiment, 40 cattle were divided into 5 groups depending on the following categories : Group 1, Normal (transferable) embryos (NE) 10 ; Group 2, 9NE5 ; Group 3, 4NE1 ; Group 4, NE=0 and Group 5, animals showed an abnormal response to superovulation treatment.

Statistical analysis

Data were transformed into square root reponse and analysis of variance (ANOVA)[1] and Student's t test[26] were used for statistical significance of differences between means.

Results

The numbers of animals belonging to each group were 7, 9, 11, 6, and 7 for Groups 1, 2, 3, 4 and 5, respectively. The mean numbers of NE were 19.1±3.8 (s.e.m), 7.2±0.4, 2.6±0.4 and 0.0±0.0 for Groups 1,2,3 and 4, respectively. The mean numbers of CL were 29.3±5.4, 12.8±1.0, 12.4±2.1 and 10.8±2.6 for groups 1,2,3 and 4, respectively. The mean numbers of total (recovered) embryos (TE) were 28.3±6.2, 9.9±1.1, 9.5±2.0, and 8.3±2.4, for groups 1, 2, 3 and 4, respectively. There were significant ($P<0.05$) differences in numbers of NE, CL and TE among the 4 groups.

Table 1 shows the $E_2/P_4\times10^{-3}$ values of 5 groups. The E_2/P_4 value of Group 5 was significantly ($P<0.05$) higher than those of Groups 1, 2, 3 and 4. Although there were no significant differences in E_2/P_4 values among Groups 1, 2, 3 and 4, the values tended to be larger as the responses of cattle to superovulation become poor.

Discussion

The result of this study indicated that the cattle with high E_2/P_4 values, namely with high E_2 and low P_4 on the first treatment day were not suitable for superovulation treatment. In other words, cattle with functional CL but not with large follicles on the first treatment day are expected to response well for superovulation, because P_4 and E_2 are well known to be secreted from CL and large follicle, respectively. The importance of functional CL on the first treatment day of superovulation in cattle was reported[11,12,20,28].

The importance of E_2 surge at estrus are known[12,14,27] in cattle. But the effect of E_2 level on the first treatment day (luteal phase) of donor cattle has not been clarified probably because the E_2 level is considered to be basal. However, as seen in Group 5 of this study, 7 out of 40 cattle showed high E_2/P_4 values, mainly due to high E_2 level. This is probably due to the coexistense of large follicles secreting E_2 with CL.

Table 1. Ratios of estradiol-17 β (E_2) and progesterone (P_4) of donor cattle on the first treatment day of superovulation

Group	Number of Cattle	$E_2/P_4\times10^{-3}$
1	7	2.05±0.48
2	9	2.90±0.46
3	11	4.64±1.67
4	6	3.40±1.55
5	7	33.09±25.80*

Mean±s.e.m.
*$P<0.05$.

The present result suggests that the high E_2/P_4 values on the first treatment day of donor cattle have a profound effect on superovulation responses causing poor result. From this result we suggest that the cattle having not only CL but also large follicle(s) on the first treatment day should either be treated (rupture the follicle) before the start of FSH treatment or their treatment should be postponed to the next proper time.

Summary

The effect of ratio of plasma concentrations of estradiol-17 β (E_2) and progesterone (P_4), on the first treatment day, on embryo production was examined in 40 superovulated Japanese Black cattle.

The cattle were superovulated by FSH-$PGF_2\alpha$ treatment and embryos were collected nonsurgically 7 or 8 days after estrus.

The result indicated that E_2/P_4 values on the first treatment day were related to embryo production. High E_2/P_4 values had a detrimental effect on donor cattle causing a poor response to superovulation.

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