

## Effect of the Ratio of Plasma Concentrations of Estradiol-17 $\beta$ and Progesterone on Embryo Production in Donor Cattle

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

(Laboratory of Animal Reproduction)

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<sup>27)</sup>.

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

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<sup>11,12,16,28)</sup>.

Therefore, the present study was conducted to examine the effect of the ratios of estradiol-17 $\beta$  (E<sub>2</sub>) and progesterone (P<sub>4</sub>), 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 30-39 mg were given twice daily over 4 or 4.5 days. The dose was decreased each day of treatment. Prostaglandin F<sub>2</sub> $\alpha$  (PGF<sub>2</sub> $\alpha$ ) analogues (pronalgon F, 40 mg, 20+10+10 mg, Upjohn CO., Japan; estrumate, 0.75 mg, 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<sup>18)</sup>.

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

---

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

and stored at  $-40^{\circ}\text{C}$  until hormone assay. The methods of  $\text{P}_4$  and  $\text{E}_2$  assay were described elsewhere<sup>11,12</sup>.

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)<sup>11</sup> and Student's t test<sup>26)</sup> 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  $19.1 \pm 3.8$  (s.e.m),  $7.2 \pm 0.4$ ,  $2.6 \pm 0.4$  and  $0.0 \pm 0.0$  for Groups 1, 2, 3 and 4, respectively. The mean numbers of CL were  $29.3 \pm 5.4$ ,  $12.8 \pm 1.0$ ,  $12.4 \pm 2.1$  and  $10.8 \pm 2.6$  for groups 1, 2, 3 and 4, respectively. The mean numbers of total (recovered) embryos (TE) were  $28.3 \pm 6.2$ ,  $9.9 \pm 1.1$ ,  $9.5 \pm 2.0$ , and  $8.3 \pm 2.4$ , 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  $\text{E}_2/\text{P}_4 \times 10^{-3}$  values of 5 groups. The  $\text{E}_2/\text{P}_4$  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  $\text{E}_2/\text{P}_4$  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  $\text{E}_2/\text{P}_4$  values, namely with high  $\text{E}_2$  and low  $\text{P}_4$  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 respond well for superovulation, because  $\text{P}_4$  and  $\text{E}_2$  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<sup>11,12,20,28)</sup>.

The importance of  $\text{E}_2$  surge at estrus are known<sup>12,14,27)</sup> in cattle. But the effect of  $\text{E}_2$  level on the first treatment day (luteal phase) of donor cattle has not been clarified probably because the  $\text{E}_2$  level is considered to be basal. However, as seen in Group 5 of this study, 7 out of 40 cattle showed high  $\text{E}_2/\text{P}_4$  values, mainly due to high  $\text{E}_2$  level. This is probably due to the coexistence of large follicles secreting  $\text{E}_2$  with CL.

Table 1. Ratios of estradiol-17 $\beta$  ( $\text{E}_2$ ) and progesterone ( $\text{P}_4$ ) of donor cattle on the first treatment day of superovulation

Group	Number of Cattle	$\text{E}_2/\text{P}_4 \times 10^{-3}$
1	7	$2.05 \pm 0.48$
2	9	$2.90 \pm 0.46$
3	11	$4.64 \pm 1.67$
4	6	$3.40 \pm 1.55$
5	7	$33.09 \pm 25.80^*$

Mean  $\pm$  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 $\beta$  ( $E_2$ ) 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.

### References

- 1) Barr, A.J., Goodnight, J.H., Sall, J.P. and Hellwig, J.T.: *SAS User's Guide* : Statistics, SAS Institute Inc., Raleigh, NC, pp 121-130, 173-177 (1982)
- 2) Brad, A., Trounson, A.O., Aarts, M.H., Drost, M., Zaayer, D.: Superovulation and non-surgical embryo recovery in the lactating dairy cow. *Anim. Prod.*, **26**, 55-60 (1978)
- 3) Crister, J.K., Rowe, R.F., Del-Campo, M.R., Ginter, O.J.: Embryo transfer in cattle : factors affecting superovulatory response, number of transferable embryos and length of post-treatment estrus cycle. *Theriogenology*, **13**, 397-406 (1980)
- 4) Donaldson, L.E.: Effect of age of donor cows on embryo production. *Theriogenology*, **21**, 963-967 (1984)
- 5) Donaldson, L.E.: Cattle breed as a source of variation in embryo transfer. *Theriogenology*, **21**, 1012-1013 (1984)
- 6) Dunn, T.G.: Relationship of nutrition to successful embryo transplantation. *Theriogenology*, **13**, 27-39 (1980)
- 7) Elsdon, R.P., Nelson, L.D., Seidel, G.E.Jr.: Embryo transfer in fertile and infertile cows. *Theriogenology*, **11**, 17-25 (1979)
- 8) Elsdon, R.P., Nelson, L.D., Seidel, G.E.Jr.: Superovulating cows with follicle stimulating hormone and pregnant mare's serum gonadotrophin. *Theriogenology*, **9**, 17-25 (1978)
- 9) Gordon, I.: Problems and prospects in cattle egg transfer. *Irish. Vet. J.*, **29**, 21-30 (1975)
- 10) Goto, K., Ezoe, K., Ohktho, S., Kajisa, O., Akuzawa, M., Nakanishi, Y., Ogawa, K., Tasaki, M., Ohta, H., Inohae, S., Tateyama, S., Kawabata, T.: Relationships between blood parameters and embryo quality in superovulated Japanese Black cattle. *Jpn. J. Anim. Reprod.*, **34**, 55-60 (1988)
- 11) Goto, K., Nakanishi, Y., Ohkutsu, S., Ogawa, K., Tasaki, M., Ohta, H., Inohae, S., Tateyama, S., Kawabata, T.: Plasma progesterone profiles and embryo quality in superovulated Japanese Black cattle. *Theriogenology*, **27**, 819-826 (1987)
- 12) Goto, K., Ohkutsu, S., Nakanishi, Y., Ogawa, K., Tasaki, M., Ohta, H., Inohae, S., Tateyama, S., Kawabata, T., Ishii, S., Miyamoto, A., Furukawa, T., Umezu, M., Masaki, J.: Endocrine profiles and embryo quality in superovulated Japanese Black cattle. *Theriogenology*, **29**, 615-629 (1988)
- 13) Greve, T.: Egg transfer in the bovine : Effect of injecting PMSG on different days. *Theriogenology*, **5**, 15-19 (1976)
- 14) Guay, P., Bedoya, M.: Effect of GnRH on blood serum hormone concentration, ovulation rates and embryo production in lacting cows treated with PMSG. *Can. J. Comp. Med.*, **45**, 352-356 (1981)
- 15) Hasler, J.F., Brooke, G.P., McCauley, A.D.: The relationship between age and response to superovulation in Holstein cows and heifers. *Theriogenology*, **15**, 109 (1983)

- 16) Hasler, J.F., McCaulery, A.D., Schermerhorn, E.C., Foote, R.H.: Superovulatory response of Holstein cows. *Theriogenology*, **19**, 83-99 (1983)
- 17) Hendricks, D.M., Lamond, D.R.: Hormonal interrelations in beef cows with induced multiple ovulations. *Nature. Lond.*, **235**, 222-223 (1972)
- 18) Kuzan, F.B.: Classification of embryos prior to freezing. In: *Bovine Embryo Transfer*, Colorado State Univ., Fort Collins, Co., pp 38-49 (1986)
- 19) Kweon, O.K., Kanagawa, H., Takahashi, Y., Yamashina, H., Seike, N., Iwazumi, Y., Aoyagi, Y., Ono, H.: Factors affecting superovulation response in cattle. *Jpn. J. Vet. Sci.*, **48**(3), 495-503 (1986)
- 20) Kweon, O.K., Ono, H., Yamashina, H., Seike, N., Mori, K., Kanagawa, H.: Relationship between serum total cholesterol and progesterone levels and the number of transferable embryos in superovulated Holstein Heifers and cows. *Jpn. J. Anim. Reprod.*, **31**, 231-234 (1985)
- 21) Massey, J.M., Oden, A.J.: No seasonal effect on embryo donor performance in the Southeast region of the USA. *Theriogenology*, **21**, 196-217 (1984)
- 22) Monniaux, D., Chupin, D., Saumande, J.: Superovulatory response of cattle. *Theriogenology*, **19**, 55-81 (1983)
- 23) Newcomb, R.: Investigation of factors affecting superovulation and non-surgical embryo recovery from lactating British Friesian cows. *Vet. Rec.* **106**, 48-52 (1980)
- 24) Schilling, E.: Ergebnisse von Superovulationsbehandlungen Variabilität deren Ursachen. *Dtsch. Tierarztl. Wschr.*, **89**, 88-92 (1982)
- 25) Shea, B.F., Janzen, R.E., McDermand, D.P.: Seasonal variation in response to stimulation and related embryo transfer procedures in Alberta over a nine year period. *Theriogenology*, **21**, 186-195 (1984)
- 26) Steel, R.G.D., Torrie, J.H.: Principles and procedures of Statistics. McGraw-Hill Book Company, Inc., New York, pp 67-87 (1960)
- 27) Wubishet, A., Graves, C.N., Spahr, S.L., Kesler, D.J., Favero, R.J.: Effects of GnRH treatment on superovulatory responses of dairy cows. *Theriogenology*, **25**, 423-427 (1986)
- 28) Yadav, M.C., Walton, J.S., Leslie, K.E.: Plasma concentrations of luteinizing hormone and progesterone during superovulation of dairy cows using follicle stimulating hormone or pregnant mare serum gonadotrophin. *Theriogenology*, **26**, 523-540 (1986)