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= Original Article =

Influence of large follicles on oestrus induction and ovulation after embryo collection in superovulated Japanese Black cows

Masayasu TANIGUCHI† and Takeshige OTOI

Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi, 753-8515, Japan
†Correspondence: masa0810@yamaguchi-u.ac.jp

ABSTRACT

This study evaluated whether large follicles (LF; greater than 18 mm in diameter) affect the return to oestrus and ovulation after embryo collection. Eleven superovulated Japanese Black cows were used as donors. Group 1 had LF (N = 6), and Group 2 had no LF (N = 5). All cows received 0.5 mg prostaglandin $F_2\alpha$ after embryo collection. No differences were observed between the two groups with regard to the superovulatory response and hormonal state at the time of embryo collection. Moreover, the interval to luteolysis, defined by plasma progesterone levels lower than 1ng/mL, did not differ between the two groups. However, the interval from embryo collection to the appearance of the dominant follicle, to oestrus and to ovulation was longer in Group 1. The present results suggest that the presence of LF prolongs the interval to the appearance of the new dominant follicle, thereby delaying oestrus and ovulation induction after embryo collection.

Key words: large follicles, after embryo collection

INTRODUCTION

Multiple ovulation and embryo transfer programs have been used worldwide to hasten genetic improvement and increase the number of offspring from highly valued donor cows. Rapid return to oestrus and regular cyclicity after embryo collection shortens the calving interval of donor cows and improves the efficiency of embryo production [2,4,5]. In superovulated cows, prostaglandin $F_2\alpha$ ($PGF_2\alpha$) (dinoplost) or its analogues (cloprostenol and fenprostalene) have been used to induce oestrus after embryo collection [1,2,5]. However, the interval from embryo collection to oestrus after $F_2\alpha$ treatment was considerably prolonged compared to normal cyclic cows [3,6]. Several strategies using $PGF_2\alpha$ after embryo collection to shorten the interval to oestrus induction have been studied. Some authors have reported the effect of different analogues of $PGF_2\alpha$ [5,6]. Others have evaluated the timing of $PGF_2\alpha$ treatment or dosage of $PGF_2\alpha$ [8,10]. Previous studies have demonstrated that 60–70% of superovulated cows exhibited oestrus within 10 days after $PGF_2\alpha$ treatment [1,2]. This study was designed to evaluate whether large follicles (LF) present at the time of embryo collection affect oestrus induction and ovulation after embryo collection in Japanese Black donor cows.

MATERIALS AND METHODS

Eleven Japanese Black cows were used as the donors in this experiment. Superovulation treatments were initiated mid-cycle (9–12 days after oestrus) by treatment with 21 Armour units of follicle stimulating hormone (Antrin-R10; Kyoritsu Seiyaku Corp., Tokyo, Japan) administered intramuscularly in seven injections of decreasing doses at 12-h intervals. On the third day, the cows received 0.5 mg $PGF_2\alpha$ (cloprostenol, Resipron-C, Asuka Seiyaku Corp., Tokyo, Japan) to induce regression of the corpus luteum and were artificially

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inseminated 60–72 h later. Embryos were collected 7 days after artificial insemination (Day 0).

All cows received 0.5 mg $F_2\alpha$ immediately after embryo collection and were allocated to one of two groups: cows (N = 6) with one or more LF greater than 18 mm in diameter (Group 1) and cows (N = 5) with no large follicles (Group 2). Oestrus was detected by visual observation performed twice daily after embryo collection. The collected ova/embryos were evaluated for fertilization and grade quality (excellent, good, fair, poor, and degenerated) according to the guidelines of the International Embryo Transfer Society [11]. Ovarian follicular populations were examined using a real-time B-mode scanner equipped with a 5.0-MHz transducer (Aloka SSD-500, ALOKA Co., Ltd., Tokyo, Japan). The size and number of ovarian follicles >5 mm were recorded, as were the size and number of follicles and the presence or absence of corpora lutea (CL). The cystic structure with the hyperechogenic luteinized wall was classified into the CL. Emergence of a follicular wave was defined as the day that the dominant follicle was retrospectively identified at a diameter of 5 mm [14]. Ovarian ultrasonographic examinations and blood sample collection were performed every day from Day 0 to next ovulation.

Just prior to ultrasonography, blood samples were collected from the coccygeal vein, put on ice for up to 1 h, and then centrifuged at 3000 rpm for 30 min at 4 °C. Plasma was separated and stored at −80 °C. Plasma progesterone (P4) and oestradiol (E2) concentrations were measured with a time-resolved fluoroimmunoassay (TR-FIA) system as previously reported [15]. The intra- and inter-assay coefficients of variation were 6.6% and 8.4%, respectively, for P4 and 10.2% and 17.4%, respectively, for E2. These measurements were taken once daily until the next ovulation. Data are expressed as mean values ± standard error of means (SEM). The mean values concerning the concentrations of hormones and ovaries after embryo collection between the two groups were analyzed using Student's t-test. The dynamic state of P4 and E2 were analysed by repeated analysis of variance using the GLM procedure of SAS (SAS for Windows, version 9.1, SAS Inst. Japan, Inc., Tokyo, Japan). The statistical model included follicle size and day and two-way interactions. When significant interactions were not observed between the two parameters, they were excluded from the model. Values of $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The numbers of collected ova/embryos between Groups 1 and 2 were not significantly different (13.5 ± 3.3 and 10.0 ± 3.3), or in transferable embryos (5.7 ± 1.7 and 4.6 ± 2.2), nor were there differences in the percentages of transferable embryos (50.0 ± 12.7% and 36.0 ± 11.4%), as previously reported [13].

Misra *et al.* [12] reported that in superovulated buffalo, an increase in the number of ovulations (>5) significantly delayed the return to oestrus. However, in this study, more than 5 ova/embryos were collected from all of the cows. In agreement with Maciel *et al.* [9], all donors returned to oestrus and ovulation occurred within 13 days after embryo collection.

As shown in Figure 1, ultrasonographic examination indicated that all follicles present at the time of embryo collection regressed regardless of their size and that induced oestrus was detected with a new dominant follicle (DF). These results are in agreement with previous reports [10]. Moreover, Lucy *et al.* [8] reported that ovarian remnants of superovulation (CL and nonovulated follicles) remain on the ovary for 25 days from the day of artificial insemination. Likewise, these follicles at the time of embryo collection could be due to the lack of ovulation.

Group 1 had more follicles ($P < 0.05$) than did Group 2 and the diameter of the largest follicle was greater ($P < 0.05$). The number of CL and the concentration of P4 at the time of embryo collection did not differ between the two groups (Table1).

The results of daily plasma P4 and E2 concentrations are shown in Figure 2. The plasma P4 concentration on day 0 were higher than other days in Group 1 and Group 2 respectively ($P < 0.01$). The effect of $PGF_2\alpha$ on luteolysis was assessed by the reduced CL sizes detected by ultrasonography and by a decrease of P4 concentration on the following days [7]. The dynamic state of plasma P4 and E2 did not different between the two groups. Moreover, the average interval from embryo collection to luteolysis (P4 < 1 ng/ml) did not differ between the two groups, as reported in previous studies [9]. However, Group 2 had longer ($P < 0.05$) intervals

Table 1. The dynamic state of hormones and ovaries after embryo collection

	Group (*n*)	
	Group 1[#] (6)	Group 2[$] (5)
Number of follicles*	9.7 ± 1.7[a]	3.8 ± 0.5[b]
Number of corpus lutea*	7.0 ± 1.6	6.8 ± 1.0
Diameter of largest follicle (mm)*	20.8 ± 1.3[a]	10.6 ± 1.9[b]
Concentration of E2 (pg/ml)*	1.2 ± 0.3	1.1 ± 0.3
Concentration of P4 (ng/ml)*	22.8 ± 4.4	13.6 ± 3.3
Days until P4 <1 ng/ml**	4.0 ± 0.7	3.6 ± 0.7
Days until appearance of the dominant follicle**	5.3 ± 0.7[a]	2.6 ± 0.7[b]
Day of oestrus**	10.0 ± 1.0[a]	7.2 ± 0.7[b]
Day of ovulation**	11.0 ± 1.0[a]	8.2 ± 0.7[b]

[#] 18 mm < [$] <18 mm
* Ovarian and hormonal status at the time of embryo collection.
** The interval from embryo collection.
[a,b] Values with different superscript letters are significantly different ($P < 0.05$).

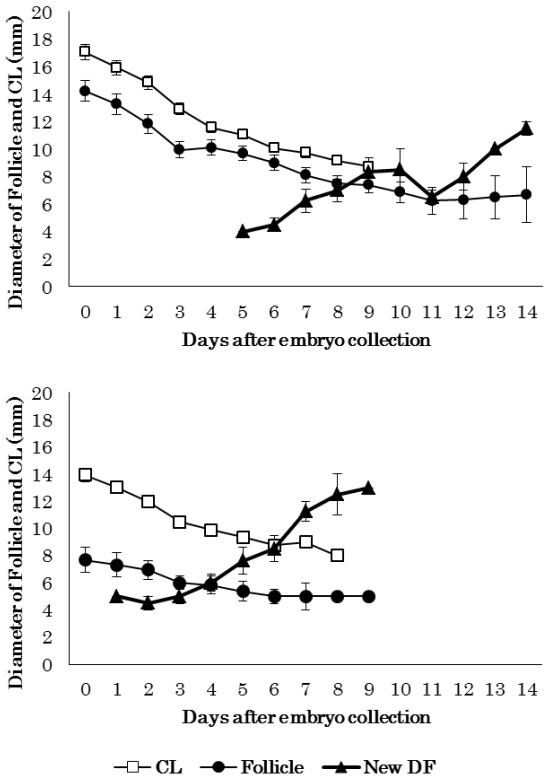

Figure 1. Mean ± SEM profiles of corpora lutea (CL:□), follicles present at the time of embryo collection (●) and new dominant follicle (DF: ▲) diameters after embryo collection to next ovulation in Group 1 (cows with one or more large follicles >18 mm in diameter) and Group 2 (cows with no large follicles).

from treatment to the first ovulation. Maciel et al. [9] suggested that the ovulating follicle started to grow 3 to 4 days after $PGF_2\alpha$ injection, and, in very few cases, those present at the time of embryo collection reached ovulation. It is reasonable to suggest that this delay to the first ovulation may be attributable to the interval from $PGF_2\alpha$treatment to the appearance of the new DF (Table1).

It has been shown that exogenous oestradiol and progesterone can control the follicular wave. The high

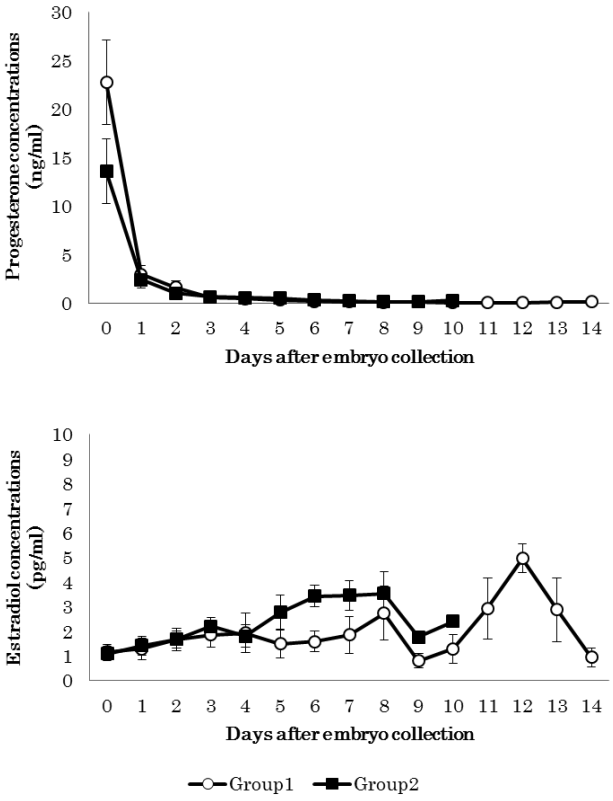

Figure 2. Mean ± SEM. profiles of plasma concentrations of progesterone (P4) and estradiol (E2) after embryo collection to next ovulation in Group 1 (○) and Group 2 (■).

concentration of oestrogen and progesterone induces regression of FSH-dependent follicles (by oestrogen) and LH-dependent follicles (by the synergistic action of oestrogen and progesterone), followed by emergence of a new follicular wave after FSH resurgence [14]. It would perhaps have been expected that the amount of oestradiol would be higher in Group 1 since their ovaries clearly had more large follicles. However, no such difference in plasma oestradiol concentration at the time of embryo collection was detected in the present study. Furthermore, it did not differ from the density of a luteal phase in normal oestrus cycle cows. Therefore, other intrafollicular factors (*e.g.*, inhibin) which suppress FSH release from pituitary [16] may prolong the interval to the appearance of the new dominant follicle.

In conclusion, the presence of LF at the time of embryo collection may delay oestrus induction and subsequent first ovulation after embryo collection, which is attributed to the interval from treatment to the appearance of the new DF. However, the underlying mechanism remains unclear. In future studies, we intend to investigate other effectors of LF.

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REFERENCES

1. **Ali Dinar M, Diskin MG, McDonagh T, Sreenan JM.** Oestrous and ovarian responses in repeatedly superovulated cows (abstract). Theriogenology. 1987; 27: 201.
2. **Chupin D, Touze JL, Procureur R.** Early rebreeding of donor cows (abstract). Theriogenology. 1984; 21: 231.
3. **Desaulniers DM, Guay P, Vaillancourt D.** Estrus induction with prostaglandin F2alpha, cloprostenol or fenprostalene during the normal estrous cycle, superovulation and after embryo collection. Theriogenology. 1990; 34: 667–682.
4. **Dorn CG, Martin LA, Farr JM, Kraemer DC.** Maintenance of calving interval in superovulated-cattle (abstract). Theriogenology. 1990; 33: 218.
5. **Halbert GW, Leslie KE, Walton JS, Betteridge KJ.** Evaluation of return to estrus in superovulated dairy heifers following prostaglandin treatment (abstract). Theriogenology. 1989; 31: 201.
6. **Jones AL, Staples TR, Page RD.** Enhanced return to oestrus in superovulated heifers using fenprostalene (abstract). Theriogenology. 1986; 25: 161.
7. **Kastelic JP, Bergfelt DR, Ginther OJ.** Relationship between ultrasonic assessment of the corpus luteum and

plasma progesterone concentration in heifers. Theriogenology. 1990; 33: 1269–1278.
8. **Lucy MC, Macmillan KL, Thatcher WW, Drost M, Tan HS.** Effect of timing of prostaglandin PGF 2 alpha injection subsequent to embryo collection on the resumption of normal follicular development following superovulatory treatment in cattle. Theriogenology. 1990; 34: 7–19.
9. **Maciel M, Gustafsson H, Rodriguez-Martinez H.** Ovarian activity in superovulated dairy cattle following cloprostenol injection on the day of embryo collection. J. Reprod. Dev. 1996; 42: 73–80.
10. **Mapletoft RJ, Bo GA, Willmott N, Pierson RA.** The effect of dose of cloprostenol on return to oestrus of superovulated donor cows (abstract). Theriogenology. 1991; 35: 237.
11. **Stringfellow DA, Seidel SM.** Manual of the international embryo transfer society (3rd ed.) International Embryo Transfer Society, Savoy, IL, 1998.
12. **Misra AK, Pant HC.** Estrus induction following PGF2alpha treatment in the superovulated buffalo (*Bubalus bubalis*). Theriogenology. 2003; 59: 1203–1207.
13. **Otoi T, Koyama N, Yamamoto K, Tachikawa S, Suzuki T.** Superovulatory responses in Japanese black beef cows following largest follicle aspiration or human chorionic gonadotrophin (hCG) treatment. J. Vet. Med. Sci. 1998; 60: 961–963.
14. **Taniguchi M, Ikeda A, Arikawa E, Shimizu R, Seki M, Karaki M,** Rajamahendran R, Otoi T. Ovarian follicular and corpus luteum changes, progesterone concentrations, estrus and ovulation following oestradiol benzoate/progesterone based treatment protocol in cross-bred cows. Anim. Reprod. Sci. 2007; 99: 389–394.
15. **Takahashi T, Hamanaka S, Imai K, Hashizume K.** A direct time-resolved fluoroimmunoassay (TR-FIA) for measuring plasma oestradiol-17beta concentrations in cattle. J. Vet. Med. Sci. 2004; 66: 225–229.
16. **Lussier J, Carruthers T, Murphy B.** Effects of bovine follicular fluid and partially purified bovine inhibin on FSH and LH release by bovine pituitary cells in culture. Reprod Nutr Dev 1993; 33: 109–119.

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