

# Superovulatory Responses in Japanese Black Beef Cows Following Largest Follicle Aspiration or Human Chorionic Gonadotrophin (hCG) Treatment

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**ABSTRACT.** The effect of largest follicle aspiration or hCG administration before induction of superovulation on the ovarian response of Japanese Black beef cows was investigated using a crossover design in which induction of superovulation was attempted in every cow. The superovulatory response of cows whose largest follicle had been aspirated from the ovaries by ultrasound-guided follicular aspiration 1 day before induction of superovulation, did not differ from the response in non-treated control cows. In contrast, in cows given 5,000 IU of hCG 3 days before induction of superovulation, the proportions of fertilized ova and transferable embryos significantly decreased compared with the other groups. — **KEY WORDS:** follicle aspiration, hCG, superovulation.

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Superovulation induced with gonadotrophin is a common method of obtaining multiple embryos from an individual cow. Although there have been many reports on dosage regimens and types of gonadotrophin preparations for ovarian superstimulation, it has been proposed that most of the variability in ovarian response to superstimulatory treatment in cattle is associated with variations in the status of follicular development at the time of treatment [1, 2]. Follicular growth during the bovine estrous cycle has been demonstrated to occur in waves with cows showing two or three waves and the emergence of a dominant follicle that either undergoes atresia or ovulates at the end of the cycle [13]. Recently, both hormonal and physical methods have been attempted to remove the dominant follicle before inducing superovulation as a means of improving ovarian response. The results of experiments to determine if the presence of a dominant follicle affects the superovulatory response have been equivocal, with some indicating that the dominant follicle affects the superovulatory response [3, 6, 7], and others showing no effect [11, 18]. Although superovulatory responses vary among animals [2], most studies have not been conducted on the basis of a crossover design with every animal receiving superovulatory treatment. On the other hand, it has been determined empirically that the optimal time to begin induction of superovulation in cattle is Days 9 to 13 of the estrous cycle, and this period brackets the beginning of the second follicular wave [5, 17]. It is difficult to identify a dominant follicle based on morphological characteristics alone since the regressing dominant follicle remains the largest in the ovaries for 3–4 days after it has passed its dominant phase [4]. Therefore, daily ultrasound examinations have been conducted to determine the status of follicular development, although it is often impractical to serially examine individual cows in a commercial setting.

In the present study, we investigated whether removal of the largest follicle, identified twice by ultrasonographic examination, by follicle aspiration or human chorionic gonadotrophin (hCG) treatment before induction of

superovulation would improve the superovulatory response in cows.

**Animals and treatment:** Five Japanese Black beef cows (5–10 years old) with regular estrous cycles were selected from our experimental herd. The experiment was conducted using a crossover design with each cow being assigned to one of three groups at 3-month intervals within a 1-year period. In the follicle aspiration group injections of follicle stimulating hormone (FSH-R; Denka Pharmaceutical Co., Ltd., Kawasaki, Japan) were initiated 1 day after removal of the largest follicle from the ovaries by ultrasound-guided follicular aspiration. In the hCG group FSH-R injections began 3 days after injection of 5,000 IU hCG (Gestron, Denka Pharmaceutical Co., Ltd.) on Day 8 of the estrous cycle. In the third group, the control group, the cow was not treated before induction of superovulation. Each cow had 2 normal estrous cycles before the start of the next attempt to induce superovulation.

**Superovulatory treatment and embryo recovery:** Superovulation was induced between Days 10 to 13 of the estrous cycle with FSH-R at a total dose of 20 mg given in decreasing doses over 3 days, and luteolysis was induced with 2 injections of 12.5 mg prostaglandin F<sub>2α</sub> (dinoprost, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) given at the time of the fifth and sixth injections of FSH-R.

Cows were examined twice daily after injection of dinoprost for signs of estrus and inseminated twice with frozen-thawed semen, about 12 hr and 24 hr after standing estrus. Seven days after the first insemination, the uterine horns of cows were flushed by a nonsurgical procedure described earlier [15, 16]. The flushings were collected into a sterile collecting bottle and filtered to remove excess flushing medium. The embryos were located using a dissecting microscope and counted and classified as described by Lindner and Wright [8]. Embryos more than 25% of whose total cell mass consisted of viable cells and whose development was not severely retarded were considered transferable. Each cow was injected with 15 mg dinoprost 3 days after embryo collection.

**Ultrasound examination and follicle aspiration:** A real-time ultrasound scanner (EUB-200 V, Hitachi Co., Ltd., Tokyo, Japan) equipped with a 5-MHz intrarectal probe (EUP-012J, Hitachi) was used to monitor ovarian status before and after hCG treatment and to identify the largest follicle at the time of the follicle aspiration and the formation of corpora lutea at the time of embryo recovery. Desired images were frozen on the screen, measurements were taken using the built-in caliper system, and a hard copy was made with a video processing unit (EZU-VP4, Hitachi). In all groups, ovaries were examined by ultrasonography 3 days and 1 day before initial injection to induce superovulation and 7 days after superovulatory estrus at the time of embryo recovery.

The largest follicle was removed by an ultrasound-guided transvaginal follicle aspiration method similar to that described for oocyte retrieval [10]. Ultrasonography with a modified 5-MHz linear transducer and an 18-gauge, 60-cm single-lumen needle was used for largest follicle aspiration. The follicle was aspirated into a 50-ml tube containing modified phosphate-buffered saline supplemented with 10 IU/ml sodium heparin by means of a regulated vacuum pump (Fujihira, Tokyo, Japan) at 150 to 250 mm Hg. The tube was taken to the laboratory and checked for the presence of an oocyte by filtering.

All data are expressed as means  $\pm$  SEM. Data were analyzed by analysis of variance and differences between groups were calculated by Tukey's test. Differences were considered to be significant at  $P < 0.05$ .

As shown in Table 1, the ovarian response did not differ between the aspiration and control group. However, the proportions of fertilized ova and transferable embryos in the hCG group were lower than in the other groups. In the hCG group, the largest follicle had an average diameter of  $10.4 \pm 0.7$  mm at the time of hCG administration 3 days before induction of superovulation, which decreased to  $6.0 \pm 2.3$  mm 1 day before induction of superovulation. However, two of the hCG-treated cows failed to ovulate the largest follicle following hCG administration. In the aspiration group, the diameter of the aspirated follicles ranged from 7 to 9 mm ( $8.0 \pm 0.5$  mm) 1 day before induction of superovulation, and the proportion of aspirated

follicles  $\geq 9$  mm in diameter was 40% (2/5). In the control group, the largest follicle had an average diameter of  $10.5 \pm 0.7$  mm 3 days before induction of superovulation, which decreased slightly to  $9.0 \pm 0.4$  mm 1 day before induction of superovulation.

Previous reports have shown that the presence of a large follicle inhibits or restricts the development of smaller follicles [9, 14], and that the presence of a dominant follicle limits the ovarian response to superstimulation [6]. Recently, Bungartz and Niemann [3] reported that removing the dominant follicle by ultrasound-guided transvaginal aspiration 2 days before induction of superovulation enhances the ovarian response. In the present study, however, removal of the largest follicle 1 day before induction of superovulation had no effect on the ovarian response to superstimulation. Guilbault *et al.* [6] used growth or stability of the largest follicle ( $>9$  mm in diameter) for at least 4 days as the criterion for the dominant follicle, while Huhtinen *et al.* [7] required 3 days beyond maximum growth. Both studies showed an inhibitory effect of the dominant follicle on the response to superstimulation. Based on the results of these studies size alone does not seem to be an adequate criterion for determining whether the largest follicle will limit the ovarian response to superstimulation. Moreover, Huhtinen *et al.* [7] suggested that a single ultrasound examination could not accurately identify the presence of a dominant follicle. In the present study, the status of follicular development was assessed twice before the day of follicle aspiration, and the proportion of the aspirated follicles  $\geq 9$  mm in diameter was 40%. Based on these results, the follicles aspirated were the largest follicles, but may not have been functionally dominant. Therefore, removal of the largest follicle before superstimulation may not have a significant effect on ovarian responses.

Rajamahendran and Sianangama [12] reported that administration of hCG on Day 7 of the estrous cycle resulted in ovulation of the dominant follicle, formation of accessory corpora lutea and significant increases in plasma and milk progesterone concentration. Moreover, Rajamahendran and Calder [11] demonstrated that induction of ovulation of the dominant follicle with hCG before induction of superovulation does not significantly affect the ovulation

Table 1. Ovarian responses and embryo yields in cows treated with hCG or follicle aspiration before induction of superovulation

Characteristic	Treatment		
	Control	Aspiration	hCG
Number of corpora lutea	$10.4 \pm 0.9$	$11.0 \pm 0.9$	$8.8 \pm 1.7$
Number of ova and embryos	$7.4 \pm 1.4$	$8.2 \pm 1.6$	$6.8 \pm 2.4$
Recovery rate (%) (ova and embryo/corpora lutea)	$70.6 \pm 9.4$	$72.6 \pm 7.6$	$73.1 \pm 16.6$
Number of fertilized ova	$6.8 \pm 1.2$	$8.0 \pm 1.4^a$	$3.2 \pm 1.8^b$
Fertilization rate (%)	$92.9 \pm 4.4^a$	$98.6 \pm 1.4^a$	$58.1 \pm 17.1^b$
Number of transferable embryos	$6.6 \pm 1.2^a$	$7.8 \pm 1.2^a$	$1.6 \pm 1.4^b$
Transferable embryos/total ova and embryos (%)	$89.6 \pm 4.3^a$	$97.1 \pm 2.9^a$	$14.5 \pm 11.3^b$
Transferable embryos/fertilized ova (%)	$96.7 \pm 3.3^a$	$98.5 \pm 1.5^a$	$19.0 \pm 13.6^b$

Values are means  $\pm$  SEM.

a)b) Values with different superscripts are significantly different ( $P < 0.05$ ).

rate or the embryo recovery rate, but tends to increase the number of transferable embryos. In the present study, the rates of ovulation and embryo recovery in the cows injected with hCG were not different from those of the other groups, but the proportions of fertilized ova and transferable embryos in the hCG-treated cows significantly decreased. The rates of fertilized ova and transferable embryos decreased (60% and 10%, respectively) even in the three cows that ovulated the largest follicle after hCG treatment. Moreover, in one of two cows which failed to ovulate following hCG administration, all recovered embryos were classified as unfertilized ova (12/12). Armstrong [2] suggested that hCG has an inhibitory effect on subordinate follicles in addition to the dominant follicle. The dose of hCG (5,000 IU) used in the present study was higher than in earlier studies [11, 12], in which 1000 IU was used. Therefore, a higher dose of hCG before superstimulation may have a detrimental effect on the developmental capacity of oocytes in subordinate follicles.

These findings indicate that removal of the largest follicle by follicle aspiration or hCG treatment before superstimulation without serial examination of follicular development, which is impractical in a commercial setting, may not improve ovarian response.

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