Use of Highly Purified Porcine FSH Preparation for Superovulation in Japanese Black Cattle

Mikio SUGANO and Sigeru WATANABE

Fukushima Animal Husbandry Experiment Station, 18 Arai-Jizouhara, Fukushima 960-21, Japan

(Received 28 May 1996/Accepted 3 December 1996)

ABSTRACT. Fifty cattle were divided into 3 groups (I, II, III) according to FSH-R dosage used to induce superovulation between days 9 and 11 of the estrous cycle. Ova/embryo recovery rate did not differ significantly between groups I and II administered total dosages of 16 and 24 mg, respectively. However, the average number of recovered ova/embryos and transferable embryos was significantly lower in group III (administered a total dosage of 30 mg) ($P<0.05$) than groups I and II. These results suggest that intramuscular administration of 16 mg FSH-R over 3 days is sufficient for inducing superovulation in Japanese Black cattle. — **KEY WORDS:** FSH-R, Japanese Black cattle, superovulation.

J. Vet. Med. Sci. 59 (3): 223–225, 1997

Several hormone preparations, including a porcine pituitary extract preparation of follicle stimulation hormone (FSH) and pregnant mare's serum gonadotrophin (PMSG) have been used to induce superovulation. In addition, several studies have investigated the use of a gonadotrophin (GTH) preparation for the same purpose with varying results [1, 6, 9, 15]. PMSG-treatment, which has a long half-life in the blood, is known to produce an antigen to the preparation after 2 or 3 treatment in cattle [16]. The FSH preparation, which has a shorter half-life, is widely used in Japanese Black cattle. Commercially available crude follicle stimulating hormone preparations (FSH-P) contain both FSH and luteinizing hormone (LH) activity [3, 5, 14]. Varying recovery rates of ova/embryos and transferable embryos in cows treated with FSH-P have been reported [7, 10, 12, 17]. Buckers [2] and Donaldson *et al.* [5] reported that these rates improved when pure porcine hypophyseal FSH (without LH) was administered.

A highly purified form of porcine FSH (FSH-R) has recently been derived from FSH-P in Japan. Isogai *et al.* [8] reported improved efficacy using FSH-R preparations in Holstein cattle. However few studies have reported the use of FSH-R in Japanese Black cattle.

The present study was performed in order to determine the optimal FSH-R dose and treatment regimen to induce superovulation.

Fifty Japanese Black cattle (aged 5 to 15 years; average

weight 495 kg) were examined for the presence of the corpus luteum (CL) by rectal palpation prior to initiating superovulation treatment between days 9 and 11 of the estrus cycle. FSH-R (Denka Pharmaceutical Co., Japan) was administered intramuscularly to each animal. Group I ($n=24$) received 2 doses of 4 mg each on the first day, 2 doses of 2.5 mg on the second day, and 2 doses of 1.5 mg on the third day for a total of 16 mg. Group II ($n=19$) received 2 doses of 6 mg each on the first day, 2 doses of 4 mg on the second day, and 2 doses of 2 mg on the third day for a total of 24 mg. In an attempt to reduce the number of administrations, group III ($n=7$) received a single dose of 10 mg on each of the 3 days, for a total of 30 mg. Prostaglandin F2 alpha (Upjohn Co., Kalamazoo, MI) was administered to each of the animals 48 and 57 hr after initiation of gonadotrophin treatment. A total dose of 35 mg was divided into doses of 25 mg in the morning, and 10 mg in the afternoon. The treated cows were inseminated artificially with frozen semen from the Fukushima Animal Husbandry Experiment Station 57 and 72 hr after the first injection of PGF_{2α}. The experimental methods are summarized in Table 1. Ova and embryos were recovered nonsurgically on day 7 or 8 after the observation of estrus (day 0=estrus) by flushing with one liter of modified Dulbecco's phosphate-buffered saline (m-PBS) containing 3 g BSA. After flushing, the medium was recovered into embryo collection dishes, and the embryos were examined

Table 1. Experimental design and treatment regimen for FSH-R-induced superovulation

Group	Treatment	No. of Cattle	Time ^{a)} of treatment						
			Day	1		2		3	
			Administra- tion time	8	17	8	17	8	17
I	FSH 16 mg PGF _{2α} -THAM 35 mg	24		4 mg	4 mg	2.5 mg	2.5 mg	1.5 mg	1.5 mg
II	FSH 24 mg PGF _{2α} -THAM 35 mg	19		6	6	4	4	2	2
III	FSH 30 mg PGF _{2α} -THAM 35 mg	7		10		10		10	10 mg
								25 mg	10 mg

a) Treatment initiated between days 9 and 11 of estrus cycle.

Table 2. Results of FSH-R-induced superovulation

Group	No. of Cattle	No. of ova and embryos collected	Proportion (%) of ova/embryos by quality					Rate of transferable embryos (%)
			A	B	C	D	E	
I	24	153	59.5	9.2	3.9	17.6	9.8	73 ^{a)}
II	19	137	54.7	5.9	2.2	24.1	13.1	63 ^{a)}
III	7	30	30.0	13.4	3.3	23.3	30.0	47 ^{b)}

A: Excellent, B: Good, C: Fair, D: Degenerated embryo, E: Unfertilized ova.

a), b) Different superscripts indicate significant differences between the means ($P < 0.05$).

Table 3. Comparison of embryo production resulting from different FSH-R treatment methods

	group I	group II	group III
No. of cattle	24	19	7
No. of ova/embryos collected	153	137	30
No. of transferable embryos (a)	111 (73)	86 (63)	14 (47)
Average No. of ova/embryos collected	6.4 ± 5.3	$7.2 \pm 4.0^c)$	$4.3 \pm 4.4^d)$
Average No. of transferable embryos	4.6 ± 5.2	4.4 ± 3.7	2.0 ± 4.1
Average No. of CL ^{b)}	8.7 ± 4.2	8.2 ± 3.8	8.2 ± 3.0

a) (No. of transferable embryos) $\div$ (No. of ova/embryos collected) $\times 100$.

b) Rectal palpation, c), d) $p < 0.05$, Mean $\pm$ SD.

under a stereomicroscope at 16 times magnification. Collected ova and embryos were transferred into m-PBS containing 20% calf serum for storage. Recovered embryos were evaluated according to morphological appearance and quality [11]. The number of CL was estimated by rectal palpation at the time of recovery. The data were analyzed using a Student's *t*-test and χ^2 -test.

In all fifty animals, ova and embryos were recovered at 7 or 8 days after onset of estrus. The results are summarized in Tables 2 and 3. In group I, which was treated twice daily for 3 days, the mean number of CL was 8.7 ± 4.2 , that of ova/embryos was 6.4 ± 5.3 , and that of transferable embryos was 4.6 ± 6.2 . The transferable embryos rate was 73%. In group II, which was treated twice daily for 3 days, the mean number of CL was 8.2 ± 3.8 , that of ova/embryos was 7.2 ± 4.0 , and that of transferable embryos 4.4 ± 3.7 . The transferable embryo rate was 63%. No significant differences were observed between groups I and II. On the other hand, in group III, which was treated once daily for 3 days, the mean number of CL was 8.2 ± 3.0 , that of ova/embryos was 4.3 ± 4.4 , and that of transferable embryos was 2.0 ± 4.1 . The transferable embryo rate was 47%. The mean number of ova/embryos in group III was significantly lower ($P < 0.05$) than that in group II. Moreover, the transferable embryo rate in group III was significantly lower ($P < 0.05$) than in the other 2 groups. The highest rate of recovered embryos judged to be "excellent" was seen in group I (59.5%), and the rates of degeneration and unfertilized ova were lowest in group I (17.6%, 9.8%, respectively). The highest number of unfertilized ova among

all the groups was seen in group III (30.0%). These factors did not differ significantly among the groups.

Isogai *et al.* reported that 36 mg FSH-R was more effective than the same dose of FSH-P for inducing superovulation in Holstein cattle [8]. Similarly, Yamamoto *et al.* [21] reported that a FSH-R dose level of 20 mg may be more effective in Holstein heifers. Furthermore McGowan *et al.* [13] studied the dose—response relationship for hMG in the range of 262.5 IU—2,100 IU for the superovulatory response in crossbred beef heifers and concluded that 1,050 IU hMG was the optimum dose of hMG. Higher doses of hMG resulted in embryos of poorer quality and an increased number of unfertilized ova.

The present study shows that superovulation can be successfully induced by administration of 24 mg of FSH-R. Further reducing this dose to 16 mg did not significantly reduce effectiveness. This suggests that FSH-R can effectively induce superovulation in Japanese Black cattle at a dose as low as 16 mg. Pawlyshyn *et al.* [18] reports that a higher dose of FSH (15 mg–60 mg) results in poorer quality embryos and an increased number of unfertilized ova. This could not be improved by increasing the FSH dosage further. Other studies have reported similar results for FSH-P administration [19, 20].

Few studies have examined the effectiveness of once a day administration of a large dose of FSH-R compared to twice daily administration of a smaller dose [4]. The present study showed that a once daily administration of a large dose of FSH-R is not as effective as twice daily administration of a smaller dose. Further, this effect can be

observed with a dose as low as 16 mg.

In conclusion, FSH-R can be used to induce superovulation in Japanese Black cattle at a 16 mg dose injected intramuscularly twice daily for 3 days.

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