Effects of Human Menopausal Gonadotrophin (hMG) for superovulation in Japanese Black Cattle

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ABSTRACT. Sixty-four Japanese Black parous cows were divided into 5 dosage groups (I, II, III, IV, V) and administered either human menopausal gonadotrophin (hMG) or FSH by intramuscular injection. No significant differences were observed between the hMG-treated groups. The average number of recovered ova/embryos in cows treated with 900 IU was significantly greater ($P < 0.05$) than in cows treated with FSH. The percentage of high-quality transferable embryos was highest in the 600 IU hMG-treated group. Also, FSH-treated cows produced a significantly larger number of unfertilized ova compared to hMG-treated cows. The results suggest that hMG treatment is sufficient for Japanese Black Cattle at a dose of 600 IU for 3 days, and that a 2-day treatment regime is also possible.—**KEY WORDS:** Cattle (Japanese Black), hMG, superovulation.

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Superovulation was first induced in cattle using pregnant mare's serum gonadotrophin (PMSG). Several hormone preparations were subsequently developed for inducing superovulation, inducing follicle stimulating hormone (FSH), which is an extract of the porcine pituitary. FSH is currently the preparation used most widely in Japan on Japanese Black Cattle. PMSG has a long half-life in the blood and only a single administration is needed in order to induce superovulation [16]. Although this is convenient from the view point of ease of administration, PMSG-treated cattle produce an antigen to the preparation after only 2 or 3 treatment. As a result, superovulation can no longer be induced in these cattle [17]. In contrast, FSH has a relatively short half-life in the blood, and twice daily injections are required for 3 or 4 days in order to induce superovulation [16]. FSH-treated cattle do not produce an antigen to the preparation. Therefore, repeated superovulation treatment is possible. However, the variability in the quantity and quality of ova/embryos following superovulation, whether induced by PMSG or FSH, remains a major limiting factor in the development of practical embryo transfer programs in cattle [1–3, 6–8, 11, 15].

Lauria *et al.* [9] previously reported that human menopausal gonadotrophin (hMG), which is a highly purified biological preparation, was effective in inducing superovulation in cattle. Compared to PMSG, hMG-induced superovulation in cattle results in less variability, thus producing a higher consistency of embryo quality and quantity [9]. The efficacy with which hMG induces superovulation in cattle is reported to be similar to that of FSH [14].

Etoh *et al.* [4] compared the effects of injecting Japanese Black Cattle with FSH and 900 IU hMG for 4 days. They reported that the number of recovered ova/embryos did not differ between hMG-treated and FSH-treated cattle [4]. However, the proportion of transferable embryos was higher in the hMG-treated group than in the FSH-treated group.

The objective of the present study was to examine the effect of four different treatment regimes, each with different doses of hMG, on the superovulatory response and quality of embryos, and to compare hMG treatment with FSH

treatment in Japanese Black Cattle.

Sixty-four Japanese Black Cattle parous cows, each weighing an average of 490 kgs and between 5 to 12 years old, were examined in this study. The cows had been used for superovulation 4 months earlier.

The cows were allowed to complete two consecutive estrous cycles before being used in the experiment. Human menopausal gonadotrophin (hMG; Mochida Pharmaceuticals Inc., Tokyo, Japan) was used for superovulatory treatment. Each 1 ml vial of hMG contained 75 IU of follicle stimulating hormone (FSH) and 75 IU of luteinizing hormone (LH). The 64 cows were divided into five groups (I, II, III, IV, V). The treatment regime for each group is shown in Table 1: hMG group I (900 IU by intramuscular injection twice daily for 4 days); hMG group II (600 IU by intramuscular injection twice daily for 3 days); hMG group III (450 IU by intramuscular injection twice daily for 2 days); hMG group IV (750 IU by intramuscular injection three times for 2 days); FSH group V (20 mg refined FSH, intramuscular injection twice daily for 3 days) (Denka Pharmaceuticals Inc., Kawasaki, Japan).

All cows were treated for superovulation on days 9 to 13 of the cycle, and hMG groups I, II, III and FSH V also received a total dose of 35 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Upjohn Co., Kalamazoo, MI), at 48 hr (25 mg) and 57 hr (10 mg) after initiation of treatment. hMG group IV also received $PGF_{2\alpha}$ at 32 hr (25 mg) and 40 hr (10 mg). The treated cows were artificially inseminated with frozen semen at 57 and 72 hr after the first injection of $PGF_{2\alpha}$ at 8:00 a.m., except for hMG group IV, which was injected with $PGF_{2\alpha}$ at 2:00 p.m. and inseminated at 51 and 66 hr.

Ova and embryos were collected nonsurgically on days 7 or 8 after the superovulatory estrus (day 0=estrus) by flushing with one liter of modified phosphate buffered saline (mPBS) containing 3 g BSA. After flushing, the medium was recovered into embryo collection dishes and embryos were examined under a stereoscopic microscope at a magnification of 16X. Collected ova and embryos were transferred to mPBS containing 20% calf serum for storage. Recovered embryos were evaluated according to their

Table 1. Treatment regime for hMG- and FSH-induced superovulation in Japanese Black Cattle

Group	Treatment	No. of cows	Day ^{a)} and hours of treatment											
			1					2					3	
			6	8	14	17	22	6	8	14	17	22	8	17
hMG I	hMG 900 IU PGF _{2α} -THAM 35 mg	15		150 IU	150 IU			150 IU		150 IU			75 IU 25 mg	75 IU 10 mg
hMG II	hMG 600 IU PGF _{2α} -THAM 35 mg	10		150	150			75		75			75 25	75 10
hMG III	hMG 450 IU PGF _{2α} -THAM 35 mg	7		150	150			75		75			25	10
hMG IV	hMG 750 IU PGF _{2α} -THAM 35 mg	7	150		150		150	150		75 25 mg		75 10 mg		
FSH V	FSH 20 mg PGF _{2α} -THAM 35 mg	25		5 mg	5 mg			3 mg		3 mg			2 mg 25	2 mg 10

a) Treatment starting on days 9–13 of the estrous cycle.

Table 2. Comparison of embryo production after hMG and FSH treatment in Japanese Black Cattle

Group	No. of cows	No. of ova/embryos			Average No. of ova/embryos	
		collected	transfer- able embryos	transferable embryo rate (%)	collected	transferable
hMG I	15	126	88	70	8.4 ± 6.4 ^{a)}	5.9 ± 4.2
hMG II	10	69	53	77	6.9 ± 5.0	5.3 ± 3.8
hMG III	7	35	27	77	5.0 ± 3.2	3.9 ± 2.8
hMG IV	7	37	31	83	5.3 ± 2.4	4.4 ± 2.0
FSH V	25	116	80	69	4.6 ± 4.5 ^{b)}	3.2 ± 3.8

a), b) $p < 0.05$.

morphological appearance and quality [10]. The data were analyzed by a Student's *t*-test and χ^2 -test.

The number of ova/embryos recovered and the proportion of transferable embryos in each group are shown in Table 2. When the ova/embryos recovered from the hMG groups (450–900 IU, 2–4 days) and FSH group were compared, the hMG groups produced a greater number of collected ova/embryos and transferable embryos. In particular, the difference in collected ova/embryos between hMG I and FSH V was significant ($P < 0.05$). In the hMG groups, the higher levels of follicular stimulation resulted in an increase in the total number of recovered ova/embryos. However, the increase in the number of transferable embryos was not significant. The average number of collected ova/embryos and transferable embryos in the 600 IU hMG-treated group tended to be less than in the 900 IU hMG-treated group, although the difference was not significant. Treatment with 450 IU twice daily for 2 days and 750 IU hMG three times daily resulted in fewer collected ova/embryos and transferable embryos than in both the 900 IU and 600 IU hMG-treated groups.

On the other hand, hMG group IV, which was treated three times per day for 2 days, showed a standing estrus

after PGF_{2α} administration and indicated by the slight increase in the number of ova/embryos. However, compared with the other hMG groups, this difference was not significant. The transferable embryo rate was highest in hMG IV (8.3%). The highest number of collected ova/embryos (8.4 ± 6.4) was seen in the hMG 900 IU group. The quality of embryos collected after hMG- and FSH-induced superovulation is shown in Table 3. The highest rate of recovered embryos judged to be "excellent" was seen in hMG II (47.8%), followed by hMG IV (45.9%) and hMG III (42.9%). The recovery rate of degenerated and unfertilized ova in hMG I was 15.1% and 15.1%, respectively. The highest number of unfertilized ova amongst all groups occurred in FSH V (22.4%) and the lowest in hMG II (8.7%) and III (8.6%).

Lauria *et al.* [9] reported that a total dose of 2,100 IU of hMG injected intravenously twice daily for 5 days was more effective in inducing superovulation in cattle than a total dose of 1,350 IU hMG administered once a day for 3 days, suggesting that the superior effect of 2,100 IU hMG treatment was due to the treatment schedule rather than to the higher dose. They also found that hMG treatment

Table 3. Quality of embryos collected after hMG- and FSH-induced superovulation

Group	No. of cows	No. of collected	Proportion (%) of ova/embryo by quality ^{a)}				
			A	B	C	D	E
hMG I	15	126	39.7	16.6	13.5	15.1	15.1
hMG II	10	69	47.8	18.8	10.2	14.5	8.7
hMG III	7	35	42.9	17.1	17.1	14.3	8.6
hMG IV	7	37	45.9	21.6	16.3	5.4	10.8
FSH V	25	116	41.4	22.5	5.2	8.6	22.4

a) Classification of ova/embryo quality-A: excellent, B: good, C: fair, D: degenerated, E: unfertilized ova.

resulted in fewer persistent follicles than MSG treatment [9]. McGowan *et al.* [14] studied the dose-response relationship for hMG in the range of 262.5 to 2,100 IU for the superovulatory response in crossbred beef heifers and concluded that 1,050 IU of hMG given twice daily for 5 days was the optimum dose of hMG. Higher doses of hMG did not result in an increase in the number of recovered ova/embryos or transferable embryos.

The present study examined the effects of four different doses of hMG with different treatment schedules. The group of cows treated with 900 IU hMG twice daily for 4 days showed the highest superovulatory response. The average number of collected ova/embryos and transferable embryos in the 600 IU hMG-treated (3-day administration) group tended to be fewer than in the 900 IU hMG-treated group, although the differences were not statistically significant. Treatment with 450 IU twice daily for 2 days and 750 IU hMG three times daily resulted in fewer collected ova/embryos and transferable embryos than in both the 900 IU and 600 IU hMG-treated groups. The average number of collected ova/embryos reported by McGowan *et al.* [14] in Hereford $\times$ Simmental crossbred heifers treated with 1,050 IU hMG for 5 days was higher than the number in Japanese Black Cattle treated with 900 IU hMG for 4 days (14.3 ± 1.8 vs 8.4 ± 6.4). However, the number of transferable embryos did not differ significantly between the two groups (7.3 ± 1.5 vs 5.9 ± 4.2). Etoh *et al.* [4] reported similar results in Japanese Black Cattle using 900 IU hMG for 4 days.

The results of this study showed that reducing the dose of hMG to 600 IU and reducing treatment from 4 days to 3 days did not adversely affect the superovulatory response. However, reducing the dosage of hMG to 450 IU and the duration of treatment to 2 days resulted in a lower superovulatory response. Injections of 750 IU hMG three times per day for 2 days did not produce a satisfactory response. McGowan *et al.* [14] reported that FSH-P-treated beef heifers showed a higher superovulatory response than hMG-treated heifers. In contrast, Etoh *et al.* [4] reported an equivalent ovulatory response and a higher rate of transferable embryos in Japanese Black cattle treated with hMG than in the cattle treated with FSH. In the present study, Japanese Black Cattle treated with 450–900 IU hMG showed an equivalent or superior ovulatory response. The reason for this difference in response between FSH-treated

and hMG-treated cattle is not well understood. It has been reported that breed, age and nutrition influence the superovulatory response to gonadotrophin [1–2, 5–8, 11–13].

In conclusion, hMG can be used to induce superovulation in Japanese Black Cattle at a dose between 600 and 900 IU injected intramuscularly twice daily for 3 or 4 days as an alternative to FSH.

It should be noted that serum levels of the steroid were not measured in the present study. In the future, blood serum levels must be measured to clarify the differences in the endocrinological response of hMG and porcine FSH.

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