Superovulation with Human Menopausal Gonadotrophin in Japanese Black Cows

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Abstract: *The aim of this study is to induce effective superovulation in Japanese Black cows with hMG and to produce high-quality embryos in a short interval of superovulation treatment. In the first superovulation treatment, a total of 750 IU hMG was used and after collection, 50 ml of iodine was infused into the uterus and a 25 ml $PGF_{2\alpha}$ intramuscular injection was also given. After confirming the return of oestrous, a second treatment was conducted with a total of 600 IU hMG. Although the total number of collected ova/embryos and transferable embryos was less in the second treatment than in the first one, the ratio of transferable and excellent embryos was considered to be high. In conclusion, it was indicated that 600 IU hMG is effective for superovulation and that iodine preparation and $PGF_{2\alpha}$ produce high-quality embryos in a short interval of superovulation treatment.*

Key words: *Japanese Black cows, Superovulation, Human menopausal gonadotrophin*

In 1982, Lauria, *et al.* [1, 2] reported the efficiency of human Menopausal Gonadotrophin (hMG) for superovulation in cows for the first time. At the present time, pregnant mare's serum gonadotrophin (PMSG) and follicle stimulating hormone (FSH) are most widely used for the superovulation of Japanese Black cows in Japan. The FSH injection needs to be given twice daily for three or four days due to its short half-life in blood. The PMSG injection has a long half-life in blood and only a single administration is needed in order to induce superovulation. But the PMSG-treated cow produces an antigen to the preparetion. It was reported that the production of embryos varies according to the kind of gonadotrophin [3–7]. As repeating superovulation treatment is possible, FSH has been more often used for superovulation in Japanese Black cows than PMSG. The purpose of the superovulation is to produce embryos in large quantities safely and stably. But the results were not favorable.

McGowan *et al.* [8] investigated the amount of hMG injected into the crossbred cows. There was also a report that hMG-treated cows produced a larger number and higher quality of embryos than FSH-treated cows [9, 10]. We previously investigated the efficacy of 450 to 900 IU hMG administered over 2- to 4-days for inducing superovulation in Japanese Black cows [11]. The result of this experiment indicated that a total dose of 600 IU hMG given twice daily for three days was the most effective [11]. The aim of this study is to induce superovulation in Japanese Black cows with hMG and to produce high-quality embryos in a short period.

As a post-superovulation treatment for the return of oestrous, cows are generally given only $PGF_{2\alpha}$ or an iodine preparation, which requires over one month, so we investigate the effect of $PGF_{2\alpha}$ along with an iodine preparation to promote the return of oestrous.

Five Japanese Black parous cows bred by the farmers in Fukushima Prefecture were used in this study. The ages of these cows ranged from 6 to 10 years with an average age of 9.2 years. They have each given birth 6 to 9 times. None of them had received superovulation treatment before. HMG (GONADRIL, Mochida Pharmaceutical Co. Ltd., Tokyo, Japan) was used for the superovulation treatment in the declining dose method of administration. All of the cows were examined for the presence of corpus luteum (CL) by rectal palpation prior to initiating superovulation treatment. The superovulation was started between days 9 and 13 of the oestrous cycle (day 0 = first day of oestrous). HMG was injected twice a day (8:00 and

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Table 1. Experiment design and regimen for superovulation with hMG

Experiment	Treatment	No. of Cows	1*		2		3		day time
			8	17	8	17	8	17	
Exp. 1	hMG 750 IU	5	150	150	150	150	75	75	
	$PGF_{2\alpha}$-THAM 35 mg						25	10	
Exp. 2	hMG 600 IU	5	150	150	75	75	75	75	
	$PGF_{2\alpha}$-THAM 35 mg						25	10	

*Administration of hMG was started 9 to 13 days after oestrous.

17:00) as shown in Table 1.

In the first superovulation treatment (Exp 1), a total of 750 IU hMG was given in 6 doses twice daily by intramuscular injection over three days. In the second superovulation treatment (Exp 2), a total of 600 IU hMG was given in 6 doses twice daily by intramuscular injection over three days. In order to induce oestrous, a total of 35 mg $PGF_{2\alpha}$ (PRONALGON F, Upjohn C., Kalamazoo, MI, USA) was given (25 mg in the morning and 10 mg in the afternoon) by an intramuscular injection 48 and 57 hours after giving hMG.

Cows showing signs of standing oestrous after the superovulation treatment were artificially inseminated twice, 57 and 72 hours after the $PGF_{2\alpha}$ injections. Frozen sperm collected from "AZUMAHIRASHIGE", raised at Fukushima Prefecture Animal Husbandry Experiment Station was inseminated immediately after thawing in 37°C warm water. The artificial insemination was carried out by a single technician.

Day 7 or 8 after the day of onset of heat (day 0), ova/embryos were collected non-surgically with a balloon catheter. One liter of modified phosphate buffered saline (EMBRYOTEC, Nippon Zenyaku Kogyo Co. Ltd., Koriyama, Japan) was used for flushing. The quality of the embryos was judged by at least two technicians based on Lindner's classification [12]. Transferable embryos were classified into three groups: A, excellent, B, good, and C, fair. Included in these classes were other degenerated embryo and unfertilized ova classes.

Just after the first collection of embryos, 50 ml of iodine preparation (2% povidone-iodine solution) was infused into the uterus of each cow and 25 ml of $PGF_{2\alpha}$ was given by intramuscular injection. The second superovulation treatment was started 9 to 13 days after the first treatment.

Analyses of the number of collected ova and embryos were done by means of the Student's t-test. The rate of transferable embryos was analysed by means of the χ^2 test.

In the first superovulation treatment with 750 IU of hMG, oestrous was observed in all cows and artificial insemination was conducted in all of them. The result of ova/embryo collection is shown in Table 2. The average number of collected ova/embryos was 18 ± 7.8 (mean $\pm$ SD). The average number of transferable embryos was 9.4 ± 3.0. The ratio of transferable embryos to all collected ova/embryos was 80% at the highest; and the average was 52.2%. As for the classification of ova/embryos, those classified as "excellent" accounted for 38.9%, unfertilized, 33.4%, and degenerated, 14.4% (Table 2).

In the second superovulation treatment with 600 IU of hMG, one cow did not show any sign of standing oestrous. Artificial insemination was conducted in the rest of the cows. The results are shown in Table 2. The average number of collected ova/embryos was 15 ± 2.2 and that of transferable embryos was 9.8 ± 2.5. As for the classification of ova/embryos, those classified as excellent accounted for the highest rate, 41.7%, unfertilized ova, second, 23.3%, and fair, third, 16.7%. The ratio of transferable embryos was 65.0%. The embryos collected from 5 cows (9 running cows) through the first and second treatments, over two months, amounted to 86, including 60 "excellent" embryos (Table 2). Differences were not statistically significant.

The length of the period from the first ovum/embryo collection to the return of oestrus was 5 days at shortest, and 10 days at longest. The average was 7.6 days. The average length of the interval between the first-time collection and second was 32.2 days.

It is said that a cow with moderate paniculus adiposus is the most suitable for superovulation or transferring of embryos, in terms of nutrition [13, 14]. As for the relationship between age or experience of giving birth and the ova/embryos collection result, Hasler *et al.* [15] investigated the ovarian response in 2 to 14-year-old Horlstein Donor cows and reported that there was no difference between them in ovarian response. Donaldson *et al.* [16] used FSH for superovulation in

Table 2. Results of hMG-induced superovulation in farm-raised Japanese Black parous cows

Cow No.	No. of ova/Embryos	No. of transferable embryos	Number of ova/embry*					Rate of transferable embryo (%)
			A	B	C	D	E	
D1	11	5	4	0	1	4	2	45.5
	13	10	4	6	0	2	1	76.9
D2	20	16	12	4	0	1	3	80.0
	18	13	9	3	1	1	4	72.2
D3	20	12	9	3	0	4	4	60.0
	14	9	6	0	3	2	3	64.3
D4	29	7	3	4	0	4	18	24.1
	15	7	6	1	0	2	6	46.7
D5	10	7	7	0	0	0	3	70.0
	Cancelled as oestrous did not start.							
Total	90	47	35	11	1	13	30	52.2
			(38.9)	(12.2)	(1.1)	(14.4)	(33.4)	
	60	39	25	10	4	7	14	65.0
			(41.7)	(16.7)	(6.6)	(11.7)	(23.3)	

Top line: 750 IU hMG administered. Bottom line: 600 IU hMG administered. *A, excellent, B, good, C, fair, D, degenerated, E, unfertilized ova. Differences between experiments were not statistically significant.

Longhorn cows to study the influence of age on cows. They reported that there was a decrease in the total number of collected ova/embryos in the case of cows over 10 years old. The donor cows in this present study included three 10-year-old cows and two cows that had given birth nine times. Although they were old, the results coincided with those in the report by Hasle, *et al.* [15].

As none of the five Japanese Black donor cows in this study had ever had superovulation treatment before, the first dose of hMG was set at 750 IU. In light of the good results of the first treatment, the dose of hMG in the second superovulation treatment was set at 600 IU. The ratio of degenerated embryos was higher in the first treatment, and that of transferable embryos was higher in the second treatment.

McGowan *et al.* [8] reported that an excessive amount of FSH-activity in gonadotrophin leads to an increase in the number of degenerated and unfertilized ova. The results of this study support their report. In order to examine the amount of hMG, the present authors also conducted an experiment in which they changed the amount of hMG from 450 IU to 900 IU, which was given in divided doses for 2–4 days [11]. The result was that a total of 600 IU hMG, given twice daily for three days, was determined to be the proper amount for Japanese Black donor cows. The results of the present experiment conducted on farms also indicate that a total amount of 600 IU given twice daily for three days is enough.

A 50 ml iodine preparation infused into the uterus of each cow and a 25 ml $PGF_{2\alpha}$ intramuscular injection given just after the first ova/embryo collection was believed to hasten the return of oestrus in donor cows. As a result, the next superovulation treatment could be conducted continuously and yield the same result as that of the first treatment. This method is considered to be effective as a technique to produce many ova/embryos in a short period.

References

1) Lauria, A., Genazzani, A.R., Oliva, O., Inaudi, P., Cremonesi, F., Monittola, C. and Aureli, G. (1982): Clinical and endocrinological investigations on superovulation induced in heifers by human menopausal gonadotrophin. J. Reprod. Fert., 66, 219–225.
2) Lauria, A., Oliva, O., Genazzani, A.R., Cremonesi, F., Crotti, S. and Barbetti, M. (1982): Improved method to induce superovulation in cattle using human menopausal gonadotrophim (HMG). Theriogenology, 18, 357–364.
3) Aoyagi, Y., Iwazumi, Y., Wachi, H., Kweon, O.K., Takahashi, Y., Kanagawa, H., Miyamoto, A., Umezu, M. and Masaki, J. (1987): Studies on superovulation with PMSG and FSH in cows—hormone level of plasma steroid and results of embryo recovery—. Jpn. J. Anim. Reprod., 33, 167–172 (in Japanese with English summary).
4) Critser, J.K., Rowe, R.F., Del Campo, M.R. and Ginther, O.J. (1980): Embryo transfer in cattle: Factors affecting superovulation response number of transferable and length of post-treatment estrous cycles. Theriogenology, 13, 397–406.

5) Elsden, R.P., Nelson, L.D. and Seidel Jr, G.E. (1978): Superovulation cows with follicle stimulating hormone and pregnant mare's serum gonadotrophin. Theriogenology, 9, 17–26.
6) Monniaux, D., Chupin, D. and Saumande, J. (1983): Superovulation response of cattle. Theriogenology, 19, 55–81.
7) Nakajima, A., Hiraizumi, S., Onodera, K. Suzuki, H., Kudo, Y. and Domeki, I. (1992): The use of bovine Anti-PMSG serum in beef cattle after PMSG-superovulation. J. Vet. Med. Sci., 54, 95–98.
8) McGowan, M.R., Braithwaite, M. and Jochle, W. (1985): Superovulation of beef heifers with Pergonal (HMG): dose response trial. Theriogenology, 24, 173–184.
9) Etou, T., Ishida, K., Hayakawa, S. and Ushijima, H. (1987): Superovulation of Japanese Black cattle with a follicle stimulating hormone and human menopausal gonadotrophin. Jpn. Anim. Reprod. Tech., 9, 121–123 (in Japanese).
10) Katsumi, A., Yamaguchi, T., Yamaguchi, C., Yamashita, Y., Ujiie, H., Onodera, M. and Ochi, T. (1994): Superovulation of Japanese Black Cattle with hMG (Pergonal). J. Vet. Med., 47, 185–189 (in Japanese with English summary).
11) Sugano, M., Takano, T., Sato, N., Ohsaki, J. and Watanabe, S. (1995): Effect of human menopausal gonadotrophin (hMG) for superovulation in Japanese Black cattle. J. Vet. Med. Sci., 57, 1113–1115.
12) Lindner, G.M. and Wright Jr R.W. (1983): Bovine embryo morphology and evaluation. Theriogenology, 20, 407–416.
13) Kinoshita, A., Kamimura, S., Imamura, H., Ookutsu, S., Yoshida, M. and Nakanishi, Y. (2001): Influence of different nutrient conditions on ovarian follicular dynamics in dairy cows. Anim. Sci. J., 72, J20–J27 (in Japanese with English summary).
14) Isogai, T. (1992): Effects of season, ageat calving, time after calving and interval of treatment on the embryo production in superovulated Holstein donors. J. Reprod. Develop., 38, j1–j6 (in Japanese with English summary).
15) Hasler, J.F., McCauley, A.D., Schermerhorn, E.C. and Foote, R.H. (1983): Superovulatory responses of Holstein cow. Theriogenology, 19, 83–99.
16) Donaldson, L.E. (1984): Effect of age of donor cows on embryo production. Theriogenology, 21, 963–967.