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Author(s)	TAKAHASHI, Yoshiyuki; KANAGAWA, Hiroshi
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INDUCTIONS OF SUPEROVULATION USING SEVERAL FSH REGIMENS IN HOLSTEIN-FRIESIAN HEIFERS

Yoshiyuki TAKAHASHI and Hiroshi KANAGAWA

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Eighty-three Holstein-Friesian virgin heifers were randomly divided into four groups and superovulated with one of the four FSH regimens as follows: Group I) twice daily injections of a constant dose of 5 mg of FSH for a period of 4 days, giving a total dose of 40 mg in 8 fractions; Group II) as in Group I with additional 5 mg of FSH on the 5th day, giving a total dose of 45 mg; Group III) twice daily injections on a decreasing dose regimen of 5, 4, 3 and 2 mg successively for 4 days with the addition of 2 mg of FSH once on the 5th day, giving a total dose of 30 mg; Group IV) similar to Group III, but a mixture of FSH and LH in the ratio of 5 : 1 was used, giving a total dose of 30 mg of FSH and 6 mg of LH in 9 fractions. Nonsurgical embryo recovery was performed 7 or 8 days after estrus. Percentage of good embryos in Group II was significantly greater than that in Group I ($P<0.05$). There was no significant difference between Groups II and III in any of the criteria analyzed. Group IV was superior to the other groups in superovulation rate and embryo yielding. Significantly greater numbers of total eggs and good embryos were observed in Group IV compared to those in Groups I and II ($P<0.05$). Percentage of the heifers with more than 3 good embryos in Group IV was significantly higher than that in Groups I, II and III ($P<0.05$).

Key words: superovulation, FSH, heifer, embryo

INTRODUCTION

Both PMSG and FSH have been widely used to induce superovulation in cattle. Some investigations comparing the superovulatory responses to PMSG and FSH reported that both gonadotrophins were equally effective,[5,15] while other workers observed higher numbers of ovulations, transferable embryos and pregnancies after using FSH.[6,10,11,16] In recent studies, it was suggested that abnormality of hormone-profiles and disruption of the physiological function of the oocyte were more frequently encountered in PMSG-treated cows than in FSH-treated animals, and that PMSG-treated cow yielded fewer viable embryos than FSH-treated cows.[7,8,12] Unsatisfac-

Department of Veterinary Obstetrics, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

tory results in the superovulation rate and embryo yielding were obtained previously by the author when PMSG was used to induce superovulation in virgin heifers.[17,18] It was thus hoped that better superovulatory results could be obtained using FSH regimens. In embryo transfer procedures, FSH injections twice a day for 4 or 5 consecutive days is a common procedure. However, different injection schedules have been used by different workers, and there several variations in each schedule. Nevertheless, there are few comparative studies of different FSH regimens.[1,2,4] In this study, comparisons of several FSH regimens in relation to the superovulation rate and embryo yielding were made.

MATERIALS AND METHODS

Eighty-three Holstein-Friesian virgin heifers, 11 to 16 months of age, were used in this study. All heifers had normal reproductive tracts, as determined by rectal palpation. The heifers were randomly divided into four groups and superovulated with one of the four FSH regimens as shown in Table 1. FSH (Denka Pharmaceutical Co., Ltd.) or a mixture of FSH and LH (Burns-Biotec) in a ratio of 5 : 1 were administered intramuscularly accordingly at approximately 9:00 and 16:00. The first injection was given 9 to 14 days after natural estrus. Forty-eight hours after the first injection of FSH, all the heifers received an intramuscular injection of 30 mg of $PGF_2\alpha$ (Upjohn Co., Ltd.) to induce luteolysis. Only the heifers showing standing estrus on the morning of the 5th day of FSH administration were assigned to the project. They were artificially inseminated twice with frozen semen at approximately 54 and 72 hours

TABLE 1 *Injection schedules of four different FSH regimens for inducing superovulation*

GROUPS	DOSE OF FSH (LH) PER INJECTION (mg)					TOTAL DOSE OF FSH (LH)
	1st	2nd	3rd	4th	5th day§	(mg)
I	5, 5	5, 5	5, 5	5, 5	–	40
II	5, 5	5, 5	5, 5	5, 5	5	45
III	5, 5	4, 4	3, 3	2, 2	2	30
IV*	5, 5 (1.0, 1.0)	4, 4 (0.8, 0.8)	3, 3 (0.6, 0.6)	2, 2 (0.4, 0.4)	2 (0.4)	30 (6)

* A mixture of FSH and LH in a ratio of 5 : 1 was used.
§ The day of estrus.
The injections were administered approximately at 9 : 00 and 16 : 00 on the 1st to 4th day, and at 9 : 00 on the 5th day. Thirty mg of $PGF_2\alpha$ was administered at 9 : 00 on the 3rd day.

after the injection of $PGF_2\alpha$. Seven or eight days after the superovulatory estrus, the ovarian response was evaluated by rectal palpation under epidural anesthesia and the corpora lutea were counted. The uterus was then flushed nonsurgically using a Foley catheter as described previously.[17,18] The eggs collected were examined under an inverted microscope at 200 to 400 X magnification. Embryos that conformed with the expected developmental stage with no signs of degeneration were judged as good embryos as described previously.[17,18]

To evaluate the superovulatory effects of each FSH regimen, the following parameters were analyzed: 1) mean number of corpora lutea; 2) mean number of total eggs recovered; 3) mean recovery rate (number of total eggs recovered / number of corpora lutea); 4) mean number of good embryos; 5) mean percentage of good embryos (number of good embryos / number of total eggs); 6) percentage of heifers with 0, 1–3 and more than 3 good embryos in each group. These mean numbers and mean percentages were subjected to t-test. The distributions among 3 classes, grouped according to the number of good embryos, were analyzed by χ^2-test.

Results

Results of superovulation, induced by the four FSH regimens using the nonsurgical method of embryo collection, are shown in Table 2.

TABLE 2 *Superovulation rate, recovery rate and number of good embryos from heifers induced by four FSH regimens using the nonsurgical embryo collection method*

GROUPS	NO. HEIFERS TREATED	NO. CORPORA LUTEA	NO. (%) EGGS RECOVERED	NO. (%) GOOD EMBRYOS	% OF HEIFERS WITH good embryos		
					0	1–3	3<
I	18	§9.2±1.2[a]	§4.8±1.1[a] (46.3±7.0)[a]	§2.8±0.7[a] (49.7±8.3)[a]	27.8	33.3	38.9[a]
II	19	8.6±1.2[a]	5.5±1.2[a] (55.9±8.1)[ab]	4.0±1.0[a] (76.1±5.9)[b]	15.8	42.1	42.1[a]
III	19	11.8±1.5[ab]	7.5±1.4[ab] (59.3±7.5)[ab]	5.1±1.4[ab] (57.1±9.1)[ab]	26.3	31.6	42.1[a]
IV	27	13.1±1.3[b]	10.2±1.2[b] (72.9±4.9)[b]	7.2±1.0[b] (69.2±5.0)[b]	11.1	14.8	74.1[b]

a, b Values in the same colume with different superscript show significant difference ($p<0.05$).

§ Values are mean±S. E..

There was no significant difference between Groups I and II in the number of total eggs recovered or the number of good embryos. However, the percentage of good embryos in Group II was significantly higher than that in Group I ($P<0.05$). A slightly higher ovulation rate and greater number of good embryos were obtained from the heifers that received a decreasing dose regimen (Group III), as compared to those in the constant dose regimen (Group II). Nevertheless, there was no significant difference between the two groups in any of the criteria analyzed. The number of total eggs recovered and the number of good embryos in Group IV were significantly greater than those in Groups I and II ($P<0.05$). A significantly higher recovery rate was found in Group IV than in Group I ($P<0.05$). The percentage of heifers with more than 3 good embryos in Group IV was significantly higher than that in the other three groups ($P<0.05$). However, there was no significant difference between Groups III and IV in the other criteria analyzed.

DISCUSSION

FSH is generally injected for a period of 4 or 5 days, though it has not been determined whether an additional injection of FSH on the day of estrus is necessary or not. It was indicated that FSH had an ovulation inducing activity in rats,[9] and the possibility that FSH might be involved in the ovulatory process in heifers was suggested.[13] It was thought that an additional injection of 5 mg of FSH on the day of estrus may have some favourable effects on fertilization and embryo quality.

Several comparative studies of superovulation with constant and decreasing doses of FSH were reported.[1,2,4] Some workers reported that superovulation induced at a constant dose of FSH increased the ovulation rate,[1,2] plasma estradiol-17β[1] and percentage of fertilized eggs[2] as compared to that induced with a decreasing dose of FSH. However, the experimental number was small ($N = 4$–5) and no data of embryo recovery was shown. In recent years, Chupin & Procureur[4] showed that twice a day injections of decreasing doses of FSH induced a significantly higher rate of superovulation and greater number of good embryos than injections of a constant dose of FSH adding up to the same total dose. However, they injected FSH twice a day for 4 days in 8 fractions and did not inject an additional dose of FSH on the day of estrus, as was done in Group I of the present study. It was found in the present study that an increase in the number of eggs occurred (Group II) when an additional dose of 5 mg of FSH was given on the 5th day. Hence the superovulation rate and embryo yielding may be significantly reduced with constant doses of FSH. Although superiority of the FSH regimen with decreasing doses was not found in the present study, it appeared that the FSH regimen with decreasing doses is as effective as that with a constant dose. Hence there is no necessity to inject FSH at exactly a constant dose.

Group Ⅳ, which was given a mixture of FSH and LH at decreasing doses, was found to be superior to the other groups in the superovulation rate and embryo yielding. This result is in agreement with that of Seidel et al.,[16] and also supports the practice of adding 20% of LH to commercial FSH preparations, which is generaly followed in North America. Recently, it was reported that the addition of a greater amount of LH to the FSH preparation in superovulation regimens appeared to reduce ovarian response and fertilization rate.[3,14] Murphy et al.[14] demonstrated that there was no significant decline in the number of corpora lutea when the ratio of FSH and LH was at 5 : 1. However, when the FSH / LH ratio was greater than 5 : 1, a significant reduction in the number of corpora lutea was noted. In their study, twice daily injections of FSH-LH over a 4-day period were performed, while in our present study, an additional dose of FSH-LH was injected on the estrous day. It was observed that the egg recovery rate and number of good embryos were higher in this group, indicating that this additional injection may have beneficial effects on embryo recovery and viable embryo yielding.

In the present study, the effects of not giving the additional dose of FSH-LH on the day of estrus and those of adding LH to the constant dose of FSH regimen were not examined, thus further investigations of this aspect need to be done.

This study was performed under the same conditions as those of our previous studies[17,18] in which virgin heifers were treated with PMSG regimens. As would be expected, the superovulatory results of the present FSH regimens are superior to those of the previous PMSG regimens. Furthermore, it was found that the FSH-LH preparation gives a much better result in inducing superovulation in virgin heifers.

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