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Citation	Japanese Journal of Veterinary Research, 32(4), 183-189
Issue Date	1984-10-20
DOI	10.14943/jjvr.32.4.183
Doc URL	<a href="http://hdl.handle.net/2115/2312">http://hdl.handle.net/2115/2312</a>
Type	bulletin (article)
File Information	KJ00002374266.pdf



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## EFFECTS OF LH-RH ANALOGUE ON THE OVULATION RATE AND EMBRYO QUALITY IN HEIFERS SUPEROVULATED WITH PMSG AND PGF<sub>2</sub> $\alpha$

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(Received for publication July 18, 1984)

Eighty-four Holstein-Friesian virgin heifers were superovulated with either 2500 or 3000 IU of PMSG in addition to 30 mg of PGF<sub>2</sub>  $\alpha$ . The heifers were divided into two groups. Approximately half of them were administered LH-RH analogue twice at 1.5 hour intervals during the superovulatory estrus (treated), and the remaining animals were not treated with LH-RH analogue (control). Nonsurgical embryo recovery was performed 7 or 8 days after estrus. In the control heifers, effects of the dose of PMSG were examined, and it was found that by increasing the dose from 2500 to 3000 IU, a significantly larger number of corpora lutea and total number of eggs were observed ( $P < 0.05$ ). However, the number of good embryos was not increased. These effects of LH-RH analogue were analyzed by comparing the treated and the untreated control heifers following the injection of the same dose of PMSG. The treatment with LH-RH analogue was effective in increasing significantly the number of ovulations and the total number of eggs and good embryos recovered ( $P < 0.05$ ) when the heifers also received 2500 IU of PMSG. The heifers received 3000 IU of PMSG did not show significant responses to LH-RH analogue, but the tendency of a higher percentage of good embryos was recorded.

Key words: Superovulation, embryo, PMSG, LH-RH analogue, heifer

### INTRODUCTION

It has been suggested that an ovulating hormone, such as HCG, injected into PMSG-treated donors at the onset of estrus had an influence on the transportation or retention of embryos, and moreover, exerted a favourable effect on the egg recovery rate.<sup>11)</sup> However, it is also evident that HCG treatment does not increase the superovulatory response to PMSG.<sup>10,11)</sup> There are several reports on the use of LH-RH (or Gn-RH) in treatments designed to study its effects on ovulation rate and embryo quality.<sup>1,6,11)</sup> In these studies, animals were treated with a fixed dose of PMSG or FSH. Hence, it was anticipated that the dose of PMSG would influence the

embryonic yield, the embryo quality and the hormonal patterns.<sup>3,5,7,14)</sup> In the present study, heifers were injected with two different doses of PMSG and then treated with LH-RH analogue twice at 1.5 hour intervals during superovulatory estrus. The effects of LH-RH analogue on the superovulatory rate and the embryo quality were analyzed.

#### MATERIALS AND METHODS

Eighty-four Holstein-Friesian virgin heifers, aged 11 to 16 months, were used in this study. All heifers had normal reproductive tracts, as determined by rectal examination. On days 8 to 14 (day 0=day of estrus) the heifers received a dose of either 2500 or 3000 PMSG (Sankyo Co., Ltd.) intramuscularly. Luteolysis was induced by a single dose of 30 mg of PGF<sub>2</sub>α (Upjohn Co., Ltd.) injected intramuscularly 48 hours after the administration of PMSG. Only the heifers that showed standing estrus within 48 hours after the PGF<sub>2</sub>α injection were injected intramuscularly with 200 and 400 μg of LH-RH analogue (Fertilerine acetate; Takeda Co., Ltd.) at 1.5 hour intervals (treated heifers). The first and the second injections were performed at 52.0 and 53.5 hours after the PGF<sub>2</sub>α injection, respectively. The remaining heifers were not treated with LH-RH analogue (control heifers). All heifers were inseminated twice with frozen semen at 53-54 hours after the PGF<sub>2</sub>α injection.

Seven or 8 days after the superovulatory estrus, the number of ovulations was determined by counting the number of corpora lutea by rectal palpation, and eggs were collected nonsurgically using a Folley catheter as previously described.<sup>16)</sup> Modified Dulbecco's PBS,<sup>17)</sup> TCM-199 and Eagle's MEM were used to flush the eggs from the uterus. All eggs recovered were individually examined under a stereomicroscope at 200 to 400 X magnification. Embryos with no signs of degeneration and those having the expected developmental stage corresponding to the age judged from after the occurrence of superovulatory estrus were classified as good embryos; on day 7, they were in a stage between late morula and blastocyst, and on day 8, blastocyst and hatched blastocyst. The numbers of corpora lutea, total eggs and good embryos were subjected to t-test. Distributions among 3 classes grouped according to the number of good embryos (0, 1-3, more than 3) were analyzed by  $\chi^2$ -test.

#### RESULTS

Data on the superovulatory responses and nonsurgical recovery are presented in Table 1.

Heifers given either 2500 or 3000 IU PMSG and not treated with LH-RH analogue (control heifers) were selected to analyze the effects of the dose of PMSG. By increasing the dose from 2500 to 3000 IU, the mean numbers of corpora lutea and eggs recovered were significantly increased ( $P < 0.05$ ). However, the mean number

TABLE 1 *Effects of LH-RH analogue on the ovulation rate and the embryo quality in superovulated heifers*

DOSE OF PMSG (IU)	LH-RH ANALOGUE	NO. HEIFERS TREATED	NO. CORPORA LUTEA	NO. (%) EGGS RECOVERED	NO. (%) GOOD EMBRYOS	% OF HEIFERS WITH		
						0	1-3	3<
						GOOD EMBRYOS		
2500	-	19	# 7.1 ± 1.0 <sup>a</sup>	# 3.8 ± 0.7 <sup>a</sup> (48.7 ± 7.7)	# 2.6 ± 0.5 <sup>a</sup> (70.7 ± 6.4) <sup>a</sup>	26.3	36.8	36.8
	treated*	22	10.8 ± 1.4 <sup>b</sup>	7.2 ± 1.0 <sup>b</sup> (66.0 ± 5.1)	4.8 ± 0.7 <sup>b</sup> (72.0 ± 5.9) <sup>a</sup>	9.1	31.8	59.1 <sup>a</sup>
3000	-	21	12.4 ± 0.9 <sup>b</sup>	6.7 ± 0.7 <sup>b</sup> (55.8 ± 5.0)	3.0 ± 0.6 (44.6 ± 6.8) <sup>b</sup>	19.0	52.4	28.6 <sup>b</sup>
	treated*	22	12.3 ± 1.7 <sup>b</sup>	5.8 ± 0.8 (51.7 ± 6.3)	3.2 ± 0.6 (58.3 ± 8.5)	27.3	22.7	50.0

\* The heifers were injected with LH-RH analogue twice at 1.5 hour intervals during estrus ; the first and the second doses were 200  $\mu$ g at 52 hours and 400  $\mu$ g at 53.5 hours after injection of PGF<sub>2</sub><sup>a</sup>, respectively.

a,b Significant difference was observed between a and b (p < 0.05).

# Values are means ± S. E.

of good embryos was not increased, and the mean percentage of good embryos was decreased significantly ( $P < 0.05$ ).

Effects of LH-RH analogue were analyzed by comparing the treated and the untreated control heifers following the injection of the same dose of PMSG. The treatment with LH-RH analogue was significantly effective in increasing the numbers of ovulations, eggs recovered and good embryos ( $P < 0.05$ ), when the heifers received 2500 IU of PMSG. Moreover, the percentage of the heifers with more than 3 good embryos was higher, and the percentage of heifers with no good embryos was lower in the treated group than in the control group. When the heifers were injected with LH-RH analogue following 3000 IU of PMSG, the mean percentage of good embryos and the distribution of the heifers with more than 3 good embryos exhibited a tendency to increase, but there were no significant differences.

#### DISCUSSION

It is well known that increase in the dose of PMSG induces rise in the superovulatory response. However, raising the dose has been found to reduce egg quality independently of the number of corpora lutea,<sup>5,14)</sup> and to decrease the pregnancy rate following the transfer of the embryos recovered.<sup>14)</sup> In general, superovulation regimens in which PMSG is used employ doses of 2000 to 2500 IU, and it is considered that the upper limit is about 3000 IU.<sup>3)</sup> In the present control heifers, significantly higher embryonic yield was recorded when 3000 IU was injected. However, the number of good embryos was not increased. The heifers which received 2500 IU had a significantly higher percentage of good embryos than those which received 3000 IU. The present results obtained from the Holstein-Friesian virgin heifers agreed with the previous conclusions.<sup>3)</sup>

Some workers reported that no statistically significant difference was noted between the cows receiving LH-RH (or Gn-RH) and the control cows in the numbers of corpora lutea palpated, total ova or transferable embryos.<sup>1,11)</sup> A single injection of LH-RH during the estrus was employed by those workers. It has been demonstrated that two injections of 100  $\mu$ g of LH-RH at 1.5 hour intervals induced a significant rise in LH release which was larger than that induced by a single injection of the same dose.<sup>2)</sup> Guay & Bedoya<sup>6)</sup> reported that the LH level was elevated significantly and that more embryos were recovered after the treatment of Gn-RH analogue, which consisted of 500  $\mu$ g divided into two injections given 2 hours apart.

In the present study, LH-RH analogue was also injected twice at 1.5 hour intervals, and its effects on embryonic yield and embryo quality were different from those mentioned above when two different doses of PMSG given. In the heifers receiving 2500 IU of PMSG, the mean numbers of eggs recovered and good embryos were significantly increased, while a higher percentage of good embryos and a larger distribution of heifers with more than 3 good embryos were recorded. However, no

significant effects of LH-RH analogue were observed when the dose of PMSG was raised to 3000 IU.

Preovulatory normal LH release is essential to yield normal embryos.<sup>4,8,9)</sup> Abnormal LH profiles, such as absence of LH surge, moderate LH surge and premature or delayed occurrence are known to have detrimental effects on oocyte maturation and fertilization.<sup>9)</sup> Although the duration, value of maximum concentration and total LH production did not correlate with the number of ovulations,<sup>12,13,15)</sup> it was indicated that the interval between the onset of estrus and the LH peak was associated with the ovulation rate: that is, shorter intervals were associated with higher ovulation rates.<sup>7,13,15)</sup>

Henricks et al.<sup>7)</sup> showed that the higher the dose of PMSG, the shorter is the interval between the onset of estrus and the LH surge. In the present control group, the heifers which received 3000 IU of PMSG must have had a shorter interval, so that they showed a greater number of corpora lutea and higher egg recovery than the heifers given 2500 IU of PMSG. It was suggested that the preovulatory normal LH surge was provoked more rapidly with the treatment of LH-RH analogue, and that the intervals in the treated heifers may have been shorter than those in the control heifers when the heifers received 2500 IU of PMSG. Therefore, significant in the ovulation rate and higher embryo yield were observed in the heifers treated with LH-RH analogue.

It has also been shown that the plasma progesterone values during estrus were significantly higher for the PMSG-treated animals than for the untreated control animals,<sup>7,8)</sup> and that the plasma progesterone levels in the animals given a higher dose of PMSG remained elevated for a longer period. Also, progesterone trough during the estrus was shorter than that in the animals given a lower dose of PMSG.<sup>7)</sup> Jensen et al.<sup>8)</sup> suggested that a higher progesterone level at estrus inhibits the LH release and impairs embryo quality. One possible explanation of reduced efficacy of LH-RH analogue after the injection of a higher dose of PMSG is that a higher progesterone level in the present heifers receiving 3000 IU of PMSG might have suppressed the LH release, which would have been provoked by the treatment of LH-RH analogue.

The present results demonstrated that the doses of PMSG administered herein will alter the hormonal patterns and the effects of LH-RH analogue on the superovulation rate and embryo quality. Further examinations of the endocrinological profiles around the time of treatment of LH-RH analogue and the appropriate time of the treatment are needed to clarify the effects of LH-RH analogue on superovulated donor animals.

#### ACKNOWLEDGMENTS

The authors wish to thank the personnel of the National Hidaka Livestock

Breeding Station, Urakawa Hokkaido, for their assistance in conducting one part of this study.

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