Superovulation Induction by Human Menopausal Gonadotrophin in Rabbits

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ABSTRACT. Superovulation induction in laboratory animals is an important technique as a means of providing eggs for embryonic research. Superovulation induction by human menopausal gonadotrophin (HMG) has not been fully established in rabbits. In the present study, the relationship between the dosage of injected HMG and the superovulatory response including fertilization rate was studied. The most satisfactory result was obtained in the group in which 30 iu of HMG was injected subcutaneously three times at 24 hr intervals. The superovulatory response by this method was considerably better that than by conventional six injections of follicle-stimulating hormone (FSH) having been widely accepted.—KEY WORDS: HMG, rabbit, superovulation.


In laboratory animals, superovulation induction constitutes an important technique for embryonic research and embryo transfer. It has been reported that the best method for inducing superovulation in rabbits is to inject follicle-stimulating hormone (FSH) twice a day for 3 days [2, 5, 9]. Recently, human menopausal gonadotrophin (HMG) has been also applied for superovulation induction in rabbits [7]. This method, however, has not been fully established.

In the present experiment, relationship between the dosage of injected HMG and the superovulatory response including fertilization rate was studied. The purpose of this study is to obtain the basic data for superovulation induction by HMG in rabbits.

Mature female Japanese white rabbits weighing between 2.8 to 3.5 kg were used in the experiment. They were purchased from Saitama Experimental Animal Supply Co., Ltd., Saitama, Japan. The rabbits were housed individually in 68 × 82 × 70 cm wire netting cages (R3 type; Okazaki Sangyo Co., Ltd., Saitama, Japan). The animal room was air-conditioned: 23 ± 2 °C, 55 ± 5% relative humidity, 15 air changes per hour, and illuminated for 14 hr (05:00-19:00) per day with 300 lux day-light fluorescent lamps. The rabbits were given commercial pellets (Lab R Stock; Nihon Nosan Kogyo Co., Ltd., Yokohama, Japan) and tap water ad libitum. Each animal had been isolated in the separate cage for at least 30 days prior to the experiment.

Five experimental groups were set up as to different dosage of HMG: 5 iu, 10 iu, 20 iu, 30 iu or 40 iu per one injection. HMG (Pergonal; Teikoku Hormone Mfg. Co., Ltd., Tokyo, Japan) was dissolved in physiological saline to make the concentration of 10 iu/ml and it was injected subcutaneously into the back of the rabbits' neck three times at 24-hr intervals.

The rabbits were mated with proven fertile males 24 hr after the last injection of HMG. After an elapse of 30 hr from the mating, the rabbits were euthanized and the number of ovulation points in each ovary was counted. The ovulated ova were collected by perfusing the excised oviducts. TCM-199 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) including 40% of inactivated normal rabbit serum was used as the perfusate. The collected eggs were examined for their development stage through a stereoscopic microscope.

The number of ovulation points in the ovaries was analysed by analysis of variance followed by Duncan's multiple range test, and fertilization rate was analysed by Chi-square test. In the both analyses, significant differences were considered when P-values were less than 0.05.

The results of this experiment were shown in Table 1. According as the dose of injected HMG increased to 30 iu, the average number of ovulation points increased remarkably. The maximum ovulation points was observed in the group of 30 iu HMG injected three times at 24 hr intervals. The mean number of ovulation points of this group was 77.8. In 40 iu HMG injected group, however, the mean number of ovulation points decreased to 56.3. It was much lower than that of the 30 iu HMG injected group.

As shown in Table 1, most of eggs recovered from all groups were in the 4-cell stage. Fertilization rate in 5 iu group was as low as 29.6%. On the other hand, satisfactory results on fertilization rate were obtained in the other groups. In 10, 30 and 40 iu groups, in particular, fertilization rates were more than 90%.

It is known that HMG has been applied to superovulation induction in cattle with satisfactory results [3, 4, 6]. In rabbits, injecting FSH six times for three days is widely accepted as the best method for superovulation induction [2, 5, 9]. In these reports, the mean number of ovulation points observed on the ovary ranged from 40 to 54.

A few reports were shown that HMG induces superovulation in rabbits [1, 7]. Onodera and Ishijima [7] thought that six injections of HMG, twice a day for three days, was necessary for superovulation in rabbits. They obtained about 40 ovulation points on average by this method. In the report, however, the best method for superovulation induction by HMG was not discussed. On the other hand, a potential that three injections of HMG might induce superovulation in rabbits have been suggested, but further discussion was not made at all [1].

In the present study, more than 75 ovulation points were observed in the 30 iu HMG injected group. This

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Table 1. Ovulatory response and development stage of recovered eggs in rabbits administered with HMG once a day for three days

| Dose of HMG per once (iu) | No. of rabbits examined | No. of ovulation points (mean±SE) | Total no. of eggs recovered | Development stage | Fertilization rate (%)
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<tbody>
<tr>
<td>5</td>
<td>4</td>
<td>22.0± 2.5*</td>
<td>71</td>
<td>45 6 15 5</td>
<td>29.6*</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>34.3±10.3*b</td>
<td>113</td>
<td>5 7 97 2</td>
<td>93.8*</td>
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<tr>
<td>20</td>
<td>4</td>
<td>70.5± 6.3*b</td>
<td>249</td>
<td>25 71 113 40</td>
<td>73.9*b</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>77.8±10.5*b</td>
<td>223</td>
<td>5 42 165 8</td>
<td>92.8*b</td>
</tr>
<tr>
<td>40</td>
<td>4</td>
<td>56.3± 6.6*</td>
<td>189</td>
<td>12 52 120 5</td>
<td>91.0*</td>
</tr>
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*Degenerating eggs.
Values with different alphabetical superscripts within each column are different (P<0.05).

Figure of ovulation points was considerable more than that of the FSH injection having been widely accepted as the best method for superovulation induction in rabbits [2, 5, 9] and that of the HMG injection previously reported by Onodera and Ishijima [7]. Comparing the results of Onodera and Ishijima [7], and those obtained in the present study, more frequent injection of HMG may decrease the ovulatory response by contraries. Fertilization rate obtained by three injections of HMG in the present study was almost equivalent compared to that by conventional six injections of FSH [2, 5, 9] or six injections of HMG [7] recently reported. There were no differences between the developmental stage of eggs recovered in this experiment and those recovered in spontaneous ovulation [8].

From the results of this study, it can be suggested that subcutaneous injection of 30 iu of HMG three times at 24 hr intervals is one of the desirable candidate for superovulation induction to collect eggs in rabbits. To reduce injection distress to the animals, authors consider that reducing the frequency of gonadotrophin injections for superovulation induction would be significant. The findings of this study seem to contribute in view of the laboratory animal care.

REFERENCES