Interval between PMSG Priming and hCG Injection in Superovulation of the Mongolian Gerbil

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Abstract: To date, a practical method for inducing superovulation in the Mongolian gerbil has not been defined; therefore, in this study, we attempted to develop a flexible superovulation protocol for this species. Superovulation can be induced in the Mongolian gerbils by using PMSG with or without hCG. The injection schedule for PMSG (and hCG) has a high degree of flexibility, but the best protocol for embryo collection for reproductive biology is 20 IU PMSG followed by 20 IU hCG 54 hours later.

Key words: Mongolian gerbil, Superovulation

Introduction

The Mongolian gerbil, a rodent belonging to the family Cricetidae, is a relatively new laboratory animal. Ovulation, fertilization, and early cleavage in the Mongolian gerbil were investigated by Marston *et al.* in the 1960s [1]. The protocol for inducing superovulation in the Mongolian gerbil has been examined by Mastron and Chang [1], Fisher and Fisher [2], and Ishijima *et al.* [3]. Mastron and Chang [1] injected mature females with 10 to 40 IU PMSG (pregnant mare's serum gonadotropin) followed by 10 IU hCG (human chorionic gonadotropin) 52 hours later. They observed a maximal ovulation of 39.0 ova per animal with a combination of 40 IU PMSG and 10 IU hCG. Fisher and Fisher [2] injected immature females with 10 to 20 IU PMSG followed by 5 to 20 IU hCG 50 hours later. They proposed that the combination of 10 IU PMSG and 10 IU hCG was the optimal dosage for superovulation in immature females. Ishijima *et al.* [3] proposed that an injection of 20 IU PMSG followed by 20 IU hCG 54 hours later was the optimal protocol for induction of superovulation in mature females, but these investigators did not mention whether copulation occurred or the cleavage rates of ovulated ova after treatment. On the other hand, some investigators followed a protocol of 10 or 20 IU PMSG followed by 10 or 20 IU hCG either 48 hours later [4] or 75 hours later [5, 6]. These investigators documented the ovulation time and examined the morphology and developmental rate of ova from superovulated mature Mongolian gerbils. Despite the aforementioned studies, a suitable protocol for induction of superovulation in this species remains unclear. This study was undertaken to confirm the optimum interval between PMSG and hCG to produce the maximum number of embryos in the mature Mongolian gerbil.

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Materials and Methods

Animals

Mature Mongolian gerbils (two to four months old) were kept in a temperature (23 ± 2°C) and light (06:00 to 20:00) controlled environment, and were given free access to food and water.

Treatment with PMSG and hCG

Virgin females were injected subcutaneously with 20 IU PMSG (Nippon Zenyaku Kogyo) without regard to the estrus cycle. After the PMSG injection, the gerbils were injected subcutaneously with 20 IU hCG (Mochida Pharmaceutical) at various times (six to 144 hours, in increments of six hours). The hCG injection was given at 16:00, and the timing of the PMSG injection was retroactively calculated from the time of the hCG

injection. The number of ovulated ova in isolated oviducts was ascertained 24 hours after the hCG injection. Some of the treated females were mated with fertile males on the night after the hCG injection. In these cases, the number of embryos in isolated oviducts was ascertained 72 hours after mating. Isolated oviducts were placed between two slide glasses and the number of ova and/or embryos was determined with light microscopy.

Treatment with PMSG alone

Virgin females were injected subcutaneously with 20 IU PMSG without regard to the estrus cycle, and the number of ovulated ova was ascertained at 24 to 144 hours after the PMSG injection. The injection of PMSG and examination for ovulation were performed at 16:00. Some of the treated females were mated with fertile males overnight at 30, 54 or 78 hours after the PMSG injection. In these cases, the number of embryos was ascertained 72 hours after mating. Observation of the numbers of ova and/or embryos was done in the above-described manner.

Treatment with hCG alone

Virgin females were injected subcutaneously with 20 or 40 IU hCG without regard to the estrus cycle. The hCG injection was given at 16:00, and the number of ovulated ova was ascertained 24 hours after the hCG injection in the above-described manner.

Statistics

The numbers of ova found in ovulated females were analyzed by One-Way ANOVA, followed by Fisher's PSLD. Values of $P<0.05$ were considered to be statistically significant.

Results

The ovulatory response of Mongolian gerbils injected with PMSG and hCG is shown in Table 1. Ovulation occurred in all experimental groups. When the interval between PMSG and hCG was 48 hours or less, the ovulation rates ranged from 30% to 90%, and mean number of ova in ovulated females ranged from 4.0 to 16.9. When the interval was increased to 54 hours or more, virtually 100% of the gerbils ovulated, and the mean number of ova in the ovulated females ranged from 26.9 to 35.0 without a statistically significant difference. The rate of abnormal ova rose when the interval between PMSG and hCG was increased to 108 hours or more.

The ovulatory response of gerbils injected with PMSG alone is shown in Table 2. The rate of ovulating females ranged from 25% to 42% at 24 to 96 hours after the PMSG injection. Thereafter, the rate of ovulating females increased according to the interval time: 67% for 120 hours; 92% for 144 hours; and 100% for 168 hours. There was a statistically significant difference in the mean number of ova in ovulated females between the group 24 to 72 hours after PMSG injection (5.0–7.5) and the group 96 hours or more after PMSG injection (26.4–31.7). The highest rate of abnormal ova (17.1%) was recorded at 168 hours after PMSG injection.

The ovulatory response of gerbils injected with hCG alone is shown in Table 3. Injection of 20 or 40 IU hCG alone could not induce ovulation in most subjects. The ovulated females had a small number of ova (20 IU: 4.5; 40 IU: 2.5), which had a major degree of abnormal morphology (20 IU: 77.8%; 40 IU: 40.0%).

Some gerbils injected with PMSG and hCG had a number of embryos in their oviducts at 72 hours after mating (Table 4). When the interval between PMSG and hCG was 30 hours, the rates of ovulated females and copulated females (the rates of females which had 2 to 4-cell stage embryos) were 50% and 20%, respectively. In these ovulated and copulated females, the mean number of ovulated ova was 13.0, and 8.5 of them (65.4%) were 2 to 4 cell-stage embryos. When the interval between PMSG and hCG was 54 hours, the rates of ovulated females and copulated females improved to 100% and 90%, respectively. These gerbils with embryos ovulated 29.0 ova on average, and 22.7 of the embryos (78.3%) were 2 to 4 cell-stage embryos. When the interval between PMSG and hCG was 78 hours, the rate of ovulated females and the mean number ova in ovulated females were 100% and 33.7, respectively, but the rate of females who copulated (70%) and the rate of embryos that developed to the 2–4 cell-stage (40.7%; 13.7/33.7) decreased under this regimen.

When females were mated at 96 or 120 hours after PMSG injection, some females had embryos that had developed into 2–4 cell stages in their oviducts 72 hours after mating (Table 5). When the gerbils mated at 96 hours after PMSG injection, the rates of ovulated females and copulated females were 90% and 70%, respectively. Conversely, the group of gerbils who mated at 120 hours after PMSG injection had a relatively lower rate of ovulated females and copulated females (60% and 40%, respectively). Ovulated and copulated females who mated at 96 and 120 hours after the PMSG injection had a mean number of 25.6 and

Table 1. Ovulation in Mongolian gerbils treated with PMSG and hCG at various intervals

Interval between PMSG and hCG	No. of animals	% of ovulated animals	No. of ova in ovulated animals (Mean ± S.E.)	% of abnormal ova
6 hr	10	70	4.6 ± 0.9^{a}	0
12 hr	10	30	4.0 ± 1.0^{ab}	0
18 hr	10	50	9.0 ± 1.6^{ab}	0
24 hr	10	40	8.3 ± 2.3^{ab}	9.1
30 hr	10	40	4.8 ± 3.4^{ab}	0
36 hr	10	40	14.5 ± 5.4^{ab}	0
42 hr	10	90	12.2 ± 3.0^{ab}	0
48 hr	10	70	16.9 ± 2.9^{b}	0
54 hr	10	100	28.0 ± 2.5^{c}	0
60 hr	10	100	30.4 ± 4.4^{c}	0
66 hr	10	100	29.7 ± 3.6^{c}	0
72 hr	10	100	31.1 ± 3.5^{c}	0
78 hr	10	100	34.8 ± 1.9^{c}	0.6
84 hr	10	100	35.0 ± 1.4^{c}	0.9
90 hr	10	100	29.9 ± 4.2^{c}	4.0
96 hr	10	100	31.5 ± 3.1^{c}	0.3
102 hr	10	100	30.3 ± 2.5^{c}	4.0
108 hr	10	100	27.7 ± 4.5^{c}	7.6
114 hr	10	90	27.4 ± 2.9^{c}	7.7
120 hr	10	100	33.4 ± 3.0^{c}	3.3
126 hr	10	100	26.9 ± 3.0^{c}	9.3
132 hr	10	100	31.9 ± 3.1^{c}	10.3
138 hr	10	100	26.9 ± 4.1^{c}	10.4
144 hr	10	100	28.8 ± 4.8^{c}	9.7

Different superscripts within columns are significantly different ($P<0.05$).

Table 2. Ovulation in Mongolian gerbils treated with PMSG alone

Interval between PMSG and autopsy	No. of animals	% of ovulated animals	No. of ova in ovulated animals (Mean ± S.E.)	% of abnormal ova
24 hr	12	33	7.5 ± 1.0^{a}	0
48 hr	12	33	5.3 ± 1.1^{a}	4.8
72 hr	12	42	5.0 ± 0.6^{a}	0
96 hr	12	25	31.7 ± 6.4^{b}	1.1
120 hr	12	67	26.4 ± 5.0^{b}	3.8
144 hr	12	92	30.6 ± 3.9^{b}	5.3
168 hr	12	100	31.3 ± 1.9^{b}	17.1

Different superscripts within columns are significantly different ($P<0.05$).

29.9 ova, respectively, and 15.7 (61.3%), and 6.3 (21.1%) of each were 2 to 4-cell stage embryos.

Discussion

Superovulation can be induced in Mongolian gerbils with PMSG with or without hCG. The injection schedule of PMSG (and hCG) has a high degree of flexibility, but 20 IU PMSG followed by 20 IU hCG 54 hours later is the best regimen for the collection of a large number of early embryos for reproductive biology.

When the interval between PMSG and hCG was 48 hours or less, the rate of ovulated females varied. This phenomenon may be caused by giving the PMSG injection without regard to the estrus cycle. Conversely, the mean number of ova obtained in ovulated females was stable at around 30 when the interval between PMSG and hCG injections was 54 hours or more. This

Table 3. Ovulation in Mongolian gerbils treated with hCG alone

Dose of hCG	No. of animals	% of ovulated animals	Mean no. of ova in ovulated animals	% of abnormal ova
20 IU	10	20	4.5	77.8
40 IU	10	20	2.5	40.0

Table 4. Mating in Mongolian gerbils treated with PMSG and hCG

Interval between PMSG and hCG/mating	No. of animals	% of ovulated animals	% of animals with embryos	Mean no. of ova in animals with embryos			
				2–4-cell	1-cell	Abnormal	Total
30 hr	10	50	20	8.5	4.0	0.5	13.0
54 hr	10	100	90	22.7	5.3	1.0	29.0
78 hr	10	100	70	13.7	19.1	0.9	33.7

Table 5. Mating in Mongolian gerbils treated with PMSG alone

Interval between PMSG and mating	No. of animals	% of ovulated animals	% of animals with embryos	Mean no. of ova in animals with embryos			
				2–4-cell	1-cell	Abnormal	Total
96 hr	10	90	70	15.7	8.6	1.3	25.6
120 hr	10	60	40	6.3	19.3	4.3	29.9

mean number of ovulated ova is comparable to the results of other investigators [1–3]. Mature female Mongolian gerbils have an estrus cycle of 4–6 days [7], a mean number of 3.9 pups (range 1–9) [8] and a mean number of ovulated ova of 6.6 [1] in their natural cycle. When the interval between PMSG and hCG was 30 hours or less, some females had oocytes within the range of natural ovulation. These oocytes may have derived from natural ovulation or from induced ovulation of naturally growing oocytes. When the interval between PMSG and hCG injections was 36 to 48 hours, there was no statistically significant difference in the number of ovulated ova; this finding was due to the unstable rate of ovulating females. Abnormal ova rarely appeared when the interval was 84 hours or less, except for 24 hours. The ratio increased when the interval was 100 hours or more. In these cases, some of the ova were found in the isthmus of the oviduct. Therefore, the increasing rates of abnormal ova may be due to the ovulation due to PMSG alone and degeneration of the resulting ova in the oviduct.

Ovulatory responses at 24 to 168 hours after PMSG injection were examined for the possibility of ovulation due to PMSG injection alone. Ovulation occurred in some females at less than 120 hours after PMS injection, and nearly all females ovulated 144 hours after PMSG injection. Up to 72 hours after PMSG injection, the number of ova in the ovulated females was comparable to that found in natural ovulation. The gerbils injected with 20 IU PMSG ovulated up to 96 hours after the injection, and at 96 hours after the PMSG injection, the mean number of ova in the ovulated females increased to approximately 30. These results are comparable to those using a regimen including hCG. The rate of abnormal ova increased at 120 hours after PMSG injection. These abnormal ova seemed to originate from degenerates of previously ovulated ova. Gerbils injected with hCG at 100 or more hours after PMSG may experience double ovulation either from PMSG alone or from the addition of hCG. The number of ova did not double when the interval was 100 hours or more. This finding is probably due to rapid transit through the oviduct of ova from the first ovulation.

Most gerbils injected with 20 or 40 IU hCG failed to ovulate. Abnormal ova in some females could have derived from the last natural estrus cycle. Injection of hCG alone cannot induce ovulation in mature gerbils.

Embryos could be harvested by the injection of PMSG followed by hCG, plus mating 30 to 78 hours after the PMSG injection. Mating exerted little influence on ovulatory responses in the treated females. Injection of PMSG and hCG at an interval of 54 hours, the regimen we advocate [3], produced the best results for ovulation

and copulation. PMSG followed by hCG 78 hours later resulted in a decline in the copulation rate. And the copulated females had many 1-cell ova which appeared to be unfertilized. This phenomenon may be due to heat near the end and aging of the oocytes, which were ovulated before mating.

When mating occurred 96 or 120 hours after PMSG injection, embryos were recovered from some females. The ovulatory response in the treated females was not influenced by mating, and mating at 96 hours after PMSG injection was selected because the number of ovulated ova reached a plateau in the experiments without mating. The copulation rate at this interval was 70%, and many 1-cell ova, which appeared to be unfertilized, were observed in the copulated females. The copulation rate and the number of embryos in the copulated females decreased when mating was assigned to 120 hours after the PMSG injection. PMSG induced prolonged ovulation, and some aging ova which ovulated beforehand may have lost fertilizability.

The females treated with PMSG alone expressed delayed plateaus in ovulatory responses when compared to the females treated with PMSG and hCG. The females injected with PMSG and hCG at an interval of 54 hours were observed for an ovulatory response 24 hours after the hCG injection. Therefore, their ovulatory responses were comparable with that in females which were injected with PMSG 78 hours earlier. The ovulatory responses in the females at 72 or 96 hours after PMSG injection were lower than those in the females injected with PMSG and hCG at the 54 hour interval. In addition, copulation rates in the females at 96 hours after PMSG injection were inferior to those in the females injected with PMSG and hCG at the 54 hour interval. The same tendency was seen in the fertilizability of ovulated ova. PMSG alone induced longer ovulation than PMSG together with hCG. A dose of 20 IU PMSG followed by 20 IU hCG 75 hours later induced ovulation in all gerbils by 12 hours after the hCG injection [6]. 20 IU hCG alone could not induce ovulation but the dose of hCG could accelerate and synchronize ovulation induced by PMSG.

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