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Fertilizability of Superovulated Eggs by Estrous Stage-independent PMSG/hCG Treatment in Adult Wistar-Imamichi Rats

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Abstract: We investigated the fertilization and developmental ability of superovulated eggs obtained from adult Wistar-Imamichi (WI) rats, by using pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) treatment. Female WI rats, 11–13 weeks of age, were divided into four groups by estrous stage (metestrus [ME], diestrus [DE], proestrus [PE], or estrus [E]). PMSG (150 IU/kg) and hCG (75 IU/kg) were injected at an interval of 48 or 55 h and the female rats were mated with mature male rats. The ovulated eggs were collected 20, 24, and 27 h after hCG injection. Regardless of the estrous stage at the time of PMSG injection, the treated rats mated and ovulated similar to the untreated spontaneously ovulated rats (S group). Although the proportion of fertilized eggs in the E- and PE-treated groups was less than the S group 20 h after hCG injection, the proportion was not different among all treated and S groups 24 h after hCG injection. The proportion of fertilized eggs using *in vitro* fertilization and the proportion of offspring obtained from 2-cell stage embryo transfer did not differ among the treated and S groups. In comparison with PMSG/hCG-treated immature rats, mating and ovulation rate of adult rats were significantly higher. The proportion of fertilized eggs obtained from mated rats did not differ between immature and adult rats. These results demonstrate that adult WI rats are good egg donors for reproductive biotechnological studies using unfertilized or fertilized eggs.

Key words: adult rat, estrous cycle, fertilization, superovulation

Introduction

Induction of superovulation is a technique to provide a large number of eggs, and it is an essential technique for reproductive biotechnology in experimental animals. There are various methods to induce superovulation in laboratory rats, such as injection of pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) [1, 2, 4, 12, 22, 25], continuous infusion of follicle stimulating hormone [5, 30], and injection of inhibin antiserum [14, 15]. Among these methods, PMSG/hCG treatment is widely used as a simple and effective method to induce superovulation [4, 28]; however, it is known that response to PMSG/hCG treatment varies by strain [1, 4, 6, 19, 20, 22, 25, 28] and with age [16, 18–20, 22, 28]. Thus, there is no universal protocol to induce superovulation with PMSG/hCG in rats. We have shown that efficient superovulation can be induced in adult (approximately 12 weeks of age) Wistar-Imamichi (WI) rats using 150 IU/kg PMSG and 75 IU/kg hCG by injection, regardless of the estrous stage at the time of PMSG injection [20]. Of note, the fertilization and

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Table 1. Timetable of PMSG/hCG injection, natural mating and egg collection

Day	Treatment	Adult 1	Adult 2	Adult 3	Immature
1	PMSG injection	10:00	10:00	10:00	10:00
	Dose (IU/kg)	150	150	150	300
	Time interval	55 h	55 h	48 h	48 h
3	HCG injection & mating	17:00	17:00	10:00	10:00
	Dose (IU/kg)	75	75	75	150
	Time interval	20 h	24 h	27 h	24–29 h
4	Egg collection & observation 1	13:00	17:00	13:00	10:00–15:00
5	Observation 2	10:00	10:00	10:00	10:00

developmental ability of the obtained eggs were not investigated in the previous study [20]. There are several reports that the fertilization and developmental ability of eggs obtained from PMSG only- or PMSG/hCG-treated rats are low in other strains [2, 3, 31, 32]. Therefore, we determined the fertilization and developmental ability of superovulated eggs obtained from PMSG/hCG-treated adult WI rats after natural mating with adult males, and using *in vitro* fertilization (IVF) and embryo transfer (ET). We also determined the superovulation and fertilization efficiency of PMSG/hCG-treated immature WI rats and compared with those of PMSG/hCG-treated adult WI rats.

Materials and Methods

Animals

Outbred WI rats obtained from the Institute for Animal Reproduction (Ibaraki, Japan) were used. These animals were housed under controlled temperature and light conditions (lights on 5:00–19:00), and supplied with food and water *ad libitum*. Adult female rats at 11–13 and 12–18 weeks of age were used as donors for superovulation treatment and recipients of ET, respectively. Adult female rats with at least two consecutive 4-day estrous cycles were used for this study. The estrous stages were detected by checking vaginal smears. Immature female rats at 3–4 weeks of age were also used as donors for superovulation. Mature male rats at 3–11 months of age were used for natural mating and IVF. Vasectomized adult male rats were used to induce pseudopregnancies in recipient female rats. The experimental protocols were approved by the Animal Care and Use Committee of the Dokkyo Medical University. The experiments were carried out under the control of the Guidelines for Animal Experimentation of Dokkyo Medical University.

Superovulation treatment

Adult female rats were divided into four groups by estrous stage, (metestrus [ME], diestrus [DE], proestrus [PE], or estrus [E]). PMSG (Serotropin; ASKA Pharmaceutical Co., Ltd.., Tokyo, Japan) and hCG (Gonatropin; ASKA Pharmaceutical Co., Ltd..) were dissolved in physiological saline at 150 and 75 IU/ml, respectively. Adult female rats received 150 IU/kg PMSG intraperitoneally (i.p.) at 10:00, followed by 75 IU/kg hCG (i.p.) 48 or 55 h later. Immature female rats received 300 IU/kg PMSG (i.p.) at 10:00, followed by 150 IU/kg hCG (i.p.) 48 h later.

Natural mating

Immediately after hCG injection, a female rat was placed in a cage with a male rat for one night. Untreated rats at the PE stage were also mated as controls for spontaneous ovulation (S group). Mating was confirmed the next morning by the presence of spermatozoa in the vaginal smears. The rats were sacrificed to collect eggs 20, 24, and 27 h after the hCG injection; some details are shown in Table 1. The oviducts were excised and eggs were flushed out with modified rat 1-cell embryo culture medium (mR1ECM) containing 110 mM NaCl and 4 mg/ml of BSA (Sigma-Aldrich Corp., St. Louis, MO, USA) instead of polyvinyl alcohol (mR1ECM/BSA) [23] containing 0.1% hyaluronidase. The collected eggs were washed with mR1ECM/BSA, the number was counted, and the eggs were classified as normal or degenerate under a stereoscopic microscope. Fertilization was determined under an inverted microscope (Leica DM IRB; Leica Microsystems GmbH, Wetzlar, Germany) based on sperm penetration into the perivitel-

Table 2. Mating and ovulation in PMSG/hCG-treated adult WI rats

Treatment[a)]	PMSG-hCG interval	Estrous stage[b)] at time of PMSG injection	No. of examined rats	No. of mated rats	(%)	No. of ovulated rats	(%)	No. of mated and ovulated rats	(%)
Adult 1,2	55 h	ME	11	10	(90.9)	11	(100)	10	(90.9)
		DE	14	11	(78.6)	13	(92.9)	10	(71.4)
		PE	12	10	(83.3)	12	(100)	10	(83.3)
		E	12	11	(91.7)	11	(91.7)	10	(83.3)
		S	10	10	(100)	10	(100)	10	(100)
Adult 3	48 h	ME	6	6	(100)	6	(100)	6	(100)
		DE	8	6	(75.0)	8	(100)	6	(75.0)
		PE	7	6	(85.7)	7	(100)	6	(85.7)
		E	8	6	(75.0)	8	(100)	6	(75.0)
		S	5	5	(100)	5	(100)	5	(100)

[a)] See Table 1. [b)] ME: metestrus; DE: diestrus; PE: proestrus; E: estrus; S: untreated spontaneous ovulation.

line space and pronucleus formation. The eggs were then incubated under 5% CO_2 in air at 37°C. The cleavage of eggs was determined under an inverted microscope at 10:00 the next day.

In vitro fertilization

IVF was carried out using the method of Toyoda and Chang [29] with a minor modification. Briefly, spermatozoa were obtained from the cauda epididymis of the male rat at 9:00. Drops of the dense mass of spermatozoa were introduced to 300 μl drops of human tubal fluid (HTF) medium [26]. Approximately 5 minutes after a portion of the sperm suspension was introduced to a new 300 μl drop of HTF, so that the final concentration of spermatozoa was 1,000/μl. The diluted sperm suspension was incubated for 5 h under 5% CO_2 in air at 37°C. Eggs were collected at 14:00 (21 h after hCG injection and 5 h sperm pre-incubation) from PMSG/hCG-treated female rats. Cumulus-oocyte complexes were dissected from the ampullae of both oviducts and introduced into the pre-incubated sperm suspension drop. The spermatozoa and eggs were incubated together for 19 h under 5% CO_2 in air at 37°C. Eggs were collected from the insemination medium and washed with mR1ECM/BSA. Subsequent evaluation and incubation of the eggs were as described above.

Embryo transfer

Fertilized eggs were collected from naturally mated female rats at 13:00 (27 h after hCG injection) and incubated in mR1ECM/BSA under 5% CO_2 in air at 37°C until 10:00 the next day. Morphologically normal 2-cell embryos were transferred into the oviducts of recipient rats which had been mated with a vasectomized male rat. Approximately 20 embryos were transferred into both oviducts of a recipient. After parturition, the number of offspring and implantation site in utero were counted.

Statistical assessment

The statistical differences of proportional data were analyzed using a chi-square test. The mean values of proportional data were subjected to an arcsine transformation in each replication, and the statistical differences of the transformed values and the other numerical data were analyzed using a *t*-test or the Tukey-Kramer test. A $P<0.05$ was considered significant.

Results

Natural mating

As shown in Table 2, PMSG/hCG-treated adult female rats were mated with male rats successfully, as were untreated spontaneously ovulated rats (S group), regardless of the estrous stage at the time of PMSG injection. The difference in the timing of hCG injection did not affect mating and ovulation efficiency. As shown in Table 3, the number of obtained eggs in the treated groups was significantly greater than that in the S group at each egg collection time. When the ovulated eggs were collected 20 h after hCG injection, the proportion of sperm-penetrated eggs (SP) in the E group was significantly less than the S group. The proportion of eggs with male and female pronuclei (PN) in the PE and E groups was significantly less than the S group, and the proportion of eggs that developed to the 2-cell stage (2-cell) in

Table 3. Fertilization and development of superovulated eggs obtained from PMSG/hCG-treated and mated adult WI rats

Treatment[a)]	PMSG-hCG interval	Egg collection time after hCG injection	Estrous stage at time of PMSG injection	No. of examin-ed rats	No. of obtained eggs	(Range)	SP% [b)]	(Range)	PN% [c)]	(Range)	2-cell% [d)]	(Range)
Adult 1	55 h	20 h	ME	5	80.4 ± 11.8[f, g)]	(65 – 96)	93.7 ± 8.1[e)]	(81.0 –100)	46.2 ± 26.0[f, g)]	(23.8 – 90.8)	51.3 ± 37.7[e, f)]	(5.9 – 93.8)
			DE	5	60.6 ± 22.9[f)]	(23 – 78)	97.9 ± 2.9[e)]	(94.5 –100)	81.0 ± 19.2[e, f)]	(56.4 –100)	70.9 ± 32.3[e, f)]	(34.6 – 95.7)
			PE	5	62.4 ± 17.1[f)]	(37 – 79)	75.8 ± 23.3[e, f)]	(36.8 – 97.3)	44.1 ± 19.4[g)]	(15.8 – 70.3)	50.2 ± 11.2[f)]	(39.1 – 66.7)
			E	5	106.2 ± 27.7[g)]	(73 –127)	45.7 ± 24.0[f)]	(19.2 – 82.3)	17.1 ± 12.7[g)]	(5.5 – 36.8)	26.8 ± 23.1[f)]	(2.1 – 57.4)
			S	5	15.0 ± 3.2[e)]	(10 – 18)	100 ± 0[e)]	(100 –100)	96.3 ± 8.4[e)]	(81.3 –100)	96.4 ± 5.5[e)]	(87.5 –100)
Adult 2	55 h	24 h	ME	5	83.6 ± 39.2[f)]	(15 –111)	94.0 ± 5.1	(85.8 –100)	87.9 ± 8.8	(75.5 –100)	70.9 ± 18.6[e, f)]	(40.4 – 87.5)
			DE	5	79.4 ± 9.7[f)]	(64 – 89)	94.3 ± 4.3	(89.9 –100)	90.0 ± 8.0	(80.0 – 98.7)	70.0 ± 21.0[e, f)]	(41.6 – 96.1)
			PE	5	67.2 ± 18.5[f)]	(43 – 86)	93.4 ± 8.6	(79.1 –100)	90.5 ± 10.0	(74.4 – 97.5)	75.5 ± 31.5[e, f)]	(21.1 – 97.5)
			E	5	105.6 ± 26.6[f)]	(81 –151)	79.7 ± 37.7	(12.6 –100)	72.3 ± 36.0	(9.9 – 97.9)	46.8 ± 29.6[f)]	(12.8 – 81.2)
			S	5	13.8 ± 2.4[e)]	(11 – 17)	96.0 ± 8.9	(80.0 –100)	96.0 ± 8.9	(80.0 –100)	96.5 ± 4.8[e)]	(90.9 –100)
Adult 3	48 h	27 h	ME	6	76.7 ± 38.3[f)]	(20 –100)	93.2 ± 6.6	(81.5 –100)	88.2 ± 7.6[e, f)]	(78.0 – 94.3)	81.9 ± 12.1[f, g)]	(61.1 – 94.0)
			DE	6	62.5 ± 18.8[f)]	(43 – 89)	87.5 ± 12.2	(69.7 –100)	85.5 ± 13.0[e, f)]	(69.7 –100)	91.9 ± 4.9[e, f)]	(83.9 – 97.7)
			PE	6	63.8 ± 20.5[f)]	(35 – 92)	71.7 ± 25.4	(22.8 – 90.8)	67.9 ± 24.5[e, f)]	(22.8 – 89.2)	87.8 ± 6.5[e,f, g)]	(81.4 –100)
			E	6	91.0 ± 24.8[f)]	(58 –116)	68.0 ± 27.1	(37.7 – 97.4)	59.3 ± 30.7[f)]	(29.3 – 97.4)	75.5 ± 13.9[g)]	(61.5 – 95.9)
			S	5	12.0 ± 3.5[e)]	(8 – 17)	98.2 ± 4.1	(90.9 –100)	98.2 ± 4.1[e)]	(90.9 –100)	100 ± 0[e)]	(100 –100)

Values shown are the mean ± SD. [a)] See Table 1. [b)]% of sperm penetrated (SP) in obtained eggs. [c)]% of eggs with male and female pronuclei (PN) in obtained eggs. [d)]% of eggs developed to 2-cell stage in sperm-penetrated eggs. [e, f, g)] Values with different superscripts within the same egg collection time, in the same column, are significantly different ($P<0.05$).

Table 4. *In vitro* fertilization and development of superovulated eggs obtained from PMSG/hCG-treated adult WI rats

Estrous stage at time of PMSG injection	No. of examined rats	No. of IVF	No. of obtained eggs	(Range)	SP % [a)]	(Range)	PN % [b)]	(Range)	2-cell % [c)]	(Range)
ME	8	5	84.3 ± 17.6[e, f)]	(66 –122)	74.9 ± 15.4	(43.5–91.6)	70.1 ± 16.8	(39.1–91.6)	91.2 ± 7.0	(81.1 –100)
DE	5	3	57.4 ± 12.4[d)]	(42 – 71)	76.5 ± 13.2	(59.5–89.1)	73.2 ± 14.5	(57.1–89.1)	91.6 ± 8.6	(77.3 –100)
PE	6	4	65.7 ± 18.1[d, e)]	(48 – 97)	89.3 ± 5.9	(78.1–94.1)	85.7 ± 5.9	(75.0–92.2)	94.6 ± 4.3	(96.4 – 97.9)
E	6	4	94.0 ± 16.6[f)]	(68 –116)	87.3 ± 10.1	(70.8–98.5)	83.3 ± 8.7	(70.8–92.6)	94.2 ± 5.5	(85.0 –100)

Values shown are the mean ± SD. [a)]% of sperm penetrated (SP) in obtained eggs. [b)]% of eggs with male and female pronuclei (PN) in obtained eggs. [c)]% of eggs developed to 2-cell stage in sperm-penetrated eggs. [d,e,f)] Values with different superscripts are significantly different ($P<0.05$).

Table 5. Full term development of embyos obtained from PMSG/hCG-treated adult WI rats

Estrous stage at time of PMSG injection	No. of donor rats	No. of recipient rats	No. of transferred embryos / recipient	% of pregnancy[a)]	No. of offspring	(Range)	(%)[b)]	No. of implanta-tion sites	(Range)	(%)[b)]
ME	3	3	18 or 20	100	8.3 ± 3.2	(6–12)	(42.8 ± 14.9)	12.0 ± 3.6	(9–16)	(61.7 ± 16.1)
DE	3	3	20	100	8.0 ± 2.0	(6–10)	(40.0 ± 10.0)	12.3 ± 1.2	(11–13)	(61.7 ± 5.8)
PE	4	4	20	100	7.8 ± 2.8	(5–11)	(38.8 ± 13.8)	13.3 ± 1.5	(12–15)	(66.3 ± 7.5)
E	3	3	20	100	11.0 ± 5.6	(5–16)	(55.0 ± 27.8)	14.0 ± 4.0	(10–18)	(70.0 ± 20.0)
S	8	4	17 or 20	100	13.3 ± 1.0	(12–14)	(69.1 ± 6.8)	16.8 ± 2.9	(13–20)	(86.6 ± 9.8)

Values shown are the mean ± SD. [a)] % in recipient rats. [b)] % in transferred embryos.

Table 6. Comparison of PMSG/hCG-treated immature and adult WI rats in mating and ovulation

Treatment [a]	Age	No. of examined rats	No. of mated rats	(%)		No. of ovulated rats	(%)		No. of mated and ovulated rats	(%)		No. of obtained eggs from ovulated rats [b]		(Range)
Immature	3–4 w	30	10	(33.3)	**	24	(80.0)	*	7	(23.3)	**	34.0 ± 33.4	**	(1–103)
Adult 3	11–13 w	29	24	(82.8)		29	(100)		24	(82.8)		67.5 ± 29.9		(14–130)

[a] See Table 1. [b] Values are the mean ± S.D. * $P<0.05$ ** $P<0.01$.

the PE and E groups was significantly less than the S group. However, when the eggs were collected 24 h after hCG injection, the proportion of SP, PN, and 2-cell in all treated groups was similar to those of the S group, except for the 2-cell in the E group. When hCG was injected 48 h after PMSG injection and the eggs were collected 27 h after hCG injection, the proportion of SP in all treated groups was similar to that in the S group; however, the proportion of PN in E group and 2-cell in E and ME groups was significantly less than that in S group.

In vitro fertilization

As shown in Table 4, the proportion of SP, PN and 2-cell after IVF was not different among all treated groups and the proportion of 2-cell was >90% in all groups.

Embryo transfer

As shown in Table 5, normal offspring were obtained by ET at the 2-cell stage in all treated groups, and the proportion of embryos that developed to offspring and implanted in the uterus was not different among all treated and S groups.

Comparison of immature and adult

Comparisons of mating rate, ovulation rate, and the number of obtained eggs in PMSG/hCG-treated adult with PMSG/hCG-treated immature WI rats were showed in Table 6. Mating and ovulation rate of adult rats were significantly higher than those of immature rats. The number of obtained eggs was significantly greater in adult than immature rats. Comparisons of the proportion of SP, PN and 2-cell obtained from mated and ovulated PMSG/hCG-treated adult rats with PMSG/hCG-treated immature rats were shown in Table 7. Those were not different between immature and adult rats.

Discussion

The main objective of this study was to assess the fertilizability and development of superovulated eggs obtained from adult rats treated by PMSG and hCG in the estrous stage. In the natural mating study, the proportion of fertilized eggs in the treated groups was less than that in the S group 20 h after hCG injection, but increased to the same level in the S group at 24 h (Table 3). These results indicate that ovulation and/or fertilization were delayed in PMSG/hCG-treated adult rats; a similar phenomenon has been described in previous studies [7–9, 21]. In our previous study [20], the PMSG-hCG interval was fixed at 55 h (hCG was injected at 17:00) because we thought the timing of the hCG injection must be adjusted to the endogenous proestrus surge of luteinizing hormone (LH surge) [27] to induce efficient superovulation in adult rats. When we used this protocol, however, we obtained fertilized eggs at 17:00 (24 h after hCG injection). It was too late to start embryo manipulation using the obtained eggs (e.g., DNA microinjection for the generation of transgenic rats). Therefore, we shortened the PMSG-hCG interval from 55 to 48 h (hCG injection was advanced from 17:00 to 10:00), and as a result, the mating, superovulation, and fertilization efficiency of the modified protocol were the same as the original protocol (Tables 2 and 3). Therefore, we considered that 1) at least 24 h after hCG injection is needed to obtain a sufficient number of fertilized eggs by natural mating, 2) the PMSG-hCG interval can be shifted from 55 to 48 h without a decrease in superovulation and fertilization efficiency; and 3) there is no need to adjust the timing of hCG injection to the timing of the endogenous LH surge for induction of efficient superovulation. These findings will help researchers to obtain unfertilized or fertilized eggs at the time they need.

In the natural mating study, the greater the number of eggs ovulated, the lower the number of eggs fertilized and divided. Such a finding has been also described by

Table 7. Comparison of PMSG/hCG-treated immature and adlt WI rats in fertilization and development of superovulated eggs

Treatment[a)]	Age of female rats	No. of examined rats	SP%[b)]	(Range)	PN%[c)]	(Range)	2-cell%[d)]	(Range)
Immature	3–4 w	7	86.9 ± 13.9	(60.0–100)	85.8 ± 14.4	(60.0–98.2)	97.6 ± 3.3	(92.1–100)
Adult 3	11–13 w	24	80.1 ± 21.4	(22.8–100)	75.2 ± 23.1	(22.8–100)	84.3 ± 11.3	(61.1–100)

Values shown are the mean ± SD. [a)] See Table 1. [b)]% of sperm penetrated (SP) in obtained eggs. [c)]% of eggs with male and female pronuclei (PN) in obtained eggs. [d)]% of eggs developed to 2-cell stage in sperm-penetrated eggs.

Ishibashi [9]. In contrast, in IVF, regardless of the number of ovulated eggs, the proportion of fertilized eggs was at the same level (Table 4). These results suggest that the cause of the low fertilization rate observed in the natural mating study was not attributed to egg quality, but attributed to environmental factors. There are studies in support of this suggestion. First, Ishibashi and Aoki [10, 11] reported that ovulation began 12 h after hCG injection and was completed 20 h after hCG injection in PMSG/hCG-treated adult rats, and ovulation was significantly longer and later than in untreated rats. Therefore, it is possible that a portion of eggs are ovulated at a sub-optimal time to encounter capacitated spermatozoa in superovulatory rats. Second, Ishibashi *et al.* [13] reported that the serum concentrations of estrogen (E2) and progesterone (P4) in PMSG/hCG-treated adult rats are significantly different from those of untreated rats. Moreover, Orihuela *et al.* [24] reported that sperm migration into and through the oviduct following artificial insemination is subject to E2 and P4 regulation. These previous studies and our present results suggest that an excessive amount of ovarian hormones is secreted in superovulated rats, which induces an abnormal environment for sperm migration, capacitation, and cleavage in the female internal reproductive tract. Based on the results of the ET study, normal offspring were born in all of the treated groups and there was no difference in the proportions of embryos developed to offspring and implanted into the uterus. These results indicate that the superovulated eggs can develop to offspring as well as spontaneously ovulated eggs.

We found that the number of eggs obtained in the E group was greater than that of the other groups. The number of eggs ovulated with PMSG/hCG treatment depends on the following factors; 1) the number of follicles which can react to PMSG/hCG in the ovary, and 2) the number of follicles which degenerate with effects of endogenous hormones. According to the Kagabu's studies [16, 17], there are strong positive correlation between the number of follicles of 250–549 μm in diameter in the ovary and the number of ovulated eggs with PMSG/hCG treatment through 3 to 17 weeks of age in WI rats. Therefore, there is a possibility that the number of follicles which can react to PMSG/hCG changes during estrous cycle in the adult WI rat. There is also a possibility that the number of follicles degenerated by endogenous hormones is less in the E group than the other groups. In any case, we must investigate the ovary and endogenous hormones in PMSG/hCG-treated adult rats to make clear why the number of ovulated eggs was greater in the E group.

We assessed the utility of adult WI rats as egg donors in comparison with PMSG/hCG-treated immature rats (Table 6 and 7) and determined that PMSG/hCG-treated adult WI rats are superior to PMSG/hCG-treated immature WI rats in mating and ovulation efficiency and in the number of obtained eggs (Table 6). The low value of mating and ovulation rate in immature rats has been reported in other strains [19, 28] and our present result consists with these previous reports. On the other hand, the fertilization efficiency of eggs obtained from mated and ovulated rats were not significantly different between immature and adult rats (Table 7). Therefore, we considered that fertilizability of ovulated eggs themselves is not different between immature and adult. Although we compared only 3–4 and 11–13 weeks of age in this study, variation with age in the numbers of ovulated eggs with PMSG/hCG in WI rats has been studied in detail by Kagabu [16, 18]. According to Kagabu's [16, 18] and our previous [20] data, efficient superovulation can be induced for at least eight weeks (10–18 weeks of age) in adult, whereas efficient superovulation can be induced for only two weeks (4–6 weeks of age) in immature rats. In comparison with other strains [1, 2, 4, 6, 19, 28], WI rats are superior to other strains in the number of obtained eggs. In addition, WI rats are also superior to other strains in handling. They are very calm and easy to handle.

In conclusion, this study has shown that superovulated eggs obtained from adult WI rats by using PMSG/hCG treatment, independent of estrous stage, had normal fertilizability and developmental ability, and we propose that adult WI rats are good egg donors for reproductive biotechnological studies using unfertilized or fertilized eggs.

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