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Effects of Superovulation Induction on Embryo Quantity and Quality in Rat

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과배란 방법이 Rat 수정란의 양과 질에 미치는 영향

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요 약

본 연구는 rat에서 PMSG 또는 FSH 처리에 의한 과배란 유도가 배란율과 수정란의 질에 미치는 영향을 알아보기 위해 호르몬 처리하고 교미시킨 후 4일령에 난관과 자궁을 세척하여 정상 8-세포기 난자와 비정상 난자를 조사하였고 각 처리에서 채란된 난자 중에 정상난자를 골라 체외 배양하여 발육율을 비교 평가하였다. 미성숙 rat와 성숙 rat에 PMSG를 주사하여 과배란을 유도한 실험에서는 미성숙 rat에서는 평균 19.1개의 수정란이 채취되었으며 성숙 rat에서는 14.2개가 채취되었고 미성숙 rat에서는 성숙 rat에 비해 더 많은 비율의 비정상적인 난자가 회수되었다. FSH와 LH-RH에 의한 방법이 PMSG와 HCG에 의한 방법보다 유의성 있게 많은 난자를 배란시켰으며, 비정상란의 빈도도 낮은 것으로 나타났다. 그러나 호르몬 처리에 의한 두 가지 방법은 자연배란에 의한 방법에 비해 훨씬 높은 비정상난자의 배란을 유도하였다(FSH, 20.1% ; PMSG, 41.2%; 자연배란, 13.4%). 또한 FSH 처리에 의해 회수된 난자가 PMSG 처리에 의해 회수된 난자보다 체외 발육율이 높은 것으로 나타났다. 그러므로 rat에서 PMSG와 FSH를 이용하여 과배란을 유도할 수 있으나 배란된 난자의 비정상율은 자연배란에 비해 훨씬 높았고, 과배란 유도시 호르몬의 종류에 따라 체외 배양율에도 영향을 미치는 것으로 나타났다.

(Key words : rat embryos, superovulation, PMSG, FSH, *in vitro* culture)

INTRODUCTION

Superovulation is one of the critical steps in embryo transfer and transgenesis of domestic animal. Superovulation induction in rats with commercial hormones often results in ovulation of low quality of embryos or no superovulatory effect. PMSG has been used in rats for superovulation earlier as in mice(Zarrow and Wilson, 1961; Husain and Saucier, 1970). However, low quality of rat embryos ovulated by PMSG injection reduced fertilization *in vivo* and *in vitro*, and developed abnormally(Walton and Armstrong, 1983; Yun *et al*, 1987). High dose of PMSG has long biological half life and dominant LH-like activity so that superovulation induction with PMSG may result in ovulation of immature oocytes(Evans and Armstrong, 1984) and excessive estrogenic and androgenic sti-

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mulation(Yun *et al.*, 1987, 1988; Leveille and Armstrong, 1989). Sex steroids levels may be closely related with quality and quantity of rat embryos. Antiandrogen(Yun *et al.*, 1988) and FSH infusion(Armstrong and Opavsky, 1988; Opavsky and Armstrong, 1989; Armstrong *et al.*, 1989) were used to eliminate PMSG-induced steroid effects. Superovulatory response can be obtained by PMSG and FSH injection, but some problems has to be solved in order to get good quality of rat embryos by superovulation induction. Researchers have still applied natural ovulation method to obtain good quality of rat embryos for their experimental purpose(Miyoshi *et al.*, 1994, 1995; Lee and Jin, 1996).

We are interested in *in vitro* culture system of rat embryos and manipulations(Lee and Jin, 1996). In this present experiment, we tried to evaluate conventional superovulation systems on rat embryo quality and quantity and discuss present problems in rat superovulation methods.

MATERIALS AND METHODS

1. Animals

Sprague-Dawley rats were housed in the light cycles schedules of 14L:10D. The animals were fed with Purina Laboratory Chow and supplied with water *ad libitum*. Immature rats were used at 28~40 days of age(55~70g body weight). Age of mature female rats were 3 month or more old(150~180g body weight).

2. Ovulation induction

1) PMSG injection

Immature female rats were injected with 20 IU PMSG subcutaneously and then mated 60 hours after PMSG injection. Mature female rats were vaginally smeared daily to evaluate stage of the estrus cycle(Baker, 1979). Mature rats with metaphase of estrus cycle were injected with 40 IU PMSG and 40 IU HCG 48 hours apart.

2) FSH infusion

A mixture of FSH(NIH FSH-S) and HCG was infused by implanting Alzet osmotic pumps (Alzet model 2001, Alza Corp, CA, USA) on the back skin to release 1 IU of FSH and 0.2 IU HCG daily(Leveille and Armstrong, 1989). One hundred microgram of leuteinizing hormone-releasing hormone(LH-RH, Sigma) was injected into peritoneal cavity 52 hours after beginning of infusion.

3) Natural mating

For natural ovulation, vaginal smear of mature female rats were checked daily and female rats with proestrus of estrus cycle were bred with male rats(Baker, 1979). Mating were confirmed by examining the presence of plug or sperm in the vagina next day.

3. Embryo evaluation

Embryos were recovered from the oviduct and uterine horn of mated rats on day 4 post coitus. Total number of recovered embryos was counted and embryo morphology were evaluated through inverted microscope(×400) just after recovery. Media used for embryos culture in this experiment were Armstrong's complete rat culture medium(ACRCM, Zhang and Armstrong, 1990) and BMOC-3(Brinster, 1971). Embryos were allocated in 50 μl microdrops of media covered with silicon oil and placed into the culture incubator under a humid atmosphere of 5% CO_2 and 95% air at 37℃.

4. Statistical analysis

Recovery rates were subjected to t-test to compare mean differences. Developmental rates and abnormality of embryos were compared by

chi-square test(Snedecor and Cochran, 1967).

RESULTS

An average of 16.2±3.9 rat 8-cell embryos was collected on day 4 from rats administrated with PMSG and HCG(Table 1). When superovulation rates by injection of PMSG and HCG were compared in immature and mature rats, immature rats ovulated more embryos than mature rats while mature rats produced less fragmented embryos. Superovulatory responses in this experiments were highly variable in both groups.

Superovulation rates of FSH infusion group or PMSG group were compared(Table 2). There were significant differences in average number of superovulated embryos and fragmented embryos between both groups. FSH infusion group produced more embryos(25.2±2.8) and less fragmented embryos than PMSG group. Both hormone-treated groups ovulated significant total number of embryos compared to natural mating group, while natural mating group produced much less fragmented embryos(FSH, 20.1%; PMSG, 41.2%; Natural mating, 13.4%).

Superovulated embryos were cultured *in vitro* to evaluate the potential effect of superovulation methods on the embryo development(Fig. 1). For *in vitro* culture normal 8-cell rat embryos were selected from superovulated embryos by morphology and cultured in Armstrong's complete rat embryo culture medium(ARCM) or Brinster's mouse culture medium-3(BMOC-3). More embryos derived from FSH infusion group developed to blastocysts than those from PMSG group regardless of culture media(Table 3). Development of embryos tends to be higher in ACRCM than BMOC-3. But there was on significant difference in the development rates wit-

Table 1. Rat superovulation rate by PMSG and HCG

Age of rats	No. rats	No. mated	No. embryos (average±se)	No. fragmented (%)
Immature[a]	12	10	191 (19.1±3.5)[c]	86 (45.0)[e]
Mature[b]	20	15	213 (14.2±4.4)[d]	68 (31.9)[f]
Total	32	25	404 (16.2±3.9)	183 (36.8)

[a] Immature rats were injected with 20 IU PMSG in the morning(8:00~9:00 AM) and then mated 60 hours after PMSG injection.

[b] Mature rats with the metaphase of estrus cycle were injected with 40 IU PMSG in the afternoon(4:00~5:00 PM) and 40 IU HCG 48 hours after PMSG injection.

[cd, ef] Different superscripts indicate significant differences($p<0.05$) between ages of rats.

Table 2. Comparison of ovulation rate in rats

Treatment	No. rats	No. mated	No. embryos (average±se)	No. fragmented (%)
FSH-LH[a]	23	18	453 (25.2±2.8)[c]	91 (20.1)[f]
PMSG-HCG[b]	30	23	421 (18.3±3.7)[d]	173 (41.2)[g]
Natural mating	16	10	89 (8.9±1.4)[e]	12 (13.4)[h]

[a] Immature rats were used for FSH mini-pump infusion method.

[b] Immature rats were used for PMSG method.

[cde, fgh] Different superscripts indicate significant differences($P<0.05$) between treatments.

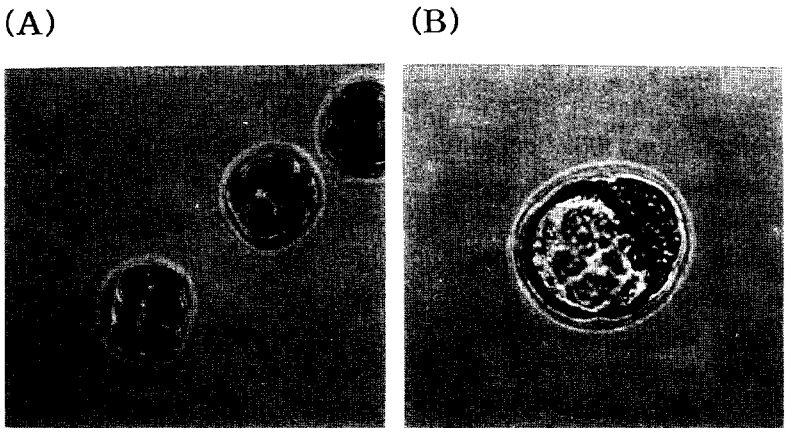

Fig. 1. Rat 8-cell embryos(A) and blasytocyst(B) following 48 hour culture *in vitro*.

Table 3. Development of rat 8-cell stage embryos to blastocysts after 48 hour culture

Source	Medium	No. embryos	No. developed (%)
PMSG	ACRCM[a]	80	32 (40.0)[cd]
	BMOC-3[b]	80	28 (35.0)[c]
FSH	ACRCM	80	45 (56.3)[e]
	BMOC-3	80	38 (48.8)[de]

[a] Armstrong's complete rat embryo culture medium.
[b] Brinster's mouse culture medium-3.
[cde] Different superscripts indicate differences($P<0.05$) between treatments.

hin the group between ACRCM and BMOC-3.

DISCUSSION

Superovulatory response in rats can be induced by PMSG or FSH. However, the yield of embryos is highly variable and recovered embryos are often abnormal, retarded or disappeared at later stage(Miller and Armstrong, 1981; Walton and Armstrong, 1981; Walton *et al.*, 1983; Yun *et al.*, 1987). Even though mature rats can be subjected to superovulation induction(Husain and Saucier, 1970), immature rats may be better for the superovulation induction with exogenous gonadotropins because they may lack endogenous gonadotropins(Zarrow and Wilson, 1961; Walton and Armstrong, 1883). In this experiment, immature rats produced more embryos than mature rats in PMSG treatment. Superovulation rates of rat 8-cell embryos induced with PMSG or FSH were lower than other reports in which one-cell embryos were collected. Some embryos may be absorbed in the reproductive track during migration. There were reports that the number of embryos recovered from superovulated rats were reduced between day 1 and day 2(Miller and Armstrong, 1981; Walton and Armstrong, 1981). It was not known why rat embryos ovulated were disappeared following superovulation induction.

Rat oocytes superovulated with PMSG resulted in reduced fertilization rate or abnormal development *in vitro* and *in vivo*(Walton *et al.*, 1983; Evans and Armstrong, 1984). PMSG-HCG group produced high proportion of abnormal embryos in this experiments(41.2%), whereas abnormal rate of embryos recovered from natural mating was low(13.4%). Developmental capability *in vitro* was significantly lower in embryos recovered from rats injected with PMSG than

FSH. Leveille and Armstrong(1989) reported that the majority embryos on day 4 exhibited some sign of degeneration following 40 IU PMSG and lower abnormal rates were observed in the embryos recovered from rats treated with 4 IU PMSG and infused with FSH. High abnormality of embryos following superovulation treatment has been shown to be related to a high maternal steroid hormone levels, especially estradiol and androgen(Yun *et al.*, 1987; Yun *et al.*, 1988; Opavsky and Armstrong, 1989; Leveille and Armstrong, 1989). However, these speculations are not whole explanation for the loss of embryos or developmental retardation because antagonist for androgen can not prevent early embryo loss or embryo development(Yun *et al.*, 1988) and FSH infusion which was reported to attribute much lower levels of estradiol and androgen affected higher abnormal rates than natural ovulation in this experiments. Further study on the superovulation induction in rats is necessary for high yield of good embryos.

SUMMARY

The induction of superovulation in rats with gonadotrophins has been reported to have a negative effect on quantity and quality of embryos. In order to examine the effect of PMSG and FSH treatments on the superovulation rates and embryo quality, rat 8-cell embryos were collected from the oviduct and uterus of rats induced by FSH-LHRH, PMSG-HCG and natural ovulation. By PMSG injection, immature rats produced more embryos than mature rats and also more abnormalities in morphology. Average embryo number per rat was higher in FSH-LHRH group than PMSG-HCG group(25.2 vs 18.3), while embryo abnormality was lower in natural ovulation group than hormone-treated groups(FSH, 20.1%; PMSG, 41.2%; natural ovulation, 13.4%). Embryos recovered from FSH-LHRH induction showed higher developmental capacity *in vitro* than those from PMSG-HCG injection. These results suggest that PMSG and FSH can induce superovulation in rats whereas they can affect embryo quality and development.

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