大韓不妊學會雜誌 : 第13卷 第1號, 1986
Kor. J. Fertil. Steril., Vol. 13, No. 1, 1986. 6.

Changes of Plasma Progesterone and Estradiol-17β Level During Early Pregnancy in Immature Female Rats Superovulated by Transplantation of a Pituitary Gland

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=국문초록=

뇌하수체 이식에 의해 과배란된 미성숙 흰쥐에서 임신초기에 혈중 progesterone과 estradiol-17β 수준의 변화

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본 연구는 한개의 뇌하수체를 이식시켜 과배란된 미성숙 흰쥐에서 혈중 progesterone과 estradiol-17β의 수준 변화를 관찰하기 위하여 시도되었다. 30일령 숫컷 흰쥐에서 뇌하수체를 제거하기 15일 전에 고환을 제거시켰으며 고환이 제거된쥐에서얻은한개의뇌하수체를실험 시작일(임신 3일전 : D-2) 오전 7시에서 10시 사이에 28일령의 암컷 흰쥐의 우측 신장 피막 아래 이식시켰다. 대조군은 같은날 오전 10시에 4 IU PMSG를 투여하였다.

실험에 사용된 쥐들은 혈중 호르몬 수준을 측정하기 위하여 임신 3일전, 2일전, 1일전, 임신 1일, 2일, 3일 및 5일에 희생시켜 채혈하였다. 임신 1일에는 교배후 estrogen의 과량분비를 차단하기 위하여 난소를 제거한 후 난소 호르몬을 투여하고 임신 8일에는 착상 상태를 조사하였다. 혈중 progesteron과 estradiol-17β 수준은 gamma counter(Packard)로 계측하였다.

본 실험에서 얻은 결과는 다음과 같다.

1. 난소를 제거하고 progesterone과 estradiol-17β를 투여한 과배란된 흰쥐는 난소를 제거하지 않고 과배란된 흰쥐나 대조군에 비하여 효과적인 착상율을 보이지 않았다($P<0.001$)
2. 과배란된 흰쥐에서 혈중 progesterone 수준은 대조군에 비해 교배후 계속적으로 높은 상승을 보였으나 교배전 수준은 대조군에 비해 낮았다($P<0.001$).
3. 과배란된 흰쥐에서 혈중 estradiol-17β 수준은 과배란 2일전부터 임신 1일까지 아주 높은 상태를 유지하였으며 임신 1일전(발정전기)에는 638±134 pg/ml으로 절정을 나타내었으나 임신 1일 이후 부터는 급격히 감소하여 임신 5일에는 10pg/ml이하로 떨어졌다.

INTRODUCTION

Superovulation can be induced successfully in immature rats not only by treatment with pregnant mare's serum gonadotrophin (PMSG) alone (Zarrow and Quinn, 1963; De La Lastra, Forcelledo and Serrano, 1972 ; Kostyk et al., 1978; Miller and Armstrong, 1981a; Yun and Kwun, 1984) but also by transplantation of a pituitary gland from male or immature female rats without any additional treatment with human chorionic gonadotrophin (HCG) (Sameshima et al., 1982). Transplantation of a pituitary gland from the orchidectomized male rat under the kidney capsule of immature female rat resulted in superovulation 3 days after grafting.

Meanwhile, excessive dose of exogenous gonadotrophins to induce superovulation may

be associated with the reduced fertility resulting from excessive follicular stimulation, which may cause loss of early embryos. It is assumed that the reason for the loss of early embryos and one cell-unfertilized ova probably caused by the early ovulation and the hypersecretion of estrogen around pre- and post-fertilization (Miller and Armstrong, 1982; Evans and Armstrong, 1984). The abnormal development and degeneration of early embryos in the superovulated rats treated with PMSG are also resulted from the abnormal ovarian hormone levels, especially the hypersecretion of estrogen after the time of fertilization (Miller and Armstrong, 1982). But there appears to be no reports until the present time indicating the changes of plasma progesterone and estradiol-17 β levels in the superovulated rats by transplantation of a pituitary gland.

Therefore the present study was undertaken to determine the plasma levels of progesterone and estradiol-17 β in immature female rats transplanted a pituitary gland into the subcapsular region of the kidney.

MATERIALS and METHODS

1. Animals

Sprague-Dawley rats, 28~30 days of age, were housed 3 or 4 per cage at room temperature (20~24℃) and 12 hrs light cycle, and were fed on a pellet diet (Samyang Co.) and tap water *ad libitum*.

2. Induction of Superovulation

A pituitary gland removed from male rats 15 days after orchidectomy (at 45 days of age) was immediately transplanted into the subcapsular area of the right kidney of 28-day-old female rats. This day of the experiment was designated as Day -2. To induce normal ovulation and gestation 4 IU PMSG(Intervet) was administered subcutaneously to the control rats at 1000 h on Day -2 (Miller and Armstrong, 1981a, 1981b).

Vaginal smears were taken daily after vaginal opening and examined microscopically (AO, 100× magnification) between 1500 and 1600 h on Day O. The animals with opened vagina and at proestrus cycle were caged with fertile proven Sprague-Dawley males (3 males and 3 females per breeding cage) at 1730 h of Day 0.

3. Replacement therapy and Ovarian Hormone Levels

At the following morning (Day 1) females were scored for the occurrence of mating between 0800 and 0900 h. A positive score denoted the presence of a copulatory plug in the vagina and/or spermatozoa in vaginal smears. Mated rats were allotted at random to groups to be sacrificed on Days 1, 2, 3, 5 and 8.

The mated rats were ovariectomized bilaterally to prevent the hypersecretion of estrogen under ether anesthesia between 1500 and 1700 h on Day 1. Thereafter, to maintain the pregnancy and to induce the nidation they were given 2 mg progesterone (Sigma) dissolved in 0.1 ml of sesame oil (Sigma) subcutaneously from the ovariectomized day to the previous day of sacrifice. Estradiol-17 β (Sigma) was administered as follow: 100 ng/kg body wt. on Days 2, 6 and 7 and 200 ng/kg body wt. in 0.1 ml of sesame oil subcutaneously on Days 3, 4 and 5, respectively. The ovariectomized rats were sacrificed between 1700 and 1800 h on Day 8 and the occurrences of implantation were examined. Blood samples were collected from a jugular vein using heparinized syringes under ether anesthesia between 2200 and 2300 h on Day -2 and between 1500 and 1600 h on Days -1, 0, 1, 2, 3 and 5. Following each blood sampling, plasma was seperated by centrifugation at 3000 rpm for 30 mintues at 4℃ and stored at -20℃ until the assay of ovarian hormones.

Table 1. Effects of progesterone and estradiol-17β on implantation in immature rats transplanted a pitui tary gland and then ovariectomized on Day 1*

Treatment	No. of rats	No. of IM sites(mean±SEM)	No. of IM occurrence		Total No. of IM sites
			BB	CR	
PGT	24	3.2±0.7[a)]	65	11	76
4IU PMSG	21	1.4±0.5[a)]	26	3	29

* : Observed on Day 8 after sacrifice.
BB : Blue bands, CR : Constriction rings, IM : Implantation.
PGT : Pituitary gland transplantation, a): $P<0.001$, The same superscripts mean significant difference from each other.

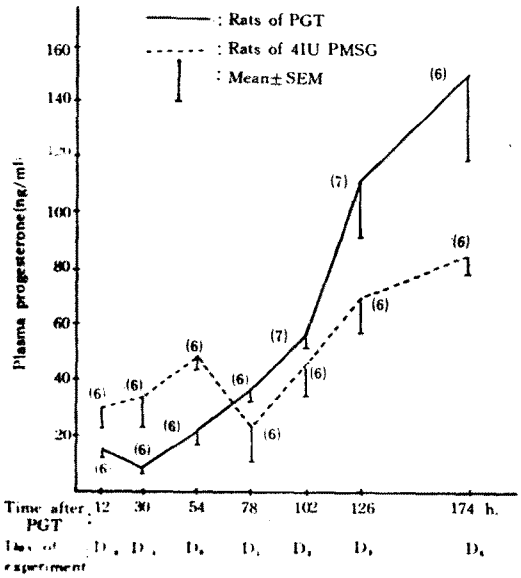

Fig. 1. Changes of plasma progesterone levels in immature rats transplanted a pituitary gland and administered 4 IU PMSG. No. in parenthesis: No. of rats, PGT: pituitary gland transplan tation

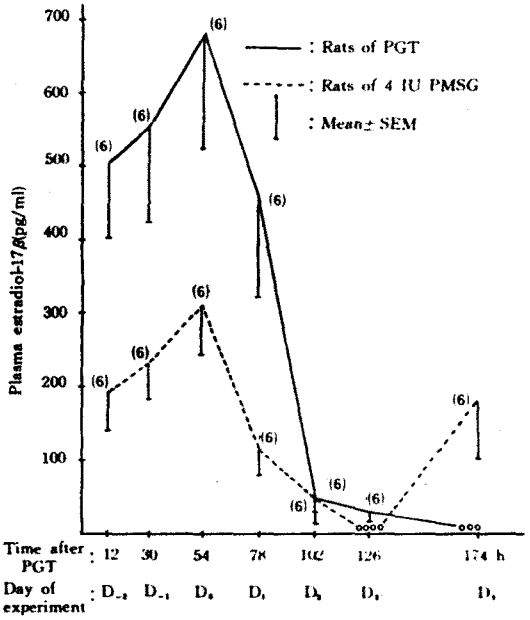

Fig. 2. Changes of plasma estradiol-17β levels in immature rats transplanted a pituitary gland and administered 4 IU PMSG. Levels below 10 pg /ml were undetectable and presented a blank circles. No. in parenthesis: No. of rats, PGT: pituitary gland transplantation.

4. Radioimmunossay of Progesterone and Estradiol-17β

The plasma progesterone and estradiol-17β levels were measured using progesterone-^{125}I (Immuchem corp.) and estradiol-^{125}I-17β kits (Radioassay System Laboratories), respectively. The detectable sensitivites of progesterone and estradiol-17β assayed in these kits were 0.15. ng/ml and 10 pg/ml, respectively.

The data of the experiments were analyzed by student's t-test and the one-way analysis of variance.

RESULTS

1. Implantation

The number of implantation sites (3.2±0.7) in the rats transplanted a pituitary gland and maintained pregnancy by the ovarian steroid hormone replacement therapy was significantly higher than those (1.4±0.5) of the rats administered 4 IU PMSG and maintained pregnancy by the ovarian steroid hormone replacement therapy as shown in Table 1.

2. Changes of Plasma Progesterone and Estradiol-17β Level

At 12 hrs after the transplantation, the mean plasma progesterone levels was 17 ng/ml, and then it was increased to about ten fold on Day 5 (151 ng/ml) as compared with the initial level. Whereas in the control rats the mean progesterone levels, which were 30 ng/ml initially, increased until Day 0 and then declined to 23 ng/ml on Day 1, but the values continued to rise again after Day 1 and reached to 86 ng/ml on Day 5. The difference in two groups was highly significant ($P<0.001$) at each day (Fig . 1).

As showen in Fig. 2, the mean plasma estradiol-17β levels in the control rats were 192 pg/ml at 12 hrs after injection of 4 IU PMSG. These levels increased to 310 pg/ml with a peak at 54 hrs, and then declined to 53 pg/ml by Day 2. In the superovulated rats the estradiol-17β levels keep high from Day -2 to 1, with a peak level of 683±134 pg/ml on Day 0. But from Day 1 on ward the levels fell drastically to those of control rats (i.e. 53 pg/ml) on Day 2. There were highly significant differences ($P<0.001$) in the plasma estradiol-17β levels between two groups on Days 0 and 1. The changes of the estradiol-17β levels between Days −2 and 2 were similar in both of the superovulated and control rats, The mean levels of plasma estradiol-17β in the control rats were not detectable on Day 3 but on Day 5 the levels rose again to 186 pg/ml. The mean plasma levels of estradiol-17β between Days 2 and 3 remained low (30~53 pg /ml) in the superovulated rats and there after the levels were not detectable on Day 5.

DISCUSSION

Miller and Armstrong (1981b) demonstrated that there were large number of follicles in 40 IU PMSG-treated rats between Days 0 and 2 and the number declined thereafter. They suggested that the principal souce of estradiol was the large, nonluteinized follicles and that of progesterone was the corpora lutea and luteinizing follicles. Estradiol was detrimental to early stage of embryo between Days 0 and 2.

The plasma levels of estradiol-17β were already very high between Days -2 and 1, especcially with a peak level at proestrus in the superovulated rats. It is suggested that the persistent high plasma estradiol-17β levels during this interval was influenced from early large follicles stimulated excessively by plasma FSH secreted from the grafted pituitary gland. It was reported that the serum estradiol levels in immature rats receiving 40 IU PMSG had two peaks first peak on Day 0 and second peak on Day 2 and remained elevated states until Day 3(Miller and Armstrong, 1981 a; Walton and Armstrong, 1981). But in this study the plasma estradiol-17β levels remained very high until Day 1, declined markedly thereafter and sustained low through the next 3 days. This indicates that the high plasma FSH of recipient released from the grafted pituitary gland is sufficient to stimulate the excessive production of large follicles and then to induce the hypersecretion of estrogen, even though for a short time (for 3 days), and that the half life of circulating FSH is shorter than that of PMSG. Niswender et al.(1774) estimated that the half life of circulatory FSH was 2 hours. Whereas Sasamoto (1962) showed that the half life of circulatory PMSG was 6 hours in the mice.

The plasma progesterone levels in the rats transplanted a pituitary gland were considerably higher than those in controls between Days 2 and 5. However, it is not clear what causes the high plasma progesterone levels in the superovulated rats (Zarrow and Gollo,1969; Bennet et al., 1980).

The regimen of progesterone and estrone given to the ovariectomized rat between Days

2 and 7 to maintain the gestation permitted a high pregnancy rate in the ovariectomized rats (Armstrong and King, 1971; Kennedy, 1980) and mice (Fiser and Macpherson, 1982) and induced as high implantation rate as the normal rats. In this investigation, the superovulated rats ovariectomized on Day 1 and then administered progesterone and estradiol-17β to prevent the hypersecretion of estrogen and the degeneration of embryos did not show an effective implantation rate on Day 8. But in the superovulated rats on Day 1, it was relatively difficult to remove the large ovaries without handling the oviducts. Therefore it can not be excluded the possibility that the loss of ova from the fimbria of oviducts in the superovulated rats is due to the surgical interference at ovariectomy. The loss of ova was at least as great as the mean loss of 42% in the control rats receiving 4 IU PMSG (Miller and Armstrong, 1982).

From this study, it was appeared in the superovulated rats that the high levels of plasma estradiol-17β secreted by the ovaries from Day −2 to Day 1 of pregnancy had a critical role in the maintenance of early pregnancy.

REFERENCES

Armstrong, D.T. and King, E.R.: *Uterine progesterone metabolism and progestational response: Effects of estrogen and prolactin. Endocrino lo logy 89 : 191-197, 1971.*

Bennett, G. R., Powell, J. G. and Cochrane, R.L.: *Maintenance of unimplanted fertilized ova in spayed rats: II. Effects of progesterone therapy and the duration of the delay in implantation. Biol. Reprod. 22 : 500-506, 1980.*

De La Lastra, M., Forcelledo, M.L. and Serrano, C.: *Influence of the hypophysis on pregnant mare's serum gonadotrophin-induced ovulation in immature rats. J. Reprod. Fert. 31 : 23-28, 1972.*

Evans, G. and Armstrong, D.T.: *Reduction in fertilization rate in vivo of oocytes from immature rats induced to superovulate. J. Reprod. Fert. 70 : 131-135, 1984.*

Fiser, P.S. and Macpherson, J.W.: *Survival of preimplantation emdryos in the uteri of mice induced to superovulate and subsequently ovariectomized. J. Reprod. Fert. 64 : 33-36, 1982.*

Kennedy, T.G.: *Estrogen and uterine sensitization for the decidual cell reaction: Role of prostaglandins. Biol. Reprod. 23 : 955-962, 1980.*

Kostyk, S. K., Dropcho, E.J., Moltz, H. and Swartwout, J.R.: *Ovulation in immature rats in relation to the time and dose of injected human chorionic gonadotropin or pregnant mare serum gonadotropin. Biol. Reprod. 19 : 1102-1107, 1978.*

Miller, B.G. and Armstrong, D.T.: *Superovulatory doses of pregnant mare serum gonadotropin cause delayed implantation and infertility in immature rats. Biol. Reprod. 25 : 253-260, 1981 a.*

Miller, B.G. and Armstrong, D.T.: *Effects of superovulatory dose of pregnant mare serum gonadotropin on ovarian function, serum estradiol and progesterone levels and early embryo development in immature rats. Biol. Reprod. 25 : 261-271, 1981 b.*

Miller, B.G. and Armstrong, D.T.: *Infertility in superovulated immature rats: Role of ovarian steroid hypersecetion. Biol. Reprod. 26 : 861-868, 1982.*

Niswender, G.D., Nett, T.M. and Akbar, A. M.: *The hormones of reproduction. In Reproduction in Farm animals, 3rd ed, pp 57-81. Ed. Hafez, E.d.E., Lea and Febiger, Philadelphia, 1974.*

Sameshima, H., Taya, K., Sasamoto, S. and Etoh, T.: *Superovulation induced by a single pituitary gland transplanted beneath the kidney capsule in adult rats. J. Endocr. 94 : 339-345, 1982.*

Sasamoto, S.: *Bioassay of PMS by induced ovulatory response in immature female mice. Jpn. J. Vet. Sci. 24 : 451 (in Japanese), 1962.*

Walton, E.A. and Armstrong, D.T.: *Ovarian function and early embryo development in immature rats given a superovulatory dose of*

PMSG, later neutralized by antiserum. Biol. Reprod. 25 : 272-280, 1981.

Yun, Y. W. and Kwun, J.K.: *Studies on the possible cause of implantation failure in immature rats given high doses of pregnant mare serum gonadotropin. Seoul Univ. J. Vet. Sci. 9:139-155, 1984.*

Zarrow, M.X. and Gallo, R.V.: *Action of progesterone on PMS-induced ovulation in the immature rat. Endocrinology 84 : 1274-1276, 1969.*

Zarrow, M.X. and Quinn, D.L.: *Superovulation in the immature rat following treatment with PMS alone and inhibition of PMS induced ovulation. J. Endocr. 26 : 183-188, 1963.*