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Effects of Antiprogesterone (RU486) and Antiestrogen (Tamoxifen) on Ovulatory Response and Oocyte Quality in Rats Primed with Pregnant Mare Serum Gonadotropin

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PMSG 전처리한 쥐에 있어서 Antiprogesterone(RU486)과 Antiestrogen (Tamoxifen)이 배란과 Oocyte에 미치는 영향

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Abstract

The effects of an antiprogesterone (RU 486) and an antiestrogen (tamoxifen) on ovulatory response and oocyte morphology were examined in pregnant mare serum gonadotropin (PMSG)-primed immature female rats (28 days of age): a comparison has been made on two different regimens primed with a "control" dose (4 IU) and a "superovulatory" dose (40 IU) of PMSG. Females for control control regimen received three consecutive injections of 1mg RU486, 1mg tamoxifen, or vehicle at 24, 36 and 48hr, and were killed at 72hr after PMSG. Animals for superovulatory regimen received 1mg RU486, 2.5mg tamoxifen, or vehicle following the injection schedule comparable to control regimen, and were killed at 60 and 72hr after PMSG. Compared to vehicle group, there was a significant reduction in ovulatory response as judged by the proportion of rats ovulating and/ or by the mean number of oocytes per rat for each treatment of RU486 and tamoxifen in both regimens. The activity of tamoxifen in inhibiting the ovulatory response was greater in control, but less in superovulatory regimen than that of RU486 based on the dose employed for each antisteroid. In both regimens, RU 486 did not have any effect on the changes in the proportion of degenerate oocytes as well as ovarian weight, while tamoxifen treatment resulted in a marked promotion of oocyte degeneration as well as a great reduction in ovarian weight, compared to each parameter of vehicle group. RU486 treatment in each regimen did not alter the serum levels of any steroid hormones observed. Howerver, tamoxifen treatment was associated with significant increases in serum 17β-estradiol and decreases in progesterone in both regimens; also significant increases in androgens in superovulatory regimen.

The results illustrate the relative inhibitory activity of RU486 and tamoxifen indicating major steroid hormone involved in PMSG-induced ovulation: 17β-estradiol for control and progesterone for superovulatory regimen. It also appears that tamoxifen-associated elevation of circulating 17β-estradiol and/ or androgens could be, in part, a contributing factor to the promotion of oocyte degeneration presumably by producing a hostile oviductal environment after ovulation.

Introduction

Pregnant mare serum gonadotropin (PMSG) is known to be an effective stimulus for induction and control of ovulation in various mammalian species. The importance of its application in superovulation procedures has been well recognized in the livestock industry. However, reproductive performance in superovulated farm animals has been disappointing because of highly variable ovarian response to PMSG and frequent recovery of retarded or abnormal embryos at the time of transfer (for review see Betteridge, 1977). It has been shown in sheep and cattle that superovulatory doses of PMSG significantly alter intrafollicular steroidogenesis leading to marked elevation of estrogen level, changes in steroid hormone ratios and perturbation of oocyte metabolism and maturation (Moor et al., 1985; Callesen et al., 1986).

Results of numerous studies using immature rat models indicate that low doses of PMSG induce a synchronized process of ovulatory response to generate "physiological" number of viable oocytes in which the sequential changes of circulating steroid hormones and endogenous gonadotropin surge are comparable to those seen in cycling adult rats (Wilson et al., 1974; Parker et al., 1976). In contrast, superovulatory doses of PMSG produce asynchronized multiple waves of ovulation with a high incidence of degenerate oocytes (Walton et al., 1983; Yun et al., 1987) and consistent recovery of premature of meiotically aberrant occytes from oviductal flushings (Yun et al., 1989). Atypical ovulations with poor quality oocytes have been ascribed to disruption of sequential changes in follicular steroid ratios and significant elevation of ovarian androgens resulting from superovulation (Yun et al., 1987; Yun et al., 1989). A study using an antiandrogen in superovulated rats provides further evidence of a significant role of androgens in controlling oocyte quality since administration of flutamide after superovulatory dose of PMSG substantially reduces oocyte and early embryo degeneration (Yun et al., 1988). In any of the above studies however, the involvement of other steroids in increasing doses of PMSG was not excluded.

In the present study, the effects of an antiprogesterone (RU486) and an antiestrogen (tamoxifen) were examined on ovulatory response and oocyte morphology in female rats primed with two different (low "control" and high "superovulatory") doses of PMSG.

Materials and Methods

1. Experimental animals

Immature female Sprague-Dawley rats were obtained from Charles River Canada Inc. (St. Constant, Quebec) at 22 days of age and maintained at a constant temperature of 21°C with lights on between 0700 and 1900 h. Water and pelleted food were supplied ad libitum. At the age of 28 days, the rats were injected with 4 IU (control dose) or 40 IU (superovulatory dose) of PMSG (Equinex, Ayerst Labs., Montreal, Quebec) between 0830 and 0900h.

Experiment 1. Twenty on rats primed with 4 IU PMSG were divided into three groups, and received three consecutive injections of 1mg RU486 (Roussel Uclaf, Romainville, France), or 1mg tamoxifen (Sigma Chemical Co., St. Louis, MO; free base), vehicle alone at 24, 36 and 48hr after PMSG.

Experiment 2. Forty one rats primed with 40 IU PMSG were divided into four groups, and received either no further injections (pre-treatment control), 1mg RU 486, 2.5mg tamoxifen, or vehicle alone following the comparable injection schedule as in Experiment 1.

PMSG and antisteroids (RU486 and tamoxifen) were administered subcutaneously in 0.4ml of 0.9% NaCl solution and sesame oil suspension containing 5% ethanol as vehicle, respectively.

2. Collection of date

All animals in Experiment 1 were killed by cervical dislocation at 72hr after 4 IU PMSG (n=7/group), and the animals in Experiment 2 were killed at 12 hr (pre-treatment control), 60hr and 72hr after 40 IU PMSG (n=5-7/group). Trunk blood was collected and stored at 4°C prior to separation of serum by centrifugation.

Ovulatory responses were assessed by counting oviductal oocytes. Oviducts were separated from uterine horns at uterotubal junctions and flushed with about 0.2ml Dulbecco's phosphate buffered saline (DPBS), as described previously (Yun et al., 1988). Clumped cumulus-oocyte masses were subsequently exposed to 0.1% hyaluronidase solution for about 5 min to facilitate counting and examination of oocyte morphology. The recovered oocytes were scored under a dissecting microscope (40X magnification), and their gross morphology was assessed under a phase-contrast microscope (100X magnification), as described previously (Yun et al., 1987). Briefly, the oocytes showing fragmentation, parthenogenesis, irregular shape or amorphous opaque mass of vitelline material, and empty zona pellucida were classified as abnormal. Ovaries were cleaned of ovarian bursae and adjacent adipose tissue, blotted, and weighed as a pair.

3. Determination of steroid hormones

Aliquots of sera were extracted twice with five volumes of diethyl ether, evaporated under nitrogen, and reconstituted with 1ml redistilled ethanol. The levels of 17β-estradiol, progesterone and androgens in serum were determined by specific radioimmunoassay using antisera kindly donated by Dr. David T. Armstrong from the University of Western Ontario (London Ontario). Cross-reactivity data and coefficients of variation for steroid assays were presented previously (Yun et al., 1987; Yun et al., 1988)

4. Stastical analysis

Experi nental data were evaluated statistically by the Student's t-test, or when appropriate by analysis of variance followed by Fisher's PLSD test. Comparisons with $p < 0.05$ were considered to be significant.
control of ovulatory response and oocyte quality after

Results

1. Experimental 1.

Effects of RU486 and tamoxifen on ovulation, oocyte normality and ovarian weight in rats primed with 4IU PMSG are presented in Table 1. All rats in vehicle group ovulated a range of 9-14 oocytes per rat. The ovulatory response to 4IU PMSG was apparently inhibited by the treatment with either RU486 or tamoxifen. In RU486-treated group, although the proportion of rats ovulating seemed to be slightly reduced by 14.3%, the mean number of oocytes recovered from each rat was significantly ($p < 0.01$) reduced by 39.3%, as compared to vehicle group. In tamoxifen-treated group, both the proportion of rats ovulating and the mean number of oocytes per rat were significantly ($p < 0.001$) reduced by 71.4% and 94.9%, respectively, as compared to each parameter observed from vehicle group of animals.

Table 1. Effects of RU486 and tamoxifen on ovulation, oocyte normality and ovarian weight in 4IU PMSG-primed rats[a]

Treatment	Proportion of rats ovulating	No. of oocytes[b]	% degenerate oocytes[c]	mg ovarian tissue[d]
Vehicle	7/7	11.7 ± 0.7	4.7 ± 2.3	43.7 ± 3.9
RU486	6/7	7.1 ± 1.3[e]	7.5 ± 4.8	50.3 ± 4.8
Tamoxifen	2/7	0.6 ± 0.4[f]	75.0 ± 25.0[g]	29.3 ± 2.0[h]

[a] Rats were treated with vehicle, 1mg RU486 or 1mg tamoxifen at 24, 36 and 48hr after injection of 4IU PMSG.
[b-d] Results obtained 24 hr after treatment are expressed as means ± SE.
[e] $P < 0.01$, [f,g] $p < 0.001$, [h] $p < 0.05$, compared to corresponding vehicle-treated group.

Thus, tamoxifen seemed to be more effective in the inhibition of ovulatory response than RU486, when administered to 4 IU PMSG primed rats at equipotent doses (three consecutive injections of 1mg per animal).

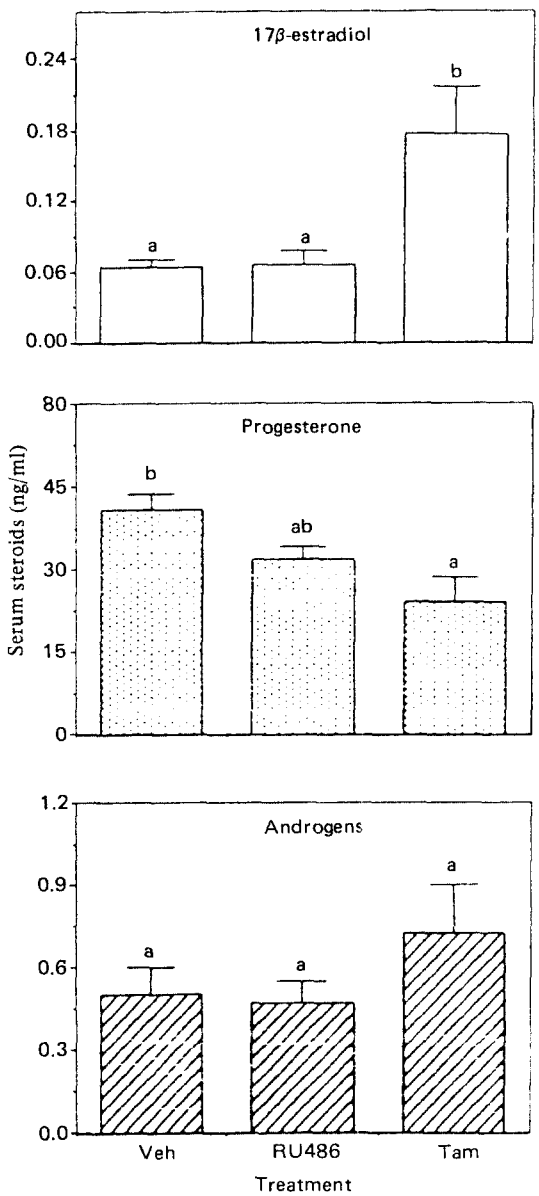

Fig. 1. Serum levels of 17β- estradiol, progesterone and androgens 24hr after tretament with vehicle, RU486 or tamoxifen in 4 IU PMSG- primed rats. Values are means ± S.E. (n=7). The means with no superscripts in common are significantly different ($p < 0.05$).

More than 95% of oocytes recovered from the rats of vehicle group appeared to be normal; only one or two degenerate oocytes were occasionally recovered from three of the seven vehicle-treated rats. No significant differences were observed in "gross normality" of the oocytes recovered from the two groups of rats treated with vehicle or RU486 after 4 IU PMSG. In contrast, tamoxifen treatment significantly ($p < 0.001$) increased the proportion of oocytes exhibiting visible signs of degeneration, as compared to that in vehicle group of animals.

Ovarian weight response to 4 IU PMSG was not affected by RU486 treatment, since there were no significant differences in the mean weights of ovarian tissue between vehicle- and RU486- treated group of rats. In contrast, tamoxifen treatment significantly ($p < 0.005$) reduced the mean ovarian weight by 33.0% as compared to that in vehicle group of animals.

The levels of serum 17β- estradiol, progesterone and androgens after the treatments with vehicle, RU486 and tamoxifen in 4 IU PMSG-primed rats are illustrated in Figure 1. There were no significant differences in the levels of all three serum steroid hormones between vehicle- and RU486- treated group of rats. However, compared to vehicle group, tamoxifen treatment was associated with a 200.0% increase in 17β- estradiol level ($p < 0.05$) and a 40.3% decrease in progesterone level ($p < 0.05$).

2. Experiment 2.

Effects of RU486 and tamoxifen on ovulation, oocyte normality and ovarian weight in rats primed with 40 IU PMSG are shown in Table 2. Although no ovulations were observed in any rats of pre-treatment control gorup, all rats in vehicle group ovulated consistently through 60 to 72hr after 40 IU PMSG. The mean number of oocytes recovered from the rats of vehicle group was 24.4 ± 1.4 oocytes per rat at 60 hr and further increased to reach 40.1 ± 2.5 oocytes per rat at 72hr. It was evident that the superovulatory response to 40 IU PMSG was inhibited by the treatment with RU486-

Table 2. Effects of RU486 and tamoxifen on ovulation, oocyte normality and ovarian weight in 40 IU PMSG- primed rats[a]

Treatment	Time after PMSG(hr)[b] 60 No. oocytes	60 % degenerate oocytes	60 mg ovarian tissue	72 No. oocytes	72 %degenerate oocytes	72 mg ovarian tissue
Vehicle	24.4 ± 1.4 (7/7)[c]	50.4 ± 9.8	147.7 ± 8.2	40.1 ± 2.5 (8/8)[c]	41.6 ± 5.1	161.0 ± 3.9
RU486	7.3 ± 2.8[d](4/7)	45.1 ± 6.7	142.9 ± 6.0	21.2 ± 4.1[e](8/9)	37.5 ± 11.8	138.1 ± 10.1
Tamoxifen	22.6 ± 1.2 (5/5)	73.2 ± 10.8	121.1 ± 4.7[f]	24.8 ± 4.1[g](5/5)	82.6 ± 8.0[h]	118.0 ± 3.9[i]

[a]Rats were treated with vehicle, 1mg RU486 or 2.5mg tamoxifen at 24, 38 and 48hr after injection of 40 IU PMSG.

[b]Results are expressed as means ± SE.

[c]Number of rats exhibiting ovulations/ total number of rats killed at each time are given in parentheses.

[d,e,g]$p < 0.01$, [f]$p < 0.05$, [h,i]$p < 0.001$, compared to corresponding vehicle-treated group.

treated group, the proportion of rats exhibiting ovulation was reduced by 42.9% at 60hr and 11.1% at 72hr, as compared to vehicle group at each time. The mean number of oocytes recovered from each rat of this group was significantly ($p < 0.01$) reduced by 70.1% at 60hr and 47.1% at 72hr, when compared to vehicle group at each time. On the other hand, tamoxifen was marginally effective for inhibition of superovulatory response, although it was administered to the 40 IU PMSG-primed rats at even higher doses (three consecutive injections of 2.5mg per animal) than RU486. When compared to vehicle group, a significant difference was noted in the mean number of oocytes only at 72hr. In this group, all rats ovulated consistently throughout the period examined.

Relatively high proportions of degenerate oocytes oocytes were observed in vehicle-treated superovulated rats: 50.4 ± 9.8% at 60hr and 41.6 ± 5.1% at 72hr There were no significant differences in the percentages of degenerate oocytes recovered from the rats in vehicle- and RU486- treated groups throughout the period examined. In contrast, tamoxifen treatment significantly ($p < 0.001$) increased the proportion of oocytes exhibiting signs of degeneration at 72hr, as compared to that in vehicle group of animals.

The weight response of superovulated ovaries after 40IU PMSG was not affected by RU486 treatment but markedly inhibited by tamoxifen treatment. Virtually no significant differences were recorded in the mean ovarian weight between vehicle- and RU486- treated group of rats. In contrast, tamoxifen treatment significantly reduced the mean ovarian weight by 18.0% at 60hr ($p < 0.05$) and 26.6% at 72hr ($p < 0.001$), as compared to that in vehicle group of animals.

The changes in serum levels of 17β- estradiol, progesterone and androgens before or after teratments with vehicle, RU486 and tamoxifen in rats primed with 40IU PMSG are presented in Figure 2. At 60hr, the levels of three serum steroid hormones in vehicle group of superovulated rats were significanlty ($p < 0.001$) elevated above those of pre-treatment controls obstained at 12hr. Compared to vehicle griup, there were no significant changes in any of the three steroid hormones throughout the period examined after RU486 treatment. However, tamoxifen treatment resulted in marked alterations in the levels of overall steroid hormones: 102.7% ($p < 0.01$) and 800.0% ($p < 0.001$) increases for 17β- estradiol, 63.9% ($p < 0.05$) and 180.6% (p K 0.001) increases for androgens at 60hr and 72hr, respectively, and a 46.2% ($p < 0.05$) decrease for proge-

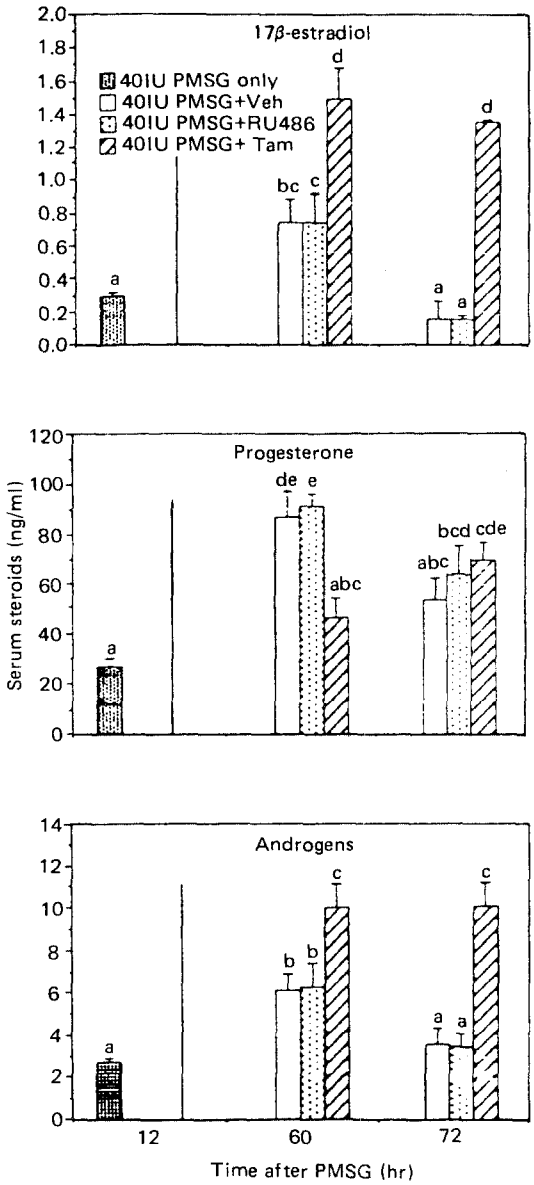

Fig. 2. Serum levels of 17β- estradiol, progesterone and androgens before or after treatment with vehicle, RU486 or tamoxifen in 40 IU PMSG- primed rats. Values are means ± S.E. (n=5-7). The means with no superscripts in common are significantly different ($p < 0.05$).

sterone at 60hr.

Discussion

Steroid hormones secreted from PMSG- stimulated ovarian tissues in rats are thought to contorl the ovulatory response and oocyte morphology. Evidence for a role of androgens in the ovulatory process has been provided by the sutdies in which a nonsteroidal antiandrogen (flutamide) was found to interfere with spontaneous gonadotropin surge and ovulation in 4 IU PMSG-primed rats (Opavsky et al., 1987) and reduce oocyte degeneration resulting from superovulation with 40 IU PMSG in rats (Yun et al., 1988). These studies suggest the important roles of androgens in the control of ovarian function and oocyte quality in PMSG- treated rats; however, they do not exclude the possibility of involvement of other steroid hormones in the process of PMSG-induced ovulation and maintenance of oocyte quality. In the present study using the rats primed with either a control dose (4 IU) or a superovulatory dose (40 IU) of PMSG, administration of an antiprogesterone (RU486) significantly reduced ovulatory response without affecting oocyte gross normality, while an antiestrogen (tamoxifen) treatment was associated with a significant decrease in ovulatory response as well as increase in the proportion of degenerate oocytes. The comparison between the treatments of RU486 and tamoxifen for the ovulation-inhibiting effects showed that the activity in its performance is greater for tamoxifen in the rats primed with 4 IU PMSG and for RU486 in the rats primed with 40 IU PMSG.

The present observation of marked inhibition of ovulatory response after RU486 and tamoxifen in PMSG- primed rats suggests potential physiological roles of progesterone and estrogen in the process of PMSG-induced ovulation. Earlier studies (Costoff et al., 1974; Parker et al., 1976) demonstrated the patterns of preovulatory surges of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in PMSG-primed rats. It appears that the rising level of preovulatory estrogens produced by PMSG exerts its stimulatory action at the hypothalamic level promoting the secretion of LH-releasing hormone (LHRH), and at the anterior pituitary to increase the responsiveness to LHRH leading to gonadotropin secretion (Labrie et al., 1978). Also, increased levels of preovulatory progesterone may synergize with estrogens to facilitate gonadotropin secretion

lowering the threshold of hypothalamus to estrogenic stimulus (Kawakami and Sawyer, 1959) and by increasing piptuitary sensitivity to LHRH (McPherson and Mahesh, 1979). The positive feedback relationship between the steroid hormones and gonadotropin secretion could be further supported by the studies using antibodies or antagonists to 17β- estradiol or progesterone (Ferin et al., 1969; Labhsetwar, 1970; Rao and Mahesh, 1986) and employing these exogenous steroid hormones in ovariectomized rats (Legan et al., 1975; McPherson et al., 1975). Therefore, the integral actions of estrogens and progesterone at hypothalamo-pituitary axis seem to be necessary for the expression of spontaneous gonadotropin surge ensuring consequent ovulation. However, in the interpretation of this mechanism, it is important to point out the differences in relative potential of each steroid hormones for the stimulation of ovulatory response between the two groups of rats primed with different doses of PMSG. The results of present study in which tamoxifen was administered to 4 IU PMSG-primed rats at equipotent dose and to 40 IU PMSG-primed rats at even 2.5-told higher dose compared to the dose of RU486 showed that the activity of tamoxifen in inhibiting ovulatory response was greater in 4 IU PMSG-primed rats, but less in 40 IU PMSG- primed rats than that of RU486. This finding indicates that the major steroid hormone involved in PMSG- induced ovulation may be different depending upon the dose of gonadotropin to stimulate ovaries: 17β- estradiol for a control dose- and progesterone for a superovulatory dose-stimulation.

It is probable that, in PMSG- primed rats, RU486 exerts its antagonism of the action of progesterone on hypothalamo-pituitary axis rather than on ovary, since it did not affect all other parameters of oocyte quality, tissue weight and steroid hormone secretion at ovarian level consistently in both groups of rats primed with 4 or 40 IU PMSG. This concept concerning the mode of action of RU486 is well in agreement with the study (Rao and Mahesh, 1986) in which RU486 treatment in 8 IU PMSG- primed immature rars and cycling adult rats significantly reduced the preovulatory surge levels of LH and FSH, but it had no effect on gonadotropin levels in immature rats 7 days after ovariectomy.

The mode of inhibitory action of tamoxifen on ovulatory response appears to be similar to that of RU 486. A number of studies demonstrated that antiestrogenic effect of tamoxifen to inhibit the secretion of preovulatory gonadotropin is mediated principally via the hypothalamo-pituitary axis rather than via ovary (Walpole, 1968; Labhsetwar, 1970; Billard and McDonald, 1973). In the above studies, direct action of tamoxifen on the ovary was negligible. However, in the interpretation for the whole mechanism of tamoxifen to modulate ovarian function, some caution is required. Current observation of a great increase in the proportion of degenerate oocytes and reduction in ovarian weight as well as marked changes in serum steroid hormones after treatment of tamoxifen may indicate the direct action of tamoxifen at the ovarian level. The effects of tamoxifen on these parameters were consistent in both groups of rats primed with 4 or 40 IU PMSG. In the present study, tamoxifen treatment was associated with a significant increase in serum 17β- estradiol and/ or androgens, and a significant decrease in progesterone in PMSG- primed rats. Evidence suggests the disparate actions of antiestrogens including tamoxifen, clomiphene citrate and nafoxidine on cultured rat granulosa cells, whereby these antiestrogens substantially augmented FSH- stimulated estrogen production and inhibited FSH- stimulated progesterone production in a dose- related manner (Welsh et al., 1984). This observation has been ascribed to enhancement of aromatase activity and suppression of pregnenolone biosynthesis via direct mediation through granulosa cell binding sites specific for antiestrogens (Sgarlata et al., 1984; Welsh et al., 1984).

Early or sustained elevation of circulating preovulatory 17β- estradiol has been found to be embryotoxic presumaby through a direct action on the preovulatory rat oocytes; the effect could be partially restored by antisera to 17β- estradiol (Butcher and Pope, 1979). Furthermore, the oviducts under the influence of high levels of ovarian and circulating 17β- estradiol have been shown to secrete a low molecular weight substance to inhibit embryo development in mice (Cline et al., 1977) and rabbit (Stone and Hamner, 1977; Stone et al.,

1977). On the other hand, it has been suggested that clomiphene citrate, related to tamoxifen in its chemical structure, may exert a direct estrogen- antagonistic effect on the intrafollicular oocyte to promote its degeneration and further to increase preimplantation embryo degeneration rates associated with reduction of developmental potential in mice (Laufer et al., 1983) and rabbits (Yoshimura et al., 1985). Similarly, an exposure of clomiphene citrate to cultured preovulatory follicles exhibited dose- dependent atretic-like changes in rats (Laufer et al., 1982). It seems, therefore, likely that the promotion of oocyte degeneration by tamoxifen treatment may be mediated by its direct antiestrogenic action in the preovulatory follicles and/ or by its elevation of circulating estrogens to produce hostile environment in the oviducts. However, in the present sutdy, the primary factor(s) leading to tamoxifen-associated oocyte degeneration between the two groups of rats primed with different doses of PMSG could be different depending upon the timing of ovulation. Time-course of ovulatory response to increasing doses of PMSG has previously been well described (Walton et al., 1983; Yun et al., 1987). Thus, in 4 IU PMSG-primed rats, the present observation of an increased proportion of degenerate oocytes after tamoxifen reflects postovulatory event at 72hr after PMSG, and suggests the impact of tamoxifen in the other hand, in 40 IU PMSG- primed rats, since ovulations were persistent between 60 and 72hr after PMSG, the tamoxifen- associated oocyte degeneration may be ascribed to both factors including the direct antiestrogenic action of tamoxifen in preovulatory follicles and its secondary impact in the oviduct. Additionally, the elevated level of circulating androgens by tamoxifen may be another contributing factor to the production of hostile oviductal environment hence promoting oocyte degeneration. It has previously been shown that a great reduction of oocyte degeneration by an antiandrogen (flutamide) in superovulated rats with PMSG has been associted with significant decrease in circulating androgens as well as 17β- estradiol (Yun et al., 1988).

In summary, both antiprogesterone (RU486) and antiestrogen (tamoxifen) exhibited inhibitory actions on ovulation in PMSG- primed rats indicating the potential physiological roles of progesterone and estrogen for active participation in PMSG- induced ovulation. The relative ovulation-inhibiting activity of the two antisteroids between two different doses of PMSG-primed rats indicates the major steroid hormone involved in PMSG-induced ovulation: 17β- estradiol for a control dose- and progesterone for a superovulatory dose-stimulation, Additionary, the deterimental effects of tamoxifen on oocyte normality has been demonstrated in PMSG- primed rats. It appears that tamoxifen- associated oocyte degeneration may be mediated by its known antiestrogenic action in preovulatory follicies and/ or its elevation of circulating 17β- estradiol and/ or androgens to produce hostile oviductal environment.

초 록

항 프로제스테론(RU486)과 항에스트로겐 (tamoxifen)이 임마성성 성선자극호르몬(PMSG)을 투여한 미성숙 암컷랫드(28일령)에서 배란반응과 난자형태에 미치는 영향을 조사하였다; PMSG는 대조용량(4IU)과 과배란용량(40IU)으로 투여한 두 실험군을 비교하였다.

대조실험군의 암컷에 RU486 1mg, tamoxifen 1 mg 그리고 무첨제를 각각 제공으로 나누어 PRSG 투여 후 24, 36 그리고 48시간에 투여하고, 72시간에 도살하였다. 과배란 실험군도 대조실험군과 유사하게 제공으로 나누어 RU486 1mg, tamoxifen 2.5mg 그리고 무첨제를 투여하고, PMSG투여 후 60시간과 72시간에 도살하였다. 암 실험군에서 무첨제 투여군과 비교할때 RU486과 tamoxifen 처치에 의해 배란된 난포의 비율과 따라서 난자수는 상이하게 배란반응에 유의성있는 감소가 있었다. Tamoxifen의 배란반응은 대체로 두 상이 대조실험군에서 더 높았으나, 과배란 실험군에서는 용량을 증가할때 RU486의 억제보다 더 낮았다. 암 실험군에서 RU486은 난소중량의 변화 난자숫의 변화에 아무런 영향을 미치지 않은 반면, tamoxifen 처리는 무첨제 처리군과 비교해서 난소중량의 유의한 증가와 난자 변성율의 증가가 나타났다. RU

486처치는 스테로이드 호르몬의 혈장치에 영향을 미치지 않았지만, tamoxifen처치는 양 실험군에서 혈청 17β-estradiol의 유의성 있는 증가와 progesterone의 감소와 관련이 있었고 과배란 실험군에서는 androgen의 유의성있는 증가도 있었다.

위의 결과는 PMSG유도 배란과 관련된 주요 스테로이드 호르몬인 17β-estradiol과 progesterone에 대한 RU486과 tamoxifen의 상대적인 억제활동을 설명한다. 또한, tamoxifen과 관련된 순환 17β-estradiol과 androgen의 상승은 배란후 부분적으로 나쁜 난관환경을 제공하여 난자변성을 촉진시키는 인자가 아닌가 추측할 수 있다.

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