

Inhibitory effect of nomegestrol acetate on steroidogenesis of cultured granulosa cells from rat ovary *in vitro*

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KEY WORDS estrogen; progesterone; cultured cells; nomegestrol; ovary

ABSTRACT

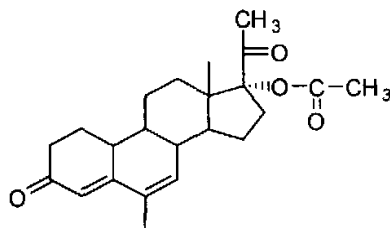
AIM: To study the effect of nomegestrol acetate, a new synthetic progesterone on granulosa cells' viability and steroidogenesis function. **METHODS:** Granulosa cells were cultured in McCoy's 5A medium. Trypan blue stain was used to measure viable cells. FSH and testosterone were added to stimulate the steroid secretion. Specific RIA assay was used to evaluate the estrogen and progesterone secretion respectively. **RESULTS:** IC₅₀ of nomegestrol acetate to damage cells is 6.85 mg/L (95 % confidence limits 5.36-8.75 mg/L). Nomegestrol acetate 0.45, 0.9, and 1.8 mg/L greatly inhibited the estrogen secretion from granulosa cells by 7.6 %, 12.5 %, 28.3 % in the presence of testosterone 0.5 μmol/L and FSH 10 U/L without affecting the number of viable cells. The secretion of progesterone were markedly decreased by 44.5 %, 53.3 %, and 62.0 % concurrently. **CONCLUSION:** Nomegestrol acetate directly inhibited the steroidogenesis of granulosa cells.

INTRODUCTION

Nomegestrol acetate is a new synthetic 19-norprogesterone derivative. Many clinical trials have demonstrated its high contraceptive efficiency with few side effects^[1-6]. Its possible mechanism may involve providing cervical blocking^[7], reducing estrogen receptor content^[8], suppressing function of hypothalamic-pituitary^[9,10], preventing follicular growth, supporting a persistent non-luteinized follicular and disrupting the endometrial architecture^[11]. According to one study in our

lab (unpublished), nomegestrol acetate inhibits regular estrus-cycle rats' ovulation two times more effectively than does megestrol acetate.

To further elucidate the pharmacological mechanism of nomegestrol acetate, we used *in vitro* culture system to investigate its direct effect on the steroidogenesis of granulosa cells. To mimic the *in vivo* microenvironment, we also added follicle stimulating hormone (FSH) and testosterone to stimulate the steroid secretion.



C₂₃H₃₀O₄
Exact Mass: 370.21
Mr: 370.48
C, 74.56; H, 8.16; O, 17.27
white powder

MATERIALS AND METHODS

Reagents Highly purified FSH was provided by professor WANG Han-Zheng (Shanghai Institute of Planned Parenthood). Nomegestrol acetate was synthesized by professor XIA Peng (Department of Organic Chemistry, School of Pharmacy, Fudan University). McCoy's 5A medium (modified without serum) was obtained from Gibco. Trypan blue stain (0.4 %) was purchased from Sigma Chemical Co. Estrogen and progesterone radioimmunoassay (RIA) kits were obtained from Shanghai Biological Engineering Center.

Animals Immature Sprague-Dawley female rats were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences (Grade II, Certificate No

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005) and were treated (im) daily with diethylstilbestrol (DES, 0.5 mg/d) beginning on d 22 to stimulate granulosa cell proliferation^[12]. The animals were given water and food *ad lib*. After five days of treatment, animals were sacrificed by cervical dislocation.

Granulosa cell culture Granulosa cells were collected and cultured as described by Erickson G and Hsueh^[13]. In brief, preantral follicles were punctured with No 4 needles and the granulosa cells were carefully expressed into McCoy's 5A medium. After washing the cells twice with McCoy's 5A medium, an aliquot was diluted with trypan blue stain and viable cells were counted with a hemocytometer. Viable cells (2×10^5) were added to 24-well Falcon tissue-culture plates containing 1 mL of McCoy's 5A medium which was supplemented with penicillin 100 μ mol/L, streptomycin 100 g/L, L-glutamine 2 mmol/L. The cells were cultured in a humidified 95 % air and 5 % CO₂ incubator at 37 °C. Nomegestrol acetate was added to the appropriate cultures in 5 μ L ethanol.

Cell viability Cell viability was checked by adding trypan blue stain to the cells and counting the percentage of cells which excluded the dye.

Steroid measurements Estrogen and progesterone secreted into the medium by the granulosa cells were measured directly without extraction, by radioimmunoassays (RIA) using RIA kits. Specific antigens were used to measure estrogen and progesterone respectively which do not crossreact with 19-norpregna.

Statistical analysis All results were subjected to group *t* test to determine whether processed group data were different from the control ($P < 0.05$).

RESULTS

Effect of nomegestrol acetate on cell viability

After 24 h treatment with nomegestrol acetate, nomegestrol acetate 1.9 mg/L did not affect the cell viability greatly. After cells were exposed to nomegestrol acetate 3.8 mg/L, the number of viable cells declined. The calculated IC₅₀ was 6.85 mg/L (95 % confidence limits; 5.36–8.75 mg/L) (Fig 1).

Effect of FSH on granulosa cell steroidogenesis Progesterone and estrogen production were almost negligible by cells cultured in medium alone compared with those of cells cultured in the presence of testosterone and FSH. Testosterone 0.5 μ mol/L and FSH 100 U/L enhanced the secretion of estrogen by 20 fold during the first day of culture and enabled secretion to continue dur-

ing the next two days but at a much lower rate than observed during the first day. However, FSH 1 and 10 U/L had little effect on the estrogen production during the first 24 h culture. During the two days of culture, FSH 1, 10, and 100 U/L enhanced estrogen secretion by nearly 8, 11, and 20 fold respectively. At the end of the three days culture, the estrogen secretion of granulosa cells was enhanced by 25, 40, and 44 fold, respectively (Fig 2).

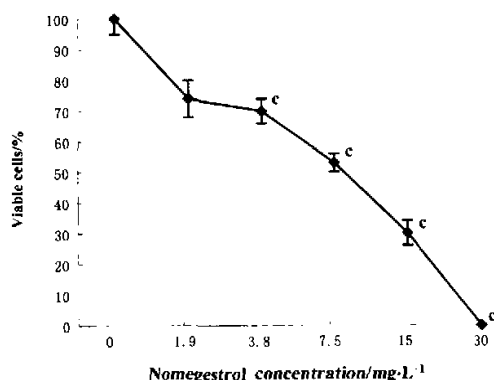


Fig 1. Effect of various concentrations of nomegestrol acetate on viable cells in culture at the end of 24 h in McCoy's 5A medium. $n = 3$. $\bar{x} \pm s$. $^*P < 0.01$ vs control (0 mg/L).

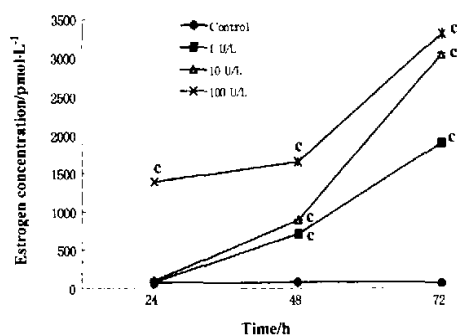


Fig 2. Effect of various concentrations of FSH and incubation time on the estrogen production of granulosa cells. Cells were cultured in FSH 1, 10, or 100 U/L and testosterone 0.5 μ mol/L for 24, 48, and 72 h respectively. $n = 3$. $\bar{x} \pm s$. $^*P < 0.01$ vs control.

As to the progesterone secretion, FSH 1, 10, and 100 U/L stimulated the secretion to about 3.4, 3.6, and 3.9 fold respectively during the first day of culture. The secretion increased further but at a little lower rate. At the end of three days culture, the secretion rose 6.5,

8.4, and 8.5 fold (Fig 3).

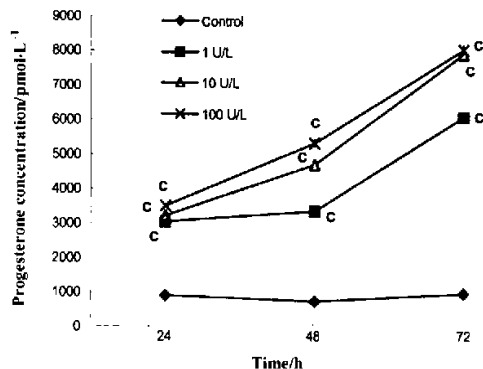


Fig 3. Effect of various concentrations of FSH and incubation time on the progesterone production of granulosa cells. Cells were cultured in FSH 1, 10, or 100 U/L and testosterone 0.5 $\mu\text{mol/L}$ for 24, 48, and 72 h respectively. $n=3$. $\bar{x} \pm s$. $^cP < 0.01$ vs control.

Effect of nomegestrol acetate on FSH-stimulated steroidogenesis in cultured rat granulosa cells Control cultures produced estrogen 3216.5 pmol/L in response to FSH 10 U/L and testosterone 0.5 $\mu\text{mol/L}$ during 72 h of the culture. When nomegestrol acetate 0.45, 0.9, or 1.8 mg/L was added to the culture, the number of viable cells was not affected and there was a decrease in estrogen production (2968.3, 2815.6, and 2307.6 pmol/L vs 3216.5 pmol/L, respectively) (Fig 4).

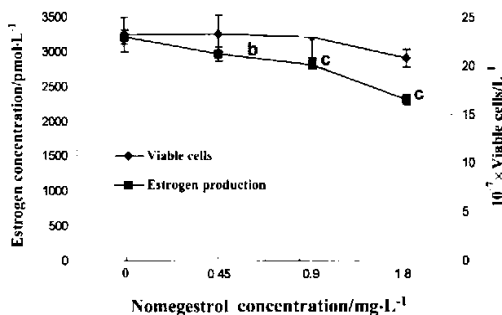


Fig 4. Effect of various concentrations of nomegestrol acetate on granulosa cells estrogen production. $n=3$. $\bar{x} \pm s$. Viable cells in all cultures at the end of 72 h incubation were counted. $^bP < 0.05$, $^cP < 0.01$ vs control.

The synthetic progestin nomegestrol acetate also inhibited the FSH and testosterone induced production of

progesterone in the cultured granulosa cells. Control cultures incubated with FSH 10 U/L and testosterone 0.5 $\mu\text{mol/L}$ produced 8007.4 pmol/L of progesterone. During a 72-h incubation, the addition of nomegestrol acetate 0.45, 0.9, and 1.8 mg/L results in approximately 45 %, 55 %, and 65 % decreases in the FSH-stimulated progesterone production, respectively (Fig 5).

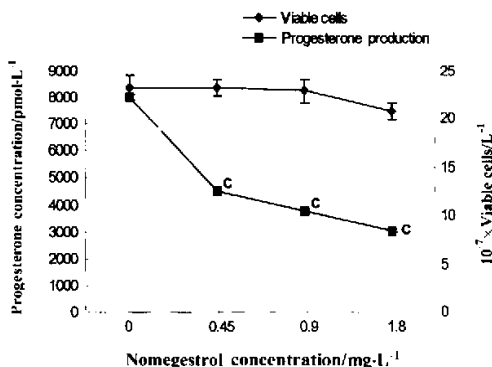


Fig 5. Effect of various concentrations of nomegestrol acetate on progesterone production granulosa cells. $n=3$. $\bar{x} \pm s$. Viable cells in all cultures at the end of 72 h incubation were counted. $^cP < 0.01$ vs control.

DISCUSSION

Two conclusions can be drawn from these data. First purified FSH can induce progesterone secretion of granulosa cells from DES-treated animals. Second nomegestrol acetate has the ability to inhibit the action of FSH greatly without reducing the viable cells number.

There are various methods of acquiring granulosa cells. Granulosa cells from pre-ovulatory follicles from DES-treated immature rats have already acquired FSH receptor^[13,14]. But the aromatase enzymes and enzymes involved in the production of estrogen and progesterone are low. Thus exogenous FSH was added to induce their activity. Testosterone 0.5 $\mu\text{mol/L}$ was also added to augment FSH-induced steroidogenesis. It was noted that differences existed with respect to the length of time required to induce the steroid secretion. The higher the FSH content, the sooner its effect appeared. This probably could be explained by important biochemical events such as the synthesis of progesterone, aromatase, and luteinizing hormone (LH) receptor do not occur synchronously.

The results reported here demonstrated that nomegestrol acetate directly inhibited the FSH-induced steroido-

genesis in cultured rat ovarian granulosa cells. The rat ovary granulosa cells contain progesterone receptor and it has been suggested elsewhere that nomegestrol acetate had high binding efficiency with progesterone receptor^[15]. So it is very likely that nomegestrol acetate exerts its effect through the progesterone receptor. This is consistent with generalized model of steroid action that steroid effects are mediated by specific receptors.

Our results are also consistent with the findings of Bazin B and Thevenot R^[16], which show that plasma LH, progesterone, and estradiol remained low in normal menstruating women who were treated with nomegestrol acetate. Their results also suggest a hypothalamic pituitary effect and an ovarian action activity are both responsible for the potentially useful contraceptive property. In addition to that, our results show a possible direct effect of nomegestrol acetate on ovarian granulosa cells.

A study in our lab (unpublished) showed that nomegestrol acetate could inhibit the ovulation of normal estrus-cycle rats. Our data suggests that one mechanism by which nomegestrol acetate blocks follicular growth may be by directly inhibiting follicle steroidogenesis.

In summary, our results indicate that nomegestrol acetate inhibit the FSH-stimulation of granulosa cell steroidogenesis *in vitro*. Such results suggest a possible mechanism whereby nomegestrol acetate exerts a direct inhibitory action on ovarian follicular development.

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诺美孕酮醋酸盐对离体培养大鼠卵巢颗粒细胞分泌甾体激素的抑制作用

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关键词 雌激素; 孕酮; 培养的细胞; 诺美孕酮;
卵巢

目的: 观察诺美孕酮对离体培养大鼠颗粒细胞分泌雌、孕激素功能的抑制作用. **方法:** 台盼蓝排斥法

进行活细胞计数. 加入 FSH 和 睾酮刺激颗粒细胞激素分泌. 放免法测定培养液中雌、孕激素含量. **结果:** 诺美孕酮杀伤细胞的 IC_{50} 为 6.85 mg/L (95% 可信限: 5.36 - 8.75 mg/L). 诺美孕酮 0.45, 0.9, 和 1.8 mg/L 在不影响活细胞数的情况下对颗粒细胞分泌雌激素的抑制率分别为 7.6%, 12.5% 和 28.3%, 对其分泌孕激素的抑制率分别为 44.5%, 53.3% 和 62.0%. **结论:** 诺美孕酮直接抑制离体培养的大鼠颗粒细胞分泌雌、孕激素.

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