Inhibitory effect of nomegestrol acetate on steroidogenesis of cultured granulosa cells from rat ovary \textit{in vitro}

QIAN Li-Hui, YANG Bo, LENG Ying, CAO Lin\textsuperscript{1}, GU Zhi-Ping (Shanghai Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China)

**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:** IC\textsubscript{90} 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 \textmu mol/L and FSH 10 U/L without affecting the number of viable cells. The secretion of progesterone was markedly decreased by 44.5%, 53.3%, and 62.0% concurrently. **CONCLUSION:** Nomegestrol acetate directly inhibited the steroidogenesis of granulosa cells.

\begin{center}
\begin{figure}
\includegraphics[width=0.5\textwidth]{nomegestrol}
\end{figure}
\end{center}

\textbf{C\textsubscript{23}H\textsubscript{30}O\textsubscript{4}}

Exact Mass: 370.21

\textit{M}r: 370.48

C, 74.56; H, 8.16; O, 17.27

white powder

**INTRODUCTION**

Nomegestrol acetate is a new synthetic 19-norprogestin derivative. Many clinical trials have demonstrated its high contraceptive efficiency with few side effects\textsuperscript{(1–6)}. Its possible mechanism may involve providing cervical blocking\textsuperscript{(7)}, reducing estrogen receptor content\textsuperscript{(8)}, suppressing function of hypothalamic–pituitary\textsuperscript{(9,10)}, preventing follicular growth, supporting a persistent non-luteinized follicular and disrupting the endometrial architecture\textsuperscript{(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 According to one study in our lab (unpublished), nomegestrol acetate inhibits regular estrus-cycle rats' ovulation two times more effectively than does megestrol acetate.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 \textit{in vitro} culture system to investigate its direct effect on the steroidogenesis of granulosa cells. To mimic the \textit{in vivo} microenvironment, we also added follicle stimulating hormone (FSH) and testosterone to stimulate the steroid secretion.

**MATERIALS AND METHODS**

\textbf{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.

\textbf{Animals}  Immature Sprague-Dawley female rats were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences (Grade II, Certificate No
005) and were treated (im) daily with diethylstilbestrol (DES, 0.5 mg/d) beginning on d 22 to stimulate granulo-
sosa 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
Hsu\(^{13}\). 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 \((2 \times 10^5)\) were
added to 24-well Falcon tissue-culture plates containing 1
mL of McCoy's 5A medium which was supplemented
with penicillin 100 \(\mu\)g/mL, streptomycin 100 \(\mu\)g/mL,
L-glutamine 2 \(\mu\)mol/mL. The cells were cultured in a
humidified 95% air and 5% \(\text{CO}_2\) incubator at 37 °C.
Noregestrol acetate was added to the appropriate cultures
in 5 \(\mu\)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 proges-
terone secreted into the medium by the granulosa cells
were measured directly without extraction, by radioim-
munoassays (RIA) using RIA kits. Specific antigens
were used to measure estrogen and progesterone respec-
tively which do not crosreact 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 noregestrol acetate on cell viability**

After 24 h treatment with noregestrol acetate,
noregestrol acetate 1.9 mg/L did not affect the cell vi-
bility greatly. After cells were exposed to noregestrol
acetate 3.8 mg/L, the number of viable cells declined.
The calculated IC\(_{50}\) 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 \(\mu\)mol/L and FSH 100 \(\mu\)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 ob-
served during the first day. However, FSH 1 and 10
\(\text{U/L}\) had little effect on the estrogen production during the
first 24 h culture. During the two days of culture, FSH
1, 10, and 100 \(\text{U/L}\) 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 noregestrol acetate on viable cells in culture at the end of 24 h in McCoy's 5A medium. \(n = 3\). \(\bar{x} \pm s\). \(\ast P < 0.01\) vs control (0 mg/L).](image)

![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 \(\text{U/L}\) and testosterone 0.5 \(\mu\)mol/L for 24, 48, and 72 h respectively. \(n = 3\). \(\bar{x} \pm s\). \(\ast P < 0.01\) vs control.](image)
8.4, and 8.5 fold (Fig 3).

![Graph showing progesterone production](image)

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 μmol/L for 24, 48, and 72 h respectively. $n = 3$. $x \pm s$. $^P < 0.01$ 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 μmol/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).

![Graph showing estrogen production](image)

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

Progesterone in the cultured granulosa cells. Control cultures incubated with FSH 10 U/L and testosterone 0.5 μmol/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).

![Graph showing progesterone production](image)

Fig 5. Effect of various concentrations of nomegestrol acetate on progesterone production granulosa cells. $n = 3$. $x \pm s$. Viable cells in all cultures at the end of 72 h incubation were counted. $^P < 0.01$ 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 μmol/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 steroidal-
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. 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, 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 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.

ACKNOWLEDGEMENTS We thank Prof. WANG Han-Zheng for generous donations of purified FSH. The technical assistance of Prof. ZHUANG Ling-Zhi is greatly acknowledged.

REFERENCES


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

钱立晖, 陈波, 冷颖, 曹霖1, 顾芝萍
（中国科学院上海生命科学研究院上海药物研究所，上海 200031，中国）

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 IC50 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
23.3%，对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。

德彪-CCRF 中国奖 (2001 年度)

“德彪-CCRF 中国奖”由中国德彪集团和中国癌症基金会于 1994 年共同创办。

德彪集团属科研发投资集团，是科研人员和制药工业间的桥梁，其宗旨是寻找和发现科学家、发明
家，以经济资助的方式，将他们的发现进一步发展。1994 年德彪公司成立中国新药开发部，以促进中国和
欧洲科研及制药工业间的交流与合作。

“德彪奖”通过与中国癌症基金会合作，鼓励抗癌及治疗老年性疾病、心血管疾病等领域的新药开发事
业。包括生物制剂、天然提取物及合成药物等，要求有新颖性和科技含量。

第一届颁奖会于 1995 年在北京举行，第二届于 1997 年在香港举行，第三届于 1999 年在兰州举行。

“德彪奖”根据项目的研究价值和新颖性颁发三项奖。一等奖：5000 瑞士法郎，并向获奖发明人或法定
代理人提供到欧洲两周业务交流的全部费用，必要时可追加一笔科研经费。二等奖：5000 瑞士法郎。三等奖：
3000 瑞士法郎。

欢迎对“德彪奖”有兴趣的个人和研究单位申请。自 2000 年 10 月起，请函秘书处报名领取申请表。
然后将申请文件挂号寄给秘书处；孙燕教授。

地址：北京朝阳区潘家园中国医学科学院肿瘤医院内科“德彪-CCRF 中国奖”秘书处
邮政编码：100021 电话：010-6778-1331，ext 8519 传真：010-6773-4107
E-mail：suy@pubmed.cicams.ac.cn

资料受理截止日期为 2001 年 4 月 25 日。