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Low Dose of Ethinyl Estradiol Can Reverse the Antiestrogenic Effects of Clomiphene Citrate on Endometrium

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Key Words
Clomiphene citrate · Ethinyl estradiol · Ovarian induction

Abstract
Fifty healthy, voluntary patients aged between 20 and 30 years with regular menstruation and plasmatic progesterone level >10 ng/ml at the midluteal phase have been enrolled in this study. They were randomly treated with clomiphene citrate (CC; group A) or CC + ethinyl estradiol (0.05 mg group B, or 0.02 mg group C). We estimated the difference in uterine artery pulsatily index, endometrial thickness and histological dating and morphometric analysis of endometrium. No significant differences in Pulsatility Index values and in the number of preovulatory follicles were noted. The difference between endometrial thickness, histological dating and morphometric analysis of the endometrium were statistically different between groups B and C vs. A. Our study shows that CC has a deleterious effect on endometrium maturity and that adding ethinyl-E_2 produces a favorable endometrial response even with very low doses.

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Introduction

Clomiphene citrate (CC) is an orally active nonsteroidal estrogen agonist/antagonist traditionally used to induce ovulation in patients with PCOS as first line therapy. In the USA, the maximum dose of CC approved for the use by the Food and Drug Administration (FDA) is 100 mg/day for 5 days [1]. Some authors, however, have found that higher doses are required, particularly in women who are overweight [2, 3]. Eventually, an increased dose of CC produces the same level of success in obese women as is attained in lean women [4, 5].

CC induces ovulation in about 60–75% of patients, although only 30–40% conceive [6–8]. The discrepancy has been attributed to a negative action of CC in the form of prolonged antiestrogenic effects on endometrial receptivity [9, 10] and interference with uterine blood flow [11]. These negative actions are augmented by the relatively long half-life of CC, which is known to be 5 days [12]. Sereepapong et al. [13] demonstrated that CC affects the endometrium of regularly cycling women, as resulted by a reduction in glandular density and an increase in the number of vacuolated cells. According to other authors, we have recently demonstrated that ethinyl E_2, at adequate dosage (0.05 mg daily for 5 days) can reverse the deleterious effects of CC [14–16].

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The aim of this study was to investigate the effects of CC alone or plus different dosages of ethinyl E_2 on endometrium and if the histological finding is correlate to endometrial thickness and uterine blood flow, both sensitive indicators of uterine receptivity [17].

Materials and Methods

Patients

A total of 50 healthy, voluntary patients were enrolled in this study. The inclusion criteria were: age between 20 and 30 years and regular menstrual cycles with plasmatic progesterone level >10 ng/ml at the midluteal phase. No patient had received estrogen or progestogen therapy for at least 6 months prior to participation. All patients gave informed consent before entering the study. The Institutional Board of Research approved the study protocol.

Treatment Protocol

All patients were randomly allocated in one of the following three groups:

Group A (n = 16): daily stimulation for 5 days with 100 mg of CC starting on day 3 (the start of the menses was designated as day 1 of treatment cycle).

Group B (n = 19): stimulation with 100 mg of CC starting on day 3. 0.05 mg of ethinyl E_2 was given daily for 5 days, beginning on day 8.

Group C (n = 15): stimulation with 100 mg of CC starting on day 3, 0.02 mg of ethinyl E_2 was given daily for 5 days, beginning on day 8.

All patients were administered 10,000 IU of hCG i.m. when the serum 17β-E_2 concentration was >200 pg per mature follicle and there was at least one follicle with a minimum diameter of 18 mm.

Laboratory Determinations and Ultrasound Scans

Plasmatic concentrations of 17β-estradiol were determined by radioimmunoassay (RIA) on blood samples on days 1, 5, 9 and 12 of the menstrual cycle. Ultrasound scans were performed daily since day 1 (to rule out ovarian cysts) until the mean follicle diameter reached 18 mm. The endometrial thickness was estimated on day 7 after ovulation. Pulsatility Index (PI) was recorded in both uterine arteries. A gynecologist experienced in transvaginal sonography (TVS) performed all examinations with a 5-MHz broadband probe.

The examiner was blinded to the patient's group. The (PI) was calculated by subtracting the peak end-diastolic shifted frequency from systolic shifted frequency and dividing the result by the mean doppler shift over the cardiac cycle. The intraobserver coefficient of variation for measurement of PI was 5.3%. All examinations were performed between 09.00 and 11.00 h to reduce the effect of circadian variation in PI [18].

Histological Dating and Morphometric Analysis of Endometrium

On day 7 after ovulation an endometrial biopsy was obtained transcervically from high anterior and posterior uterine walls using a Novak curette. Specimens were then fixed in 10% formal-saline, embedded in paraffin, and 4-μm sections were stained with hematoxylin and eosin for dating according to the criteria of Noyes et al. [19]. The same pathologist who was blinded to the patients' protocol regimen interpreted biopsies. Histological dating that was >2 days out of phase was considered abnormal.

According to Johannisson et al. [20], we considered some parameters to be specifically sensitive to tissue differentiation in order to evaluate the endometrium morphology: the number of complete and incomplete glands per field, average glandular diameter, glandular epithelial height in the glands and the number of vacuolated cells per 1,000 glandular cells.

Statistical Analysis

Statistics were performed with the SPSS statistical package (Sigmastat and Sigmaplot, Microsoft Corp., Redmond, Wash., USA). The χ^2 and Fisher t test were used. Significance was defined as a $p < 0.05$.

Results

There were no statistically significant differences (SSD) in mean (±SD) age and body mass index between the three groups (data not shown). There were no SSD in follicular development as measured by the number of preovulatory follicles and no SSD was found in progesterone plasma level measured 7 days after ovulation (data not shown).

The endometrial thickness was estimated 7 days after ovulation. In all cases it was >6 mm. It was 6–9 mm in 4 cases in group A (30%), in 2 cases in group B (10.5%), in 2 cases in group C (13.3%); it was 9–12 mm in 10 cases in group A (62.5%), in 16 cases in group B (89.4%), in 13 cases in group C (86.6%); it was >15 mm in 2 cases in group A (12.5%), in 1 case in group B (5.2%), in 0 case in group C (0%). The differences between groups A vs. B and vs. C were statistically significant. No statistical differences were noted between groups B and C (fig. 1).

PI values (±SD) were not different between groups (fig. 2).

The results of histological dating of endometria are shown in table 1.

The mean number of glands per mm^2 and the average glandular diameter were higher in groups B and C than in group A. The height of glandular surface epithelium was not different between the groups. There were more vacuolated cells per 1,000 glandular cells in group A than in groups B and C. No statistical differences were noted between groups B and C (table 2).

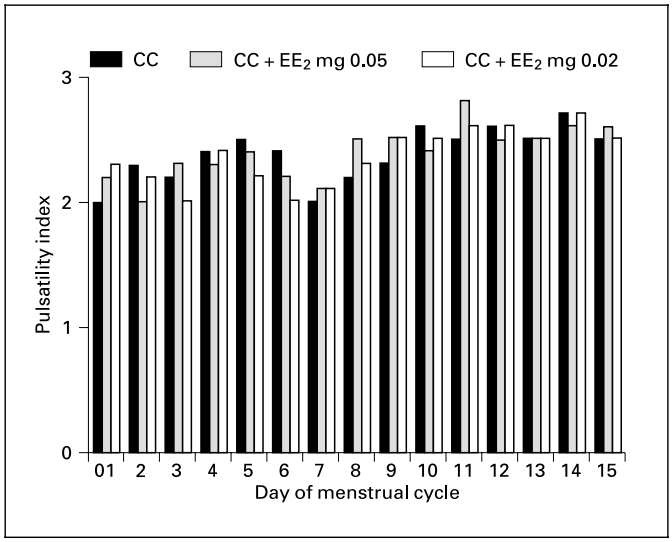

Fig. 1. Percentage distribution of endometrial thickness on day 7 after ovulation.

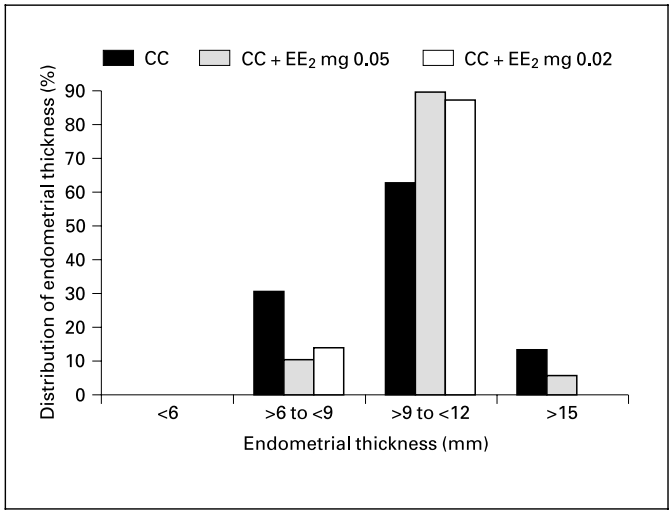

Fig. 2. Mean (+ SE) pulsatility index for patients undergoing ovarian stimulation with CC alone or CC plus ethinyl E_2 at different dosages.

Table 1. Histological dating of endometrium in CC-treated cycles (group A), CC plus EE_2 0.05 mg (group B) and CC plus EE_2 0.02 mg cycles (group C)

Histological dating	Group A		Group B		Group C	
	n	%	n	%	n	%
In phase (± 2 days)	6	37[a, b]	16	84[a]	13	86[b]
Delayed growth (<2 days)	10	63[c, d]	3	16[c]	2	14[d]
Accelerated growth (>2 days)	0		0		0	

[a, b, c, d] $p < 0.05$; values within rows with the same superscript were significantly different.

Table 2. Histological parameters studied in the endometrium in CC-treated cycles (group A), CC plus EE_2 0.05 mg (group B) and CC plus EE_2 0.02 mg cycles (group C)

Histological parameters	Group A	Group B	Group C
Glands per mm^2	26.2 ± 4.5[a, b]	29.7 ± 5.2[a]	30.1 ± 4.7[b]
Glandular diameter, µm	86.4 ± 11.2[c, d]	105.2 ± 13.9[c]	102 ± 10.3[d]
Height of glandular epithelium, µm	20.2 ± 2.3	21.3 ± 1.9	20.9 ± 2.3
Stromal cell count	60.2 ± 4.5	61.5 ± 2.8	62.2 ± 3.2
Vacuolated cells per 1,000 glandular cells	50.2 ± 20.1[e, f]	29.2 ± 19.3[e]	30.5 ± 25.1[f]

Values are mean ± SD.
[a, b, c, d, e, f] $p < 0.05$; values within rows with the same supercript were significantly different.

Discussion

Clomiphene citrate is the most commonly used drug in the treatment of infertility and its beneficial effect in patients with anovulation has not been disputed but, on the other hand, the number of pregnancies achieved after ovulation induction is much lower than expected [21]. This discrepancy has been attributed to a negative action of CC, in the form of antiestrogenic effects on endometrial receptivity [9]. Actually, histological studies have shown the estrogen-antiagonist effect of CC on the endometrium [22–25]. Ideally, glandular and stromal com-

partments of the endometrium should be synchronous in their development and maturity; the importance of synchronizing endometrium has been demonstrated in various animal models where pregnancy rates can only be maintained if embryo transfer occurs within a 72-hour window during the early luteal phase (days 16–19) [26].

According to Hosie and Murphy [27], our study showed that there is a disparity in the development and maturity of the endometrial compartments after exposure to CC, but this glandular-stromal disparity can be abolished with addition of estrogens. In a previous study [14], we have showed that the addition of 0.05 mg of ethinyl E_2 (given daily per 5 days) to CC increases endometrial thickness and decreases the risk of spontaneous abortion. In this study we found, according to Sereepapong et al. [13], a reduction of glandular density and an increase in the number of vacuolated cells in the endometrium of patients exposed to CC but these adverse effects can be prevented by administering ethinyl estradiol E_2 to both attempted dosages. Furthermore, the number of the glands per mm^2 and the diameter of glands were lower in CC-treated cycles than in the group with ethinyl E_2 supplementation. This shows that if, on the one hand, CC reduces the glandular density of endometrium, on the other ethinyl E_2 can reverse this deleterious effect. We have not found a connection between endometrial thickness and histological parameters. This discrepancy can be attributed to stromal edema as occurs in patients treated with tamoxifen. So, unlike other authors [17], we think that the endometrial thickness should not be considered a sensitive indicator of the uterine receptivity in patients undergoing ovarian induction with clomiphene citrate.

In conclusion, we demonstrated that CC has a deleterious effect on endometrium maturity and that addition of ethinyl E_2 produces a favorable endometrial response.

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