

The Effect of Consecutive Cycles of Clomiphene Citrate Therapy on Endometrial Thickness and Echo Pattern

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Objective: To determine whether successive cycles of clomiphene citrate affect endometrial thickness.

Methods: Thirty-four women presenting for treatment of anovulation, oligoovulation, or follicle maturation defects were given the smallest dose of clomiphene citrate necessary to attain a mature follicle. If no pregnancy ensued, the same dose was continued if a follicle 18–24 mm in diameter and a serum estradiol (E2) level greater than 200 pg/mL were achieved. Ethinyl E2 was supplemented for poor cervical mucus only. Endometrial thickness and echo patterns were measured each cycle at peak follicular maturation.

Results: There was no difference in mean endometrial thickness during the first six cycles of therapy, nor was there a trend for thickness to increase or decrease with successive cycles with or without the addition of ethinyl E2. There was no change in the distribution of echo patterns with successive cycles of clomiphene citrate. Post-treatment measures of thickness and echo pattern did not differ from baseline pre-treatment values. The homogeneous hyperechogenic pattern was the rarest. Mean serum E2 and progesterone levels at mid-cycle did not change with successive cycles.

Conclusion: One proposed mechanism for the dichotomy between ovulation and pregnancy rates after clomiphene citrate therapy is that the drug adversely affects the endometrium. If clomiphene citrate does affect implantation adversely, the mechanism does not seem to be related to thinning the endometrium or causing an echo pattern that indicates a poor prognosis. The data also suggest that estrogen supplementation does not influence endometrial thickness and would best be used exclusively for hostile cervical mucus. (*Obstet Gynecol* 1995;86:341–5)

One study¹ suggested a lowered pregnancy rate after embryo transfer if there is a thin endometrium or a

homogeneous hyperechogenic pattern preceding hCG injection when the controlled ovarian hyperstimulation regimen is clomiphene citrate–human menopausal gonadotropins (hMG). Another study² found an adverse relationship of thin endometrium or homogeneous hyperechogenic pattern before a rise in progesterone with assisted reproductive technology, even when clomiphene citrate was not used. At least two studies^{3,4} also demonstrated a reduced pregnancy rate with thin endometria, even in cycles without assisted reproductive technology. It has been suggested that clomiphene citrate treatment may be associated with reduced pregnancy rates, even when apparent successful ovulation has been achieved, because of an antiestrogen action adversely affecting the endometrium and impairing subsequent implantation.⁵ Eden et al⁶ found that clomiphene citrate inhibited the mid-cycle endometrial thickness compared with natural cycles. These findings were confirmed by Lenz and Lindenberg⁷ in clomiphene citrate–hMG cycles.

Although these previous studies demonstrated a statistically decreased mean endometrial thickness of clomiphene citrate–treated patients compared with untreated controls, the differences were not so great that one is forced to conclude that a slightly thinner endometrium would affect implantation adversely. Many of these studies were on first cycles of clomiphene citrate.

Some data suggest that clomiphene citrate has a cumulative adverse effect on cervical mucus in succeeding cycles related to its antiestrogenic action.⁸ We hypothesized that clomiphene citrate therapy might adversely affect the endometrial thickness similarly when used in consecutive cycles. This study evaluated both endometrial thickness and echo patterns in patients taking low-dose clomiphene citrate therapy for a minimum of four cycles.

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Materials and Methods

Thirty-four women presenting for treatment of ovulatory dysfunction were included in this study. Subjects ranged in age from 21–42 years (mean \pm standard deviation [SD] 32.3 ± 5.4). Seven women were treated for anovulation (defined as the failure to attain a progesterone level greater than 3 ng/mL), and four women were oligoovulatory with infrequent ovulation occurring in at least 6-week intervals. Twenty-three patients had immature follicular development (failure to develop a follicle 18–24 mm in diameter in conjunction with a serum estradiol (E2) level greater than 200 pg/mL) despite regular menses (late-luteal-phase biopsies dating more than 2 days out of phase). Patients with luteal-phase defects but making mature follicles were excluded because they did not need clomiphene citrate based on our previous data⁹ showing that progesterone supplementation alone produced better pregnancy rates in women with luteal-phase defects and mature follicles than did follicle-maturing drugs. All the women completed at least four cycles of clomiphene citrate therapy. Inclusion also required normal baseline FSH levels (less than 15 mIU/mL for our assay); above this level, we have noticed a decreased pregnancy rate.

The clomiphene citrate protocol required that patients begin with 50 mg on day 5 of their cycle and continue the medication for 5 days. If the cervical mucus was poor, 20–50- μ g ethinyl E2 could be administered beginning the day after the clomiphene citrate tablets were stopped and continuing until peak follicular maturation. This period varied in patients from 2–6 days. If in the preceding cycle, a minimum of one follicle with an average diameter of at least 18 mm in conjunction with a serum E2 level at least 200 pg/mL was not attained, then the dose of clomiphene citrate could be increased by 50 mg/day in the next cycle. However, a maximum dose of 100 mg for 5 days was used in this study. Nineteen (55.9%) patients were maintained at the 50-mg daily dose throughout treatment; eight (23.5%) started at 50 mg and increased to 100 mg; three (8.8%) started at 50 mg and had at least one cycle in which the dose was reduced to 25 mg; and four (11.8%) required 100 mg for each of their cycles. Twenty of the 34 patients (58.8%) were given ethinyl E2 in at least one of their clomiphene citrate cycles for the treatment of poor cervical mucus.

Sonographic studies were performed throughout the follicular phase to monitor follicular maturation, endometrial thickness, and echo pattern using an ATL Ultramark 4 Unit (Advanced Technology Laboratories, Bothell, WA) equipped with a 5-MHz vaginal transducer. Endometrial thickness was measured in millimeters by placing electronic calipers on the outer walls of

the endometrium in the longitudinal axis of the uterine body. The endometrium was graded as either triple-line, isoechogenic, or hyperechogenic, based on the echo pattern. A triple-line pattern appeared as a multi-layered endometrium in which hyperechogenic outer lines and a well-defined central echogenic line were visualized with hypoechogenic areas seen between these lines.² An isoechogenic endometrium was an intermediate pattern in which the echogenicity of the endometrium was the same as the myometrium, with a poorly defined central echogenic line.² A hyperechogenic endometrium had an echo-dense pattern compared with the myometrium, with no central echogenic line visualized.² All endometrial assessments were performed by one sonographer who was blinded to the nature of the study but not to the medications that the patients were taking. Endometrial thickness and echo pattern used for this study were those obtained at peak follicular maturation.

At peak follicular maturation, we measured serum E2 and progesterone levels by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). We recorded the day of the cycle on which peak follicular maturation was attained and the number of mature follicles observed. Baseline serum FSH levels were measured by the Amerlex assay (normal range 1.3–15 mIU/mL) (Amersham-Amerlex; Eastman Kodak Company, Rochester, NY). Baseline FSH levels were determined before 10 AM on day 2 or 3 of the cycle in patients with regular menses and at initial consultation for those with oligomenorrhea. Baseline measurement of thickness, echo pattern, and serum levels were obtained in the cycle before clomiphene citrate therapy was initiated.

Analysis of variance with repeated measures was used to test the null hypothesis that endometrial thickness was the same after clomiphene citrate therapy against the alternative that the thickness changed as a result of clomiphene citrate therapy. The repeated measures model included subject-to-subject variability. The compound symmetry of the correlation matrix was tested using the Greenhouse-Geisser epsilon test. Analysis of variance was also used to compare the change in serum E2 and progesterone levels for the duration of clomiphene citrate therapy. Independent *t* tests were used to compare the endometrial thickness between patients using ethinyl E2 and those not supplemented with estrogen in each cycle. The change in the distribution of echo patterns after clomiphene citrate therapy was evaluated using McNemar test. $P < .05$ was considered statistically significant. Power analysis showed that a study having 80% power to detect a change of at least 1 mm in the endometrial thickness at the $P < .05$ level of significance required 34 subjects.

Table 1. Hormone Levels by Cycle of Clomiphene Citrate Therapy*

Cycle (n)	Estradiol (pg/mL)	Progesterone (ng/mL)
1 (34)	371 ± 213	0.6 ± 0.5
2 (34)	427 ± 223	0.6 ± 0.5
3 (34)	405 ± 181	0.7 ± 0.6
4 (34)	411 ± 187	0.6 ± 0.4
5 (23)	405 ± 193	0.5 ± 0.4
6 (12)	519 ± 262	0.7 ± 0.5
7† (7)	561 ± 363	0.7 ± 0.5

Data are presented as mean ± standard deviation.

* $P > .05$, analysis of variance repeated measures for first six cycles (not significant).

† Cycle 7 not included in analysis of variance.

Table 2. Endometrial Thickness by Duration of Clomiphene Citrate Therapy

Cycle (n)	Mean* (mm)	SD (mm)	Median (mm)	Minimum (mm)	Maximum (mm)	Mean ± SD change from cycle 1 (mm)
1 (34)	10.7	2.1	11	7	14	
2 (34)	10.8	2.5	10	7	18	-0.12 ± 2.3
3 (34)	10.0	2.3	10	6	16	-0.65 ± 2.3
4 (34)	10.5	2.5	10	7	17	-0.21 ± 2.1
5 (23)	10.8	2.8	11	7	18	-0.35 ± 2.8
6 (12)	10.6	2.3	11	7	14	-0.83 ± 2.5
7† (7)	10.4	1.5	10	9	13	-0.71 ± 2.0

SD = standard deviation.

* $P > .05$, analysis of variance for first six cycles (not significant).

† Cycle 7 not included in analysis of variance.

Results

Thirty-four women completed a minimum of four cycles of clomiphene citrate therapy; 23 completed five cycles, 12 completed six cycles, and seven completed seven cycles. The results of cycle 7 are presented for descriptive purposes only because there were insufficient cases to include this cycle in the statistical analysis. Because of missing data in cycles 5 and 6, the analysis of variance for repeated measures was performed separately for cycles 1-4 ($n = 34$), cycles 1-5 ($n = 23$), and cycles 1-6 ($n = 12$).

On average, peak follicular maturation was reached on day 14 in cycles 1-4, day 13 in cycle 5, day 15 in cycle 6, and day 13 in cycle 7. The mean number of mature follicles achieved in each cycle at peak follicular maturation was 1.3. The mean serum E2 and progesterone levels at peak follicular maturation were the same throughout the cycles (Table 1).

The mean endometrial thickness in the cycle before starting clomiphene citrate therapy was 10.2 ± 2.1 mm (median 13, range 7-13). After clomiphene citrate therapy, the endometrial thickness ranged from 6-18 mm. The mean endometrial thickness did not vary by more than 1 mm throughout the duration of the clomiphene citrate therapy, nor did it differ significantly from pre-treatment baseline levels (Figure 1, Table 2).

At the end of four cycles of clomiphene citrate therapy, the endometrial thickness was within 1 mm in 21 (61.8%) patients, had increased by more than 1 mm in eight (23.5%), and had decreased by more than 1 mm in five (14.7%) ($P > .05$, McNemar test). Within each cycle, the mean endometrial thickness was compared for those patients taking ethinyl E2 against those who were not. There was no difference in the mean (\pm SD) endometrial thickness by use of ethinyl E2 in any cycle: cycle 1, 10.5 ± 2.0 mm; cycle 2, 11.1 ± 2.8 mm; cycle 3, 10.0 ± 2.1 mm; cycle 4, 10.9 ± 2.5 mm; cycle 5, 9.7 ± 2.6 mm; and cycle 6, 10.7 ± 2.2 mm.

Throughout the course of clomiphene citrate therapy, the triple-line and isoechogenic echo patterns were observed in 83-91% of the cycles. The homogeneous hyperechogenic pattern was observed in only 9-17% of all cycles. Table 3 presents the distribution of echo patterns for each cycle of clomiphene citrate therapy. The distribution is the same for the first six cycles of therapy ($P > .05$, χ^2). Seven patients had at least one cycle with the homogeneous hyperechogenic pattern. Evaluating the subset of 27 patients who did not have a hyperechogenic pattern, 16 women had the same echo pattern in all their cycles (either the triple-line or isoechogenic pattern) and 11 had a combination of the triple-line and isoechogenic patterns in their cycles. The following is the distribution of echo patterns in

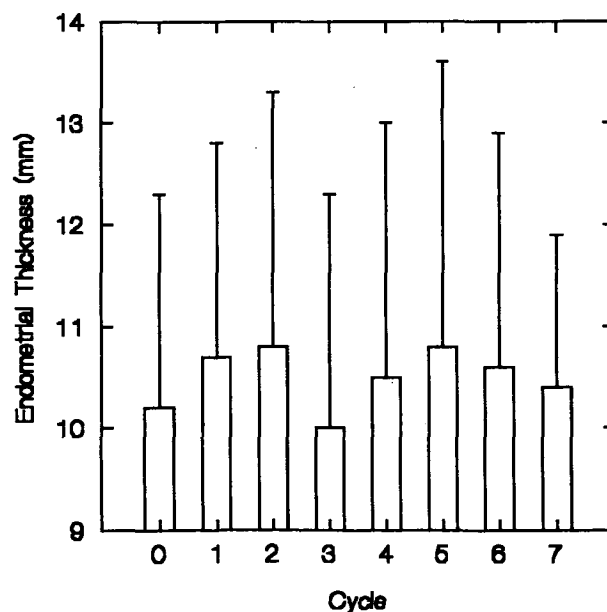


Figure 1. Mean endometrial thickness by cycle of clomiphene citrate therapy. Cycle 0 is baseline data in cycle preceding therapy.

Table 3. Echo Patterns by Cycle of Clomiphene Citrate Therapy*

Cycle	Triple-line	Isoechogenic	Hyperechogenic
1	17 (50.0%)	13 (38.2%)	4 (11.8%)
2	17 (50.0%)	14 (41.2%)	3 (8.8%)
3	12 (35.3%)	18 (52.9%)	4 (11.8%)
4	12 (35.3%)	17 (50.0%)	5 (14.7%)
5	7 (30.4%)	14 (60.9%)	2 (8.7%)
6	3 (25.0%)	7 (58.3%)	2 (16.7%)
7†	3 (42.8%)	3 (42.8%)	1 (14.2%)

* $P > .05$, χ^2 , for first six cycles (not significant).

† Cycle 7 not included in analysis of variance.

which ethinyl E2 was administered: triple-line 35.9%, isoechogenic 48.1%, and homogeneous hyperechogenic 16.0%, as compared with 46.1, 49.2, and 4.6%, respectively, when ethinyl E2 was not administered ($P > .05$).

Discussion

The antiestrogen activity of clomiphene citrate has resulted in an increased frequency of poor postcoital tests.⁸ An antiestrogenic effect on endometrial proliferation has been suggested,^{6,7,10} and poor endometrium for implantation has been hypothesized as one of the mechanisms to explain the discrepancy between the high ovulation rates but lowered conception rates (25–43% over 6 months) after clomiphene citrate therapy.^{5,6,11}

However, our data did not show any effect of clomiphene citrate on endometrial thickness or echo pattern, even with use in consecutive cycles. Furthermore, our study was the only one to use the clomiphene-treated patients as their own controls. More support for the concept that clomiphene citrate does not make the endometrium less appropriate for implantation was provided by a study¹² evaluating pregnancy rates in patients who had ovulatory defects treated randomly with hMG or clomiphene citrate; no differences in pregnancy rates were found.

There have been two conflicting reports^{10,13} concerning the efficacy of supplemental estrogen to improve endometrial thickness in patients taking clomiphene citrate. Our data corroborates the study by Ben-Ami et al,¹³ in which no improvement in endometrial thickness was found, despite supplemental estrogen therapy.

There appeared to be a trend for higher E2 levels with successive cycles, especially when cycle 1 was compared with cycle 7. Because there was no statistical difference between the means, these differences may not be real. However, if future studies prove that there is a progressive rise in mean serum E2 levels with successive cycles, one possible mechanism may be that as

pregnant patients are eliminated, the percentage of those with polycystic ovaries increases. This may have been evidenced by the fact that in later cycles the patients remaining had a higher percentage of multiple mature follicles (23.5, 35.3, 23.5, 29.4, 26.0, 41.7, and 57.1% for cycles 1–7, respectively). Data from in vitro fertilization cycles have shown that a patient with polycystic ovary syndrome tends to produce higher serum E2 levels; however, the oocytes may be immature and thus have much lower fertilization rates.

Partially based on the present study and previous data,¹² we believe that the treating physician should not necessarily conclude that patients should be switched from clomiphene citrate to gonadotropin therapy if no pregnancy ensues after a few cycles of clomiphene citrate therapy. Nevertheless, a randomized comparison of the two therapies in women failing to conceive after three cycles of clomiphene citrate therapy would be interesting. Of course, this study would necessitate properly timed intrauterine insemination when mucus is hostile and therapy to correct luteal-phase defects and luteinized unruptured follicles when needed.

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