

Endometrial Thinning after Ovarian Stimulation using Letrozole or Clomiphene Citrate: A Randomized Trial

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ABSTRACT

Objective: To compare endometrial thickness after three consecutive cycles of ovulation induction with clomiphene citrate versus letrozole.

Methods: Eighty-four women with normal menstrual interval who attended the university infertility clinic from June 2016 to March 2017 were eligible for the study. After the endometrial thickness of baseline cycle was recorded, all participants were randomized into two groups of clomiphene citrate or letrozole treatment for three consecutive cycles. Endometrial thickness and estradiol level were measured when at least one follicle reached 17 mm in diameter. The differences in endometrial thickness relative to baseline of the two groups were compared.

Results: A total of 62 patients completed three cycles of ovarian stimulation. Both drugs resulted in significantly thinner endometrium compared with the baseline cycle thickness. The mean endometrial thickness was significantly decreased in the clomiphene citrate group compared with letrozole group (7.46 ± 1.71 vs 8.88 ± 2.34 mm, $p = 0.029$). Estradiol level on the day of induced ovulation was significantly higher in the clomiphene citrate group than in the letrozole group (706.0 (207.9, 2209.0) vs 168.7 (30.0, 401.8), $p < 0.001$). The number of the follicles reaching 17 mm on the day of induced ovulation was higher in the clomiphene citrate group (1.9 ± 0.8 vs 1.4 ± 0.5 , $p = 0.002$).

Conclusion: Letrozole had less effect on endometrium thinning after three consecutive cycles of induced ovulation compared with clomiphene citrate.

Keywords: Clomiphene citrate; letrozole; ovulation induction; endometrial thickness; consecutive cycles (Siriraj Med J 2018;70: 335-342)

INTRODUCTION

Ovulation induction combined with intrauterine insemination (IUI) provides a treatment for infertility caused by anovulation or unexplained infertility. It is safe, efficient and inexpensive. However, the fecundity rates varies from 15-22 percent,¹ and optimal rates require repeated treatments. Clomiphene citrate (CC) is the major medicine used for ovulation induction. It is a racemic mixture of zuclomiphene and enclomiphene.² Zuclomiphene is the most anti-estrogenic component

and mildly estrogenic, whereas enclomiphene is entirely anti-estrogenic. Clomiphene citrate competitively binds to the estrogen receptor in the hypothalamus and pituitary, leading to the alleviation of the negative feedback exerted by endogenous estrogen, and alters the characteristics of pulsatile gonadotropin releasing hormone secretion,³ which results in the elevation of production and secretion of follicle-stimulating hormone (FSH) from pituitary. Unfortunately, this antagonistic effect on estrogen receptors does not only exist in the hypothalamus, but

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Received 10 May 2018 Revised 4 June 2018 Accepted 7 June 2018

doi:10.14456/smj.2018.53

also throughout the body, especially in the endometrium and endocervix. As a result, the estrogen antagonist effect of CC disturbs endometrial growth and maturation.⁴

The metabolism of CC varies between individuals and is related to body weight.⁵ Women weighing 45-59 kg have become pregnant using 50 mg/day CC.⁶ The circulating half-life of CC ranges from 27.5-38.5 days, and serum concentrations of zuclophene remain at least 10% of peak levels after 28 days post-ingestion of a single 50 mg tablet.⁷ The effect of repetitive administration of a 50 mg tablet at 28-day intervals is cumulative, resulting in a 50% increase in basal CC levels per month.⁷ Accumulation of CC or metabolites in endometrium may affect the pregnancy outcome and preclinical abortions.²

Letrozole was proposed to be an alternative to CC for ovulation induction.⁸ It is an aromatase inhibitor that prevents the conversion of androgen to estrogen. The resulting elevated level of intra-ovarian androgen is positively associated with the expression of FSH receptor mRNA in small antral follicles,⁹ which have enhanced sensitivity to FSH stimulation. The circulating half-life of letrozole is only 42 hours, which is not affected by age.¹⁰ Pharmacodynamic analysis suggested that letrozole had no effect on endometrial thickness and cervical mucus, but improved folliculogenesis.¹¹ A systematic review and meta-analysis indicated that a significant increase in live birth and pregnancy rates was observed in letrozole-treated compared with CC-treated polycystic ovarian syndrome patients.¹² In 2016, the American Congress of Obstetricians and Gynecologists recommended that letrozole be considered a first-line therapy for ovulation induction in polycystic ovarian syndrome patients.¹³ The different pregnancy rates between the CC and letrozole groups may due to drug effects on endometrial thickness.^{14, 15}

Some researchers found that thin endometrium caused lower pregnancy rates.^{16,17} No pregnancy occurred if the endometrial thickness was less than 6 mm.¹⁷ This suggested that endometrial thickness is one of the key factors involved in successful insemination cycles. Several studies reviewed the effect of CC and letrozole on endometrial thickness, but the results were controversial.¹⁸⁻²² Some evidence indicated that repeated use of CC decreased endometrial thickness.¹⁷ However, no study has compared the cumulative effect of letrozole versus CC on endometrial thickness. Therefore, the main objective of this study was to compare endometrial thickness after three stimulation cycles using CC or letrozole. Secondary objectives were to compare endometrial thickness on the day of induced ovulation between the third and baseline cycle of each drug; and to compare estradiol levels and the number

of follicles ≥ 17 mm on the day of induced ovulation in each cycle.

MATERIALS AND METHODS

Subjects and procedures

The study was conducted between June 2016 and March 2017 at the university infertility clinic and was approved by the Hospital Institutional Review Board (Si 371/2016). Recruited patients were aged 18-42 year old and recommended for IUI and had a normal menstrual cycle interval (defined as 21 to 35 days per cycle or at least nine menstrual cycles per year) and no previous treatment. Those with endometrial lesions or bilateral fallopian tube obstruction were excluded. Patients who had used oral contraceptive drugs within one month, or depot medroxyprogesterone acetate/GnRH agonist depot within six months before enrollment were also excluded. Informed consent was obtained before the trial.

The baseline blood test included day 3 hormones (FSH, estradiol, luteinizing hormone (LH), and prolactin), serologic blood test (Anti-HIV, VDRL, HBsAg) and hemoglobin typing. Tubal patency was confirmed by hysterosalpingography. Transvaginal ultrasound was performed to screen endometrial abnormalities, such as lesions in the uterine cavity. Serial ultrasound was performed by one trained gynecologist who was blinded to the protocol, to check for the dominant follicle every other day starting from day 10 ± 1 of the menstrual cycle. When the dominant follicle reached 17 mm in diameter, serum estradiol levels were assessed, ovulation was induced on the same day, and endometrial thickness and number of follicles above 17 mm were subsequently recorded. Urine pregnancy tests were performed 14 days after IUI or timed intercourse. Patients whose follicle failed to reach 17 mm in diameter on day 21 of the menstrual cycle, or those pregnant at the baseline cycle, were excluded. The remaining participants were randomized into two groups at the beginning of the second cycle, using computer generated randomization numbers allocated at a ratio of 1:1 by the research nurse and sealed in individual envelopes. The first group received 50 mg/day CC (Ovomit[®], Remedica Ltd., Cyprus), and the other group received 2.5 mg/day letrozole (Letov[®], Cadila Healthcare Limited, India). The medication was commenced on the third day of the menstrual cycle and continued for five days. Participants then had transvaginal ultrasound, hormonal assessment and ovulation induction, as described above in the baseline cycle. Data was analyzed for participants who received medication for three consecutive cycles.

Transvaginal ultrasound

Endometrial thickening was measured using a 7.5 MHz transvaginal transducer (Prosound α 7, Aloka Co., Ltd, Tokyo, Japan). Endometrial thickness was measured from the echogenic junction between endometrium and myometrium, from one side to the opposite side at the point of maximal thickness, with the uterus in a central longitudinal axis. The depth magnification was adjusted until the uterus covered about two-thirds of the sonographic view. Follicular diameter was documented by calculating the mean of the two perpendicular diameters measured at the largest plane of the follicle.

Sample size

Sample size was calculated based on the study of Angel et al.,²³ who reported the endometrial thickness was 7.47 mm and 8.56 mm in the CC and letrozole groups, respectively. Therefore, using 5% and 20% type I and II errors, respectively, and a 20% dropout rate, each group required a sample size of 45 patients.

Statistical analysis

Statistical analysis was performed using PASW Statistics 18. Continuous data was expressed as mean \pm SD for a normal distribution, or median with range if non-normal. Categorical data was expressed as percent. The unpaired T-test or Man-Whitney U test were used as appropriate. Differences in endometrial thickness between two groups were calculated using the linear mixed model, using intention to treat analysis. All tests of significance were two-tailed with p value < 0.05 .

RESULTS

One hundred and thirty-two participants were screened, and 97 participants were eligible to undergo baseline evaluation (Fig 1). After the baseline evaluation, 13 cases were excluded. The remaining 84 patients were randomized into two groups. After further exclusion because of loss of follow-up or pregnant participants during the first 2 cycles, a total of 31 cases in each group continued receiving the third cycle of stimulation.

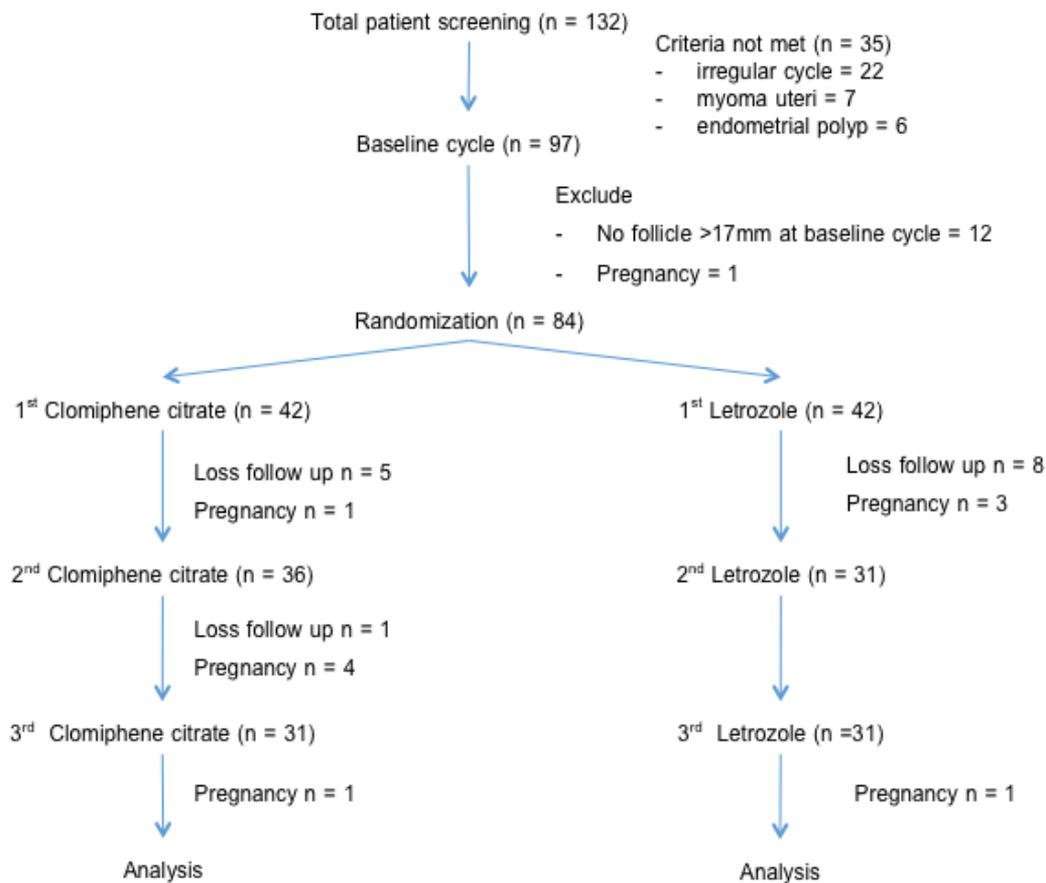


Fig 1. Flow and randomization of participants.

A total of 132 patients were screened, 97 were eligible for baseline evaluation. Thirteen patients were excluded before randomization due to pregnancy or no presence of dominant follicle. Eighty-four patients were equally randomized into two groups, the clomiphene citrate group (CC) and the letrozole group. In the CC group, 6 and 5 patients were excluded from the study at cycle 1 and 2, due to loss follow up or pregnancy. In the letrozole group, 11 patients were excluded from the first cycle because of loss follow up and three pregnancies. There were 31 patients left in each group at cycle 3.

The baseline characteristics of participants are displayed in Table 1. There was no difference in mean age, BMI, cycle interval, duration of marriage, type of infertility, and baseline FSH, LH and estradiol levels between the two groups. Baseline cycle characteristics are shown in Table 2. The endometrial thickness on the day of induced ovulation, the number of dominant follicles and serum estradiol levels were not statistically different between groups.

Endometrial thickness on the day of induced ovulation for each menstrual cycle of both groups is shown in Fig 2. The CC group seemed to have a progressive reduction in endometrial thickness when CC was used repeatedly. During letrozole treatment, endometrial thickness was reduced compared with the baseline cycle, but the level of reduction remained constant over the three stimulation cycles. Overall, endometrial thickness for each group in all stimulation cycles was significantly reduced compared with the baseline level. There was no difference in endometrial thickness between the CC and letrozole group for the first and second stimulation cycles (Table 2). However, in the third stimulation cycle, endometrial thickness was significantly reduced in the CC group compared with the letrozole group (7.5 ± 1.7 and 8.9 ± 2.3 cm, respectively, $p = 0.008$). The difference remained significant when adjusted for other potential factors (i.e., age, BMI, type of infertility, and duration of marriage) (Table 3).

There were seven participants (22.6%) in the letrozole group, and 12 participants (38.7%) in the CC group exhibiting endometrial thickness less than 7 mm on the day of induced ovulation in the third stimulation cycle ($p = 0.27$).

There were significantly higher levels of estradiol and more follicles with > 17 mm diameter in the CC group for every stimulation cycle as shown in Table 2.

DISCUSSION

Endometrial thickness is one of the crucial factors affecting pregnancy rate.^{16,17} Thinner endometrium has a reduced functional layer, resulting in an implantation site closer to the spiral arteries of the basal layer, which contains high blood flow and oxygen tension. This situation facilitates the production of reactive oxygen species (ROS), leading to interrupted embryonic development and implantation.^{24,25} In addition, the resistance index of uterine and radial artery blood flow was high in endometrium of reduced thickness, so glandular epithelium growth was impaired and expression of endometrial vascular endothelial growth factor was reduced, limiting endometrial growth.²⁶ Thin endometrium may result from several factors, including anti-estrogenic effects of CC,²⁷ adhesion after endometrial curettage,²⁸ long-term (>10 years) combined oral contraceptives²⁹ and idiopathic causes.

TABLE 1. Baseline characteristics between clomiphene citrate (CC) and letrozole groups.

	CC (n=31)	Letrozole (n=31)	P-value
Age (years)	34.9 ± 3.2	36.2 ± 3.1	0.105
BMI (kg/m ²)	21.4 ± 3.0	22.6 ± 4.9	0.249
Cycle interval (days)	29.3 ± 2.9	29.7 ± 3.0	0.496
Duration of marriage (years) †	4 (1-14)	4.5 (1-12)	0.297
Characteristics of infertility ‡			
Primary	27 (87.1%)	27 (87.1%)	1.000
Secondary	4 (12.9%)	4 (12.9%)	
Baseline FSH (mIU/mL)	7.4 ± 1.8	7.6 ± 3.3	0.685
Baseline LH (mIU/mL)	4.6 ± 1.9	5.3 ± 2.4	0.226
Baseline E ₂ (pg/mL)	39.8 ± 19.9	34.8 ± 18.3	0.311

Data shown as mean ± SD

†shown as median (minimum-maximum)

‡shown as n (percent)

TABLE 2. Effects of ovulation induction in clomiphene citrate (CC) and letrozole groups. Most data show as mean \pm SD.

Baseline cycle	CC (n=31)	Letrozole (n=31)	P-value
Endometrium thickness (mm)	9.4 \pm 2.1	10.0 \pm 2.2	0.205
Number of follicle \geq 17 mm	1.0 \pm 0.2	1.0 \pm 0.18	0.561
Estradiol level [†] (pg/ml)	252.4 (5.0 - 808.5)	238.2 (13.4 - 1195.0)	0.795
Size of dominant follicle (mm)	18.6 \pm 3.0	17.8 \pm 1.5	0.198
1st cycle stimulation			
Endometrium thickness (mm)	8.3 \pm 2.5	8.9 \pm 2.0	0.301
Number of follicle \geq 17 mm	1.8 \pm 0.8	1.2 \pm 0.4	<0.001*
Estradiol level [†] (pg/ml)	825.0 (211.0 – 1997.0)	173.9 (32.1 – 360.8)	<0.001*
Size of dominant follicle (mm)	20.0 \pm 2.9	19.9 \pm 3.6	0.954
2nd cycle stimulation			
Endometrial thickness (mm)	8.1 \pm 2.0	9.0 \pm 2.4	0.119
Number of follicle \geq 17 mm	2 \pm 1.0	1.3 \pm 0.6	0.003*
Estradiol level [†] (pg/ml)	938.9 (234.8 - 1887.0)	146.0 (48.0 - 336.6)	<0.001*
Size of dominant follicle (mm)	20.3 \pm 3.0	19.3 \pm 5.2	0.362
3rd cycle stimulation			
Endometrial thickness (mm)	7.5 \pm 1.7	8.9 \pm 2.3	0.008*
Number of follicle \geq 17 mm	1.9 \pm 0.8	1.4 \pm 0.5	0.002*
Estradiol level [†] (pg/ml)	706.0 (207.9 – 2209.0)	168.7 (30.0 – 401.8)	<0.001*
Size of dominant follicle (mm)	19.7 \pm 3.2	20.9 \pm 3.0	0.142

[†] Data shown as median (min-max)

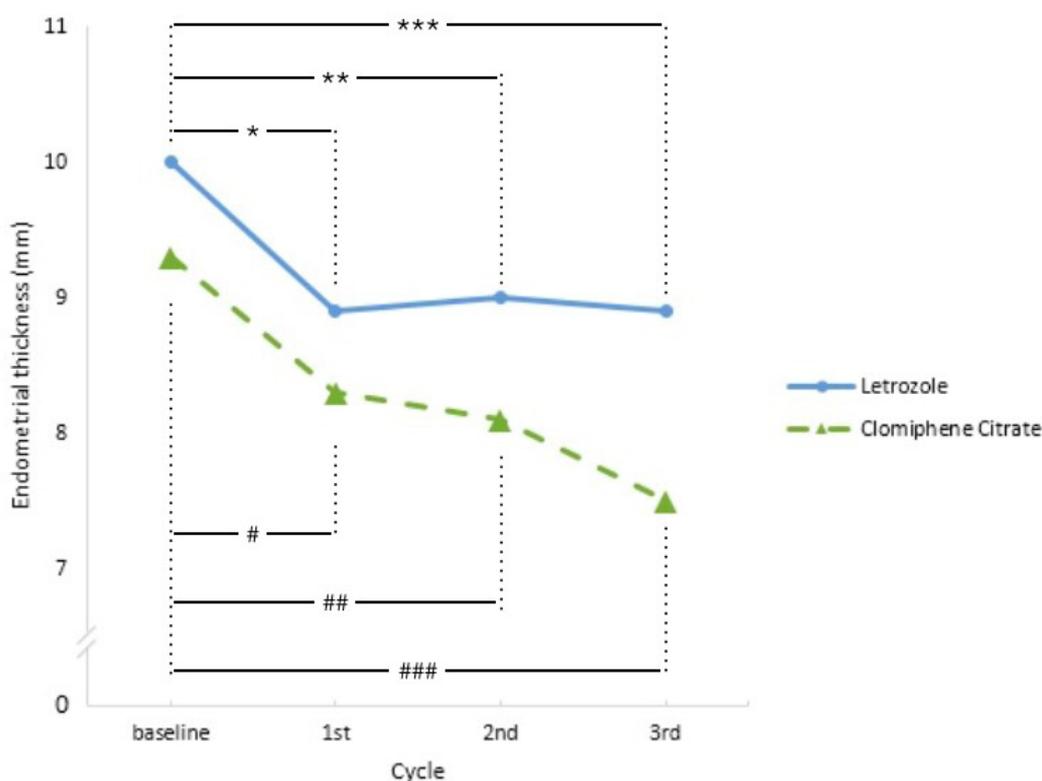
*Statistically significance

TABLE 3. Difference of endometrial thickness at the end of the 3rd cycle stimulation of both drugs.

Endometrial thickness (mm), mean (SD)	Baseline	Cycle 1	Cycle 2	Cycle 3	Adjust MD [†]	95% CI	P-value
CC (n=31)	9.4 (2.1)	8.3 (2.5)	8.1 (1.9)	7.5 (1.7)	0.97	(0.1,1.84)	0.029*
Letrozole (n=31)	10.1 (2.2)	8.9 (2.1)	9.0 (2.4)	8.9 (2.3)			

[†] Adjusted by age, BMI, type of infertility, duration of marriage

* Statistically significance



* p= 0.029, ** p=0.043, *** p= 0.021,
p= 0.042, ## p= 0.03, ### p= 0.01

Fig 2. Relation between endometrial thickness between each menstrual cycle of both drugs.

The CC group had a continuous reduction of endometrial thickness when used repeatedly. In the letrozole group, the endometrial thickness was diminished compared with the baseline cycle; however the reduced thickness level remained constantly over the three stimulation cycles. Overall, endometrial thickness for each group in all stimulation cycles was significantly decreased compared with the baseline level.

In the present study, we found that endometrial thickness was significantly decreased after three consecutive stimulation cycles in the CC compared with letrozole group. The decrease remained significant after adjustment for other potential factors. Some randomized studies reported a similar finding in a single cycle treatment,^{20,30} although this result was contrary to other earlier findings.³¹⁻³³ A systematic review and meta-analysis found no significant difference in endometrial thickness between CC and letrozole administration for unexplained infertility.¹⁸ In that analysis, six randomized studies were included, although only four studies reported endometrial comparisons.^{20,22,30,31} The cumulative effect of these treatments on the endometrium during consecutive ovarian stimulation had not been previously examined. In this study, an accumulated deteriorative effect on endometrial thickness was detected in the CC group after three repeated stimulations. This may be due to the cumulative effect of the antagonist function of CC on estrogen receptors. The alpha and beta estrogen receptors are expressed differently in various tissues. Estrogen receptor β (ER β) is the prevalent estrogen receptor in brain and cardiovascular systems, while estrogen

receptor α (ER α) predominates in the uterus, especially during the proliferative phase of the menstrual cycle.³⁴ In the uterus, CC acts as an estrogen agonist/antagonist via ER α , in an estrogen concentration-dependent manner; whereas it acts as a pure estrogen antagonist via ER β .³⁵ Therefore, CC enhances the secretion of pituitary gonadotropins, but attenuates the estrogen effect on ER α in the uterus and cervix. Young et al.,³⁶ showed the rise of serum zuclomiphene citrate levels through four consecutive cycles of CC administration. The long half-life of zuclomiphene may cause endometrium thinning by antagonistic effects on ER α , especially when used repeatedly.³⁶ Accordingly, the thinning of endometrium in the CC group is likely caused by the anti-estrogenic effect of zuclomiphene.

In the letrozole group, endometrial thickness was also reduced in stimulation cycles compared with the baseline cycle. Considering the half-life or clearance rate of letrozole (around 42 hours), it takes approximately 10 days for letrozole metabolization and clearance from serum.¹⁰ Therefore, during a normal menstrual cycle interval (21-35 days), serum estradiol levels became low on the day of induced ovulation compared with the

baseline cycle, leading to endometrial thinning. Letrozole is effectively metabolized before the commencement of the next cycle, resulting in a smaller cumulative effect. Therefore, endometrial thickness in the stimulation cycles was lower than the baseline value, but remained at a similar level in all three consecutive cycles.

The definition of thin endometrium varies from less than 5 to 8 mm.³⁷ The recommended follicular phase endometrial thickness, which is positively associated with pregnancy outcome, is 7 mm.^{37,38} Past research found no pregnancy occurred when endometrial thickness was less than 6 mm.¹⁷ In the current study, an equivalent number of participants had endometrial thickness less than 7 mm in the CC and letrozole groups (12 (38.7%) vs 7 (22.6%), respectively). Surprisingly, one pregnancy in the CC group occurred despite an endometrial thickness of only 4.9 mm, resembling a similar case that conceived via IUI with an endometrial thickness of 4 mm.³⁹ Though endometrial thickness is one of the key factors involved in pregnancy success during insemination cycles, it may not be the only important factor to consider.

This is the first study to compare the effect of consecutive cycles of CC and letrozole on endometrial thickness. Our study has some limitations to consider. Firstly, only one dose of each drug was examined, and the longer-term effect of higher doses, especially for CC, remains unknown. Secondly, the primary objective of the study was endometrial thickness, and pregnancy and live birth rates were not assessed. Further study should be encouraged to evaluate the later outcomes. Third, endometrial receptivity in thin endometrium was not studied. More molecular studies are required to discover the underlying mechanisms.

In conclusion, CC had a more cumulative detrimental effect on endometrial thickness than letrozole after repetitive administration. Letrozole may be considered instead of CC in later cycles to limit the degree of endometrial thinning.

ACKNOWLEDGMENTS

The study was financially supported by a Siriraj Grant for Research Development, Faculty of Medicine Siriraj Hospital, Mahidol University (Grant number: R015932042).

Disclosure statement: The authors declare no conflict of interest.

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