# Do the Dose and Starting Time of Clomiphene Citrate in Induction Protocol Affect the Clinical and Endocrinological Response?

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## Abstract

**Objective:** To compare the standard ovulation induction protocols with the protocol of starting CC on the first day of cyclus and usage of a short time.

**Materials and Methods:** A total of 59 patients (age range 20-31 years) were randomized into two groups. In group I the patients had received CC 50 mg/day for three days starting on the first day of their cyclus. The patients in group II had received CC 50 mg/day for five days starting on the fifth day. Main outcome measures were estradiol level on the 11st and estradiol and progesterone levels on the 22nd day, endometrial thickness, ovulation rate, pregnancy rate, and miscrriage rate.

**Results:** In group I, serum levels of estradiol and progesterone and the rate of ovulation were found to be lower, and the rate of abortus was found to be higher.

**Conclusion:** Starting therapy with CC on the first day of cyclus with short duration has not given any additional benefit to endocrinological and clinic outcomes.

**Key words:** clomiphene citrate, ovulation induction, pregnancy outcome

## Özet

### Klomifen Sitratın Dozu ve Tedaviye Başlama Zamanı Klinik ve Endokrinolojik Yanıtı Değiştirir mi?

**Amaç:** Standart ovülasyon indüksiyon protokolünü klomifen sitratı siklusun birinci gününde başlayarak kısa süreli verilmesi ile karşılaştırmak.

**Materyal ve Metot:** S.B. Ankara Eğitim ve Araştırma Hastanesi, Kadın Hastalıkları ve Doğum Kliniği, İnfertilite Polikliniği'nde toplam 59 hasta iki gruba randomize edildi. Yaşları 20 ile 31 arasında değişen WHO Class II anovülasyon tanısı konulmuş ve herhangi bir infertilite tedavisi almamış hastalar seçildi. Grup I hastalar siklusun birinci gününden itibaren toplam üç gün 50 mg/gün klomifen sitrat aldı. Grup II hastalar siklusun beşinci günü başlayarak beş gün boyunca 50 mg/gün klomifen sitrat aldılar. Ana ölçüt olarak siklusun 11. ve 22. günü estradiol ve progesteron düzeyi, endometrial kalınlık, ovülasyon oranı, gebelik oranı ve düşük oranı alındı.

**Sonuçlar:** Grup I'de estradiol, progesteron değerleri ve ovülasyon oranları daha düşük, abortus oranı daha yüksekti.

**Tartışma:** Klomifen sitratın siklusun birinci gününden itibaren kısa süreli uygulanması endokrin ve klinik sonuçlara ek bir katkıda bulunmamıştır.

**Anahtar sözcükler:** klomifen sitrat, ovülasyon indüksiyonu, gebelik sonuçları

## Introduction

An evolution has occured in reproductive endocrinology with the use of clomiphene citrate (CC) in the clinical area. Some points still remain to be clarified for this drug about the mechanism of action, various effects on different parts of the reproduction axis, even administration dose and starting time.

Clomiphene citrate is the most widely used drug in ovulation induction. It is well established that pregnancy rates obtained with CC therapy (25 to 99%) are less than expected compared to the rates of successful ovulation (55 to 99%) (1). The discrepancy between the rate of ovulation after the use of CC and relatively low pregnancy rate is explained in part by the adverse effect of CC on the uterine cervix (2-4), vaginal epithelium (2-4) and endometrium (5,6) during the implantation period. Some studies have shown that the use of ethynyl estradiol during the follicular phase can reverse these effects of CC, but the results are far from satisfactory.

The administration dose and timetable of CC has been determined empirically. It may be reasonable to decrease the dose and/or starting the drug earlier in order to decrease the unwished antiestrogenic effect especially during the sensitive preovulatory period.

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In this study we have started CC on the first day of cycle with the thought of probable maintenance of satisfactory levels of FSH during follicular recruitment and selection, and because of low lewels of clomiphene citrate in the preovulatory phase the antiestrogenic effect could be lowered. The clinical and endocrinological results were compared with those of conventional method.

## Materials and Methods

Fifty-nine patients admitted to infertility clinic were enrolled in the study. This study was carried out between April 1996 and February 2000. All couples diagnosed as infertile have undergone a complete serum hormonal evaluation, hysterosalpingography, laparoscopy and sperm analyses. From these patients, the ones who had not received any infertility treatment and who had a Class II ovarian dysfunction according to the World Health Organisation criteria were accepted into the study.

All patients gave informed consent before they entered the study. The beginning of menses, either spontaneous or induced by progesterone was designated as day 1 of the treatment cycle. All patients were randomly enrolled in one of the two groups.

In Group I (n=30), stimulation began on day 1 with the administration of 50 mg clomiphene citrate (Serophene®; Serono, İstanbul, Turkey) daily for three days. This group of patients has been followed for 72 cycles.

In group II (n=29), induction began on day 5 with the administration of 50 mg CC daily for three days. This group of patients has been followed for 64 cycles. If ovulation and a normal luteal phase could not be achieved in the first cycles, the dose of CC has been increased by 50 mg doses per day to a maximum of 150-200 mg/day.

Plasma levels of estradiol were measured on day 11 and 22; plasma progesterone levels were measured on day 22 by radio immunosorbent assay (RIA) (Diagnostic Products Corp, LA, USA).

Transvaginal ultrasonography was performed on day 9, 11 and 13 of the cycle for the assessment of follicular growth and endometrial thickness measured from the echogenic interface of the endometrium-myometrium junction in transverse fundal sections. All sonographic evaluations were performed with 5 MHz vaginal probe by the same researcher in the study.

The number of follicles with a diameter of 18 mm or greater were recorded before human chorionic gonatotrophin (hCG) administration. When the diameter of leading follicle was 18 mm or greater, 10 000 IU of hCG was administered to the patients for the exact timing of ovulation. Determining the collapse of the dominant follicle and presence of free fluid in the pouch of Douglas during sonographic scans and serum progesterone levels greater than 5 ng/mg were regarded as positive signs for ovulation (8-10).

Durations of follicular and luteal phases were measured with respect to the day of ovulation and uterine bleeding. Patients with one day menstrual retardation or after 7 days of progesterone measurement were tested for the presence of a possible pregnancy by measuring β-hCG levels by RIA method (Diagnostic Corp, LA, USA).

Results were expressed in mIU/mL. When serum β-hCG levels were high, the presence of a clinical pregnancy was confirmed later by means of transvaginal ultrasonography.

The statistical analyses were performed with the help of MS Excel 97 for Windows statistics program. Students's t test was used for comparison of results. The level of significance was defined as $p<0.05$.

## Results

In both groups, mean age, mean duration of infertility, gravida and body mass indexes were identical, as shown in Table 1.

Estradiol levels were similar in both groups on day 11. But on day 22, estradiol and progesterone levels were significantly higher in Group II. Plasma β-hCG levels were similar in both groups. Plasma hormone levels of the treatment groups are shown in Table 2.

The mean duration of luteal phase, the numbers of follicles with diameter 18 mm or greater and the endometrial thickness measured on day 13 were similar in both groups, but the mean follicular phase of Group I patients was significantly shorter than those of Group II patients (Table 3).

The ovulation rate was 63.88% in Group I, but 78.11% in Group II. The difference was statistically significant. At the same period, the percentages of pregnancy were similar in both groups (Table 4). Eleven pregnancies were achieved in Group I patients, however eight pregnancies were achieved in Group II patients. The miscarriage rates were 36.36% in Group I and 25% in Group II.

## Discussion

The discrepancies between the ovulation rate and pregnancy rate induced by CC caught the attention of many

**Table 1.** Characteristics of the patients in the treatment groups

| | Group I (n=30) | Group II (n=29) |
|---|---|---|
| Age (year) | 25.6 | 25.7 |
| BMI* (kg/m$^2$) | 23.7 | 24.0 |
| Mean duration of infertility (Mo) | 42.1 | 36.9 |
| Gravida | 0.65 | 0.54 |

*Body mass index.

**Table 2.** Plasma hormone levels in the treatment groups

| | Group I | Group II (n=72) | P value (n=64) |
|---|---|---|---|
| Estradiol at day 11 (pg/mL) | 265.59±7.57 | 255.46±8.86 | NS |
| Estradiol at day 22 (pg/mL) | 228.70±7.14 | 247.75±8.54 | <0.05 |
| Progesterone at day 22 (ng/mL) | 10.99±0.76 | 13.36±0.75 | <0.05 |
| β-hCG at day 29 (mIU/L) | 166.90±11.71 | 145.50±13.12 | NS |

NS: not significant.

**Table 3.** Comparison of mean follicular phase, mean luteal phase, number of follicle and endometrial thickness in the treatment groups

| | Group I (n=72) | Group II (n=64) | P value |
|---|---|---|---|
| Mean follicular phase (day) | 12.26±0.11 (n=46) | 12.96±0.14 (n=50) | <0.05 |
| Mean luteal phase (day) | 13.69±0.14 (n=46) | 13.54±0.13 (n=50) | NS |
| The number of follicle ≥18 mm | 0.88±0.075 (n=72) | 0.95±0.078 | NS |
| Endometrial thickness (mm) | 10.91±0.206 | 10.75±0.166 | NS |

**Table 4.** Percentage of ovulation and pregnancy rates in the treatment groups

| | Group I | Group II | P value |
|---|---|---|---|
| Ovulation rate (%) | 63.88 | 78.11 | <0.05 |
| Pregnancy/ovulatory cycles (%) | 23.9 | 16.0 | NS |

researchers. This discrepancy was attributed to the antiestrogenic effect of CC and researches were concentrated on adding estrogen to the treatment in order to eliminate this unwished effect (11-13). In a recent study, to decrease the peripheral antiestrogenic effect of CC, 50 mg of CC was used for three days and some remarkable results were obtained (14).

In standard ovulation protocols, no differences have been observed in the rates of ovulation, pregnancy or spontaneous abortion whether CC was started on day 2, 3, 4 or 5 (15). In our study, when CC was started on the first day and continued for three days, the follicular phase shortened, the ovulation rate decreased and the abortion rate increased.

The ovulation induction effect of CC is secondary to increased gonadotropin secretion (16). There is a rapid increase in serum FSH and LH levels on the second and third days of the therapy and those start to decrease after the last dose of CC treatment.

The increase in serum FSH levels are observed 2-3 days after CC reaches sufficient level for folliculogenesis. Once it is initiated, it will progress to ovulation as in spontaneous cycles (17).

According to current physiology knowledge (18,19), FSH should have increased in the follicular recruitment period in Group I patients. The plasma half-life of CC is long and estimated to be 5 days (20). If the long half-life of CC is taken into account, FSH increase continues during the follicle selection period of the cycle. The discrepancy between ovulation rate and pregnancy rate cannot be explained by the antiestrogenic effect of CC solely. Too early increase of FSH and following poor folliculogenesis and probable premature LH peak may help to explain this discrepancy.
The levels of progesterone and estradiol on day 22 in Group I patients was found to be significantly low. This finding can also be attributed to the poor folliculogenesis or the negative efect of CC on corpus luteum (21).

The relation between abortions and plasma progesterone levels could not be proven in researches. Successful pregnancy outcomes were reported with low progesterone levels (22,23). In our study, higher rate of abortion in Group I patients can be explained with desynchronized endometrial proliferation due to the lack of harmony in the levels of estradiol and progesterone than progesterone levels only (24).
Though the ovulation rate was lower in Group I patients, the pregnancy rate of each ovulatory cycle was similar to the pregnancy rates obtained in standard ovulation induction and spontaneous cycles (25-27).

As a result, starting treatment with CC on day 1 and three days course in order to eliminate the peripheral antiestrogenic effect is not to superior to standard ovulation techniques.

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