



Journal of Medical Sciences

ISSN 1682-4474

science
alert

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JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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Comparison of Clomiphene Citrate Plus Estradiol, with Tamoxifen Citrate Effects in Induction of Ovulation and Pregnancy in Poly Cystic Ovarian Syndrome Patients

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The aim of this study was to compare the rates of ovulation and pregnancy after tamoxifen citrate, clomiphene citrate and after adding estradiol (E₂) to clomiphene citrate among anovulatory women with infertility. This study was a randomized double blind clinical trial conducted from Aug. 2006 to Aug. 2007 in present Infertility Clinic of Imam Education and Research Hospital, Sari, Iran. The patients were 157 anovulatory women under 35 years of age undergoing ovulation induction. Of total, 144 cases completed the study. They were assigned randomly to receive either tamoxifen citrate or clomiphene citrate on cycle days 3-9 and/or clomiphene citrate on cycle days 3-9 plus E₂ on cycle days 8 until all patients undergo HCG injection. Rates of ovulation and pregnancy for the three treatment modalities were measured. Although not statistically significant, the ovulation rates was higher in TAX group compared with clomiphene citrate group or clomiphene citrate plus E₂ group (77.1 vs. 60.4 vs. 58.3% p = 0.1). The overall pregnancy rates was significantly higher in women who received tamoxifen citrate compared with those who received clomiphene citrate or clomiphene citrate plus E₂ (52.1 vs. 33.3 vs. 22.9%, p = 0.01). This study demonstrated Hormonal supplementation with oral Estradiol following clomiphene citrate therapy in comparison with clomiphene citrate or tamoxifen citrate could not increase ovulation and pregnancy rate.

Key words: Clomiphene citrate, tamoxifen citrate, estradiol, induction of ovulation endometrial thickness

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INTRODUCTION

Ovulation disorders are one of the most common causes of infertility. It can prevent pregnancy in its severe form (anovulation) or simply be a contributing factor (oligo ovulation). Disorders of ovulation account for about 30 to 40% of all cases of female infertility (Berek, 2007; Speroff and Fritz, 2005). Polycystic ovarian disease is one of the most common problems in relationship with ovulatory dysfunction affecting 7% of women of reproductive age. It is a very common problem that presents in a variety of clinical manifestations, including hirsutism, chronic anovulation, irregular menstruation, obesity and infertility (Berek, 2007; Speroff and Fritz, 2005).

Due to relationship between Poly Cystic Ovarian Syndrome (PCOS) and infertility, it leads to recurrent visits, depression and anxiety in patients. Therefore, achieving appropriate treatment to increase ovulation and pregnancy is very important in PCOS patients (Berek, 2007). Clomiphene Citrate (CC) is a nonsteroidal triphenyl ethylene derivative which is widely used as a first line treatment for ovulatory dysfunction in these patients.

According to earlier studies, ovulation occurs in 80% of CC induced cycles while pregnancy is found in half of them. This may be due to antiestrogenic effects of the drug on the endometrium and cervical mucus (Speroff and Fritz, 2005; Goldstein *et al.*, 2000). Tamoxifen citrate (TAX), is another drug in this group which has more estrogenic activity on the endometrium and cervical mucus (Steiner *et al.*, 2005; Wu, 1997). Therefore, it is expected that pregnancy rate would be higher in the TAX group in comparison with CC group.

In earlier studies there were some controversies between TAX and CC in ovulation and pregnancy rate and some of them reported higher pregnancy with TAX (Messinis and NilLius, 1982; Nardo, 2004; Boostanfar *et al.*, 2001; Elkind-Hirsch *et al.*, 2002). Now, there is a question: whether higher rates of pregnancy with TAX in comparison with CC, would be due to its effect on cervical mucous and endometrial thickness or other factors are contributing to this difference?

This study was designed to compare fertility rate between CC and TAX and to find out if there is a difference between them, whether adding estradiol (E_2) to CC would make CC more effective or other factors including hormonal alteration levels are interfering?

MATERIALS AND METHODS

This was a randomized double blind clinical trial study conducted from Aug. 2006 to Aug. 2007 in Infertility Clinic of Imam Khomeini Education and

Research Hospital, Sari, Iran. One hundred and forty four women with Polycystic ovarian syndrome (PCOs) were enrolled in this study.

All of them met inclusion and exclusion criteria of the study. In the first visit, a detailed medical history was taken and their past medical history was reviewed. Physical examination was performed to rule out of uterine, kidney, liver and thyroid disease. Pelvic sonography was performed to rule out of uterine anomalies and uterine leiomyoma.

Laboratory test including CBC, urine analysis, semen analysis (for rule out of male factor) and hystrosalpingogram to verify tubal patency was performed. Demographic characteristics of patients including age, weight, height and BMI were recorded.

In addition to abnormal hystrosalpingogram and semen analysis, exclusion criteria included female older than 35 years, secondary infertility, duration of infertility more than 10 years, hyperprolactinemia, hyper or hypothyroidism, $FSH > 12 \text{ mIU mL}^{-1}$ in the 3rd day of cycle, $BMI > 30 \text{ kg m}^{-2}$, clomiphene resistance, previous exposure to any ovulation induction agents, interval of earlier treatment with ovulatory agents less than 6 months and contraindication to one the drugs.

The study entirely explained for the participants and they were given informed consent. All study protocols were approved by medical ethical committee of Mazandaran medical university.

All patients, who met inclusion criteria underwent a transvaginal sonography on the day 3 of menstrual cycle to rule out of ovarian cysts, defined as any sonoluscent structure measuring $>10\text{mm}$ in mean diameter. Candidates were then screened on cycle day 3 with an endocrine profile (FSH-LH-Estradiol).

Subjects were randomly divided into 3 groups to receive either clomiphene (oral tab 50 mg, ferdos company, Iran) plus estradiol (oral tab 2 mg, aboraihan company, Iran), (group A), clomiphene (group B) and tamoxifen (group C) (oral tab 10 mg, ferdos company, Iran), clomiphene (100 mg day^{-1}) was administered to group A and B between cycle days 3-9 and group A also received estradiol.

Estradiol was administered 2 mg daily from cycle day 8 and was continued until HCG injection day.

Patients in group C received tamoxifen 20 mg daily between cycle days 3-9 then all patients in three groups underwent HMG injection (IM), on cycle days 8 and 9.

All participants in three groups received placebo (same color and size, designed in School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran). A vaginal ultrasound was performed on cycle day 10 and then repeated sequential until the largest follicle diameter reached more than 18 mm. The number of follicles larger

than 14 mm was recorded. Serum LH and E₂ was checked and endometrial thickness was measured and then HCG ampoule 10,000 IU, IM injected at the same day.

Patients was asked to have Intercourse 8 h after HCG injection and then two nights after sequence.

Thirty-six hours after HCG injection a transvaginal sonography was performed for evaluation of ovulation (follicular disappearance with fluid in cul de sac) and if there was not evidence of ovulation sonography was repeated the day after. Pregnancy was confirmed by detection of β-HCG two week later and subsequent transvaginal sonography 3-4 weeks after HCG injection.

Data were analyzed by two-way Analysis of Variance (ANOVA) and for quantitative data and χ² for qualitative data. p<0.05 was considered statistically significant. This study had the ability (at 80% power and α of 0.05) to detect a 20% difference in ovulation and pregnancy rate.

To analyze serum concentrations of Estradiol, FSH, LH and progesterone a competitive Chemiluminescent immune assay was used (Kit's of Biomerieux Company, FRANCE, with Enzyme Linked Fluorescent Assay ELFA).

RESULTS

Of 157 women initially enrolled in the study, 13 (8.2%) excluded before study due to ovulation failure. One hundred and forty four patients completed the study cycle and under double blinding conditions were randomized in 3 groups. there was 48 women in each study group.

The groups were matched by duration of infertility (≥5 years, <5 years), BMI (19-52, 26-30, 30-35) and age (20-25, 26-30, 31-35 years old). There were no significant differences between the study groups. Demographic characteristics of the study population (N = 144) are shown in Table 1.

There was no difference between three groups in the number of antral follicles on cycle day 3 and number of mature follicles (≥18 mm) in HCG injection day.

Also LH, FSH and E₂ levels in 3rd day of cycle (Table 2) and LH and E₂ in HCG injection day and progesterone levels on day 7 after HCG injection was not significantly different in all groups (Table 3).

Estradiol and LH levels and endometrial thickness in the day of HCG injection and variation of them and progesterone levels 7 days after HCG injection and the day of cycle on which HCG was injected shown in Table 3.

LH levels in the HCG injection day and variation of its levels (the difference between LH in HCG injection day and LH in 3rd day of cycle) were significantly higher in patients receiving TAX compared with CC or CC plus E₂ (p = 0, p = 0.001) (Table 3).

Table 1: Demographic characteristic of the study population

Variables	CC	TAX	CC+E ₂	p-value
No. of women	48.0	48.0	48.0	
Age (years)	27.6±4.50	27.1±4.1	27.3±5.1	0.8
Duration of infertility (years)	4.2±2.70	4.2±2.3	3.5±2.7	0.20
BMI (kg m ⁻²)	25.4±2.03	9.8±3.0	25.9±2.7	0.42

Table 2: Base line endocrine parameters in 3rd day cycle

Variables	CC	TAX	CC+E ₂	p-value
LH (mIU mL ⁻¹)	7.2±4.50	7.3±4.60	6.4±3.60	0.50
FSH (mIU mL ⁻¹)	5.2±2.70	5.8±2.90	5.6±1.80	0.55
Estradiol (pg mL ⁻¹)	68.6±55.6	50.2±39.7	51.9±43.6	0.10

Table 3: Hormonal variations and endometrial thickness in treatment cycle

Variables	CC	TAX	CC+E ₂	p-value
LH in HCG injection days (mIU mL ⁻¹)	21.8±17.6	31.20±23.5	13.9±8.05	0.000
LH variation (mIU mL ⁻¹)	16.3±21.8	31.10±47.5	7.2±7.50	0.001
E ₂ in HCG injection days (pg mL ⁻¹)	621.2±428.8	465.70±642.3	674.7±435.4	0.059
E ₂ variation (pg mL ⁻¹)	552.9±435.8	413.80±465.9	640.7±432.2	0.001
Endometrial thickness (mm) on 3rd day	7.9±4.02	9.82±2.17	8.4±2.40	0.007
Variation of endometrial thickness (mm)	3.6±1.60	6.10±1.90	4.9±2.20	0.003
HCG injection days (day)	13.2±1.90	3.90±2.30	12.5±1.70	0.002
Progesterone (ng mL ⁻¹)	13.3±13.3	15.60±11.2	16.7±15.4	0.450

There was significant difference in the variation of E₂ (the difference between E₂ in HCG injection day and LH in 3rd day of cycle) in patients receiving CC plus E₂ compared with CC or TAX alone (p = 0.045) (Table 3).

Endometrial thickness in HCG injection day and its variations were higher in patients receiving TAX compared with CC or CC plus E₂ (p = 0.007, 0.003) (Table 3).

The duration of follicular growth was statistically shorter in CC plus E₂ group compared with other groups.

The overall rate of ovulation in the CC group was 29 of 48 cycles (60.4%) and in the TAX group it was 37 of 48 cycles (77.1%) and in the CC plus E₂ group was 28 of 48 cycles (58.3%). There was no significant difference between groups in ovulation rate (p = 0.1).

At the end of study period, the pregnancies recorded were 33.3% in the CC group and 52.1% in the TAX group and 22.9% in the CC plus E₂ group. All the pregnancies were normal and single in three groups. Figure 1 shows data of ovulation rate per cycle and pregnancy rate per ovulatory cycle in women receiving treatment.

No side effects and symptoms or signs of ovarian hyper stimulation were reported in each group.

Of the pregnancies in the CC group, one patient ended in spontaneous miscarriage and of the 25 pregnancies in the TAX group, one patient was resulted to blighted ovum and needed to D and C and of 11 pregnancies with CC plus E₂, one patient had missed abortion and needed to D and C. There was no significant difference in abortion rate between three groups.

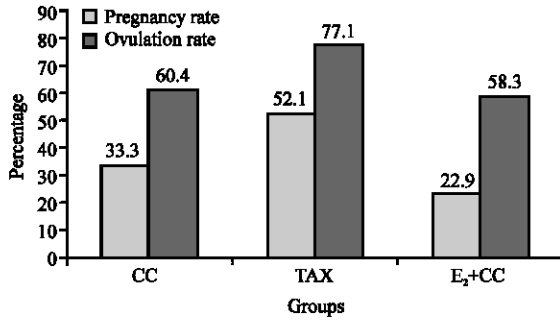


Fig. 1: Data of ovulation rate per cycle and pregnancy rate per ovulatory cycle in women receiving treatment

DISCUSSION

Based on antiestrogenic effects of Clomiphene Citrate (CC) on endometrium and cervical mucus, we suggested that adding Estradiol (E₂) to CC, the same as or more than tamoxifen (TAX) leads to increase pregnancy rate (Elkind-Hirsch *et al.*, 2002, 2005; Nakamura *et al.*, 1997), therefore, we compared pregnancy rate between TAX and CC and found no significant difference between CC and TAX in ovulation rate but pregnancy rate was higher with TAX which was shown in some of the earlier studies (Boostanfar *et al.*, 2001; Elkind-Hirsch *et al.*, 2002). There was no significant change in Estradiol (E₂) levels between HCG injection day and third day of menstrual cycle. However, endometrial thickness was significantly higher in TAX group.

Nakamura *et al.* (1997) in his study about the effects of CC on the endometrial thickness and echogenic pattern of the endometrium, showed that serum E₂ levels were significantly higher during the CC cycle compared with pretreatment cycle but E₂ receptor contents in the endometrium were significantly lower during the CC cycle, so, despite of no difference in serum E₂ levels at HCG injection days the endometrial thickness was higher in TAX group in our study. And adding E₂ to CC the endometrial thickness did not increase significantly.

On the other hand, adding E₂ to CC left out the difference between CC and TAX statistically.

According to these findings despite decreasing E₂ receptor contents (Nakamura *et al.*, 1997), present study revealed that endometrium in CC treated cycle could respond to higher dosage of estrogen.

Check *et al.* (1995) in their study about the effects of CC on endometrial thickness showed that the adverse effect of CC on implantation is not related to the thinning of the endometrium or its echo pattern.

In contrast with present study they found that exogenous estrogen does not have effect on endometrial

thickness and should be used just in special situations and for quality improvement of the cervical mucous (Check *et al.*, 1995), contrary to results, Unfer *et al.* (2001) and Gerli *et al.* (2000), who used 0.02 and 0.05 mg of ethinyl estradiol showed that low dose of ethinyl estradiol can reverse the antiestrogenic effects of CC on endometrium.

In a study by Elkind-Hirsch *et al.* (2002) and Elkind-Hirsch *et al.* (2005) reported that the effects of CC with or without hormonal supplementation on morphology and endometrial receptivity, estrogen and progesterone was used and in contrast to present results, they found out hormonal treatment in CC treated cycle may improve endometrial receptivity and ultimately yield higher pregnancy rates. In their study, endometrial thickness at mid-cycle was not different among the treatment groups; higher rate of pregnancy in their study was may be due to effects of administered progesterone (Nakamura *et al.*, 1997).

In this study, mid-luteal progesterone levels were similar in all three groups and there were no significant difference between them.

In this study, there was no difference in the number of preovulatory follicles between groups which is also shown by Unfer *et al.* (2001) and Gerli *et al.* (2000).

In this study the follicular growth rate was significantly higher in patients receiving CC plus E₂ which was similar to the study of Elkind-Hirsch *et al.* (2005). In his study LH surge happened earlier in patients received estrogen.

This is an important point which can be used to increase follicular growth in patients with slow follicular growth or resistant to treatment.

Adashi *et al.* (1981) reported that CC or E₂ both independently can increase the sensitivity of the gonadotroph cells to GnRH and thereby FSH and LH secretion. TAX unlike CC, inhibits LH secretion via decreasing sensitivity of the gonadotroph to E₂.

So, we expected, adding E₂ to CC leads to higher levels of LH in HCG injection day. but in our study, LH levels in TAX treated cycle was higher than the other groups and the cause of higher rates of pregnancy with TAX in present study, is may be due to higher levels of LH levels prior to HCG injection.

Costello *et al.* (1998) reported that influence of LH surge prior to HCG injection on the pregnancy rate, showed that occurrence of LH surge prior to HCG injection, increases pregnancy rate and decreases miscarriage (Annapurna, 1997). In this study, there was no significant difference in miscarriage rate among any of the treatment groups.

According to present results, it is suggested that difference between CC and TAX in pregnancy rate, is not related to their difference in endometrial thickness and adding E₂ to CC does not increase pregnancy rate when compared with CC alone and it can also leads to lower pregnancy rate with change in endometrial quality and luteal phase defect.

It is also suggested that follicular growth rate is higher in CC plus E₂.

Elkind-Hirsch *et al.* (2005) found that the occurrence of LH surge in patients who received estrogen, happened earlier. Moreover, this is a critical point that can be helpful to increase follicular growth rate in resistant patients and patients with slow follicular growth.

CONCLUSION

According to present results, it is believed that pregnancy rate with TAX is higher than CC and adding E₂ to CC does not increase endometrial receptivity. Higher pregnancy rate with TAX is may be due to higher increasing of LH levels in TAX treated cycles which probably leads to increasing corpus luteum quality and pregnancy rate. Due to several controversies, it is important to evaluate the effect of LH levels and endometrial thickness in ovulation induction, independently in order to clarify whether endometrial thickness is effective or LH levels.

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