Article

Ovulation induction with tamoxifen and alternate-day gonadotrophin in patients with thin endometrium

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Abstract

Tamoxifen has been reported to be oestrogenic on the lower genital tract. To evaluate its potential positive effect on the endometrium, and consequently early miscarriage and ongoing pregnancy rate, a prospective study was employed in patients for intrauterine insemination who failed to develop an adequate endometrial thickness in a previous ovulatory cycle. Ovarian stimulation was initiated with tamoxifen 40 mg/day from day 3 of the menstrual cycle for 7 days or clomiphene 100 mg/day for 5 days, in combination with 150 IU of human menopausal gonadotrophin on alternate days starting on day 4. Human chorionic gonadotrophin (HCG) was administered when at least one leading follicle was larger than 20 mm. Intrauterine insemination was accomplished 24–36 h after HCG injection and luteal phase supplement was achieved with micronized progesterone 200 mg transvaginally per day. It was found that tamoxifen-treated patients required more stimulation days and used more gonadotrophin, but recruited less follicles larger than 14 mm than clomiphene-treated patients. However, a significantly increased endometrial thickness ($P < 0.001$) and pregnancy rate ($P = 0.015$), decreased early miscarriage rate ($P = 0.001$) and thus improved ongoing pregnancy ($P < 0.001$) rate were noted in tamoxifen-treated patients. These results suggest that although tamoxifen may not be a first-line treatment in patients with adequate endometrium, it may be a promising alternative for patients with thin endometrium.

Keywords: *clomiphene, tamoxifen, thin endometrium, ovulation induction, pregnancy*

Introduction

Clomiphene citrate (CC) has been used in the treatment of anovulatory infertility since its introduction in 1956 (Greenblatt *et al*., 1961). By depleting the oestrogen receptors, CC acts as an anti-oestrogen on the central nervous system. This increases the pulse frequency of FSH and LH, giving a moderate gonadotrophin stimulus to the ovary, and thus overcoming ovulatory disturbances and increasing the number of follicles reaching ovulation (Adashi, 1984; Dickey and Holtkamp, 1996). Over the years, evidence has accumulated indicating that CC is successful at inducing ovulation in 50–75% of patients, but the pregnancy rate achieved after ovulation induction is much lower than expected (Drake *et al*., 1978; Gysler *et al*., 1982; Wu and Winkel, 1989). The discrepancy has been attributed to CC's prolonged peripheral anti-oestrogenic effects on cervical mucus and the endometrium (Gonen and Casper, 1990; Massai *et al*., 1993; Sereepapong *et al*., 2000). Particularly, its effect on the endometrium may explain a larger part of the lower pregnancy rate in assisted reproduction cycles (Hammond *et al*., 1983; Thatcher *et al*., 1988; Gonen and Casper, 1990). Moreover, Hsu *et al*. (1995) demonstrated that CC also interferes with uterine blood flow. Nevertheless, in order to decrease the gonadotrophin dose required for optimal stimulation, co-treatment of CC

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with gonadotrophin therapy has already been an increasingly utilized method of ovulation induction for patients in whom CC treatment was unsuccessful (Dickey *et al.*, 1993a). For these reasons, a simple, inexpensive and safe alternative of CC to be used in combination with gonadotrophin in ovulation induction may be required.

Clomiphene citrate and tamoxifen citrate (TMX) are both non-steroid selective oestrogen receptor modulators. CC is part of the triphenylethylene family of compounds. It has two isomeric forms, *cis* and *trans*, which in the current nomenclature correspond to zuclomiphene and enclomiphene respectively (Sovino *et al.*, 2002). The action of zuclomiphene is mainly anti-oestrogenic, whereas enclomiphene has oestrogenic effects. TMX is also a triphenylethylene that closely resembles CC. In addition to their structural homology, both agents had been shown to be effective for ovulation induction. Klopper and Hall (1971) were the first to describe successful results with TMX for the induction of ovulation in women suffering from secondary amenorrhoea. Various researchers have described the value of TMX in cases of corpus luteum insufficiency (Fukushima *et al.*, 1982) and in conditions of inadequate cervical mucus secretion (Roumen *et al.*, 1984). However, only a few clinical trials with TMX for the induction of ovulation have been reported (Gerhard and Runnebaum, 1979; Ruiz-Velasco *et al.*, 1979; Messinis and Nillius, 1982; Boostanfar *et al.*, 2001). These reports demonstrated that the overall ovulation and pregnancy rates were similar in both groups (Steiner *et al.*, 2005). Other studies have suggested that TMX may be superior to CC in that it does not appear to have an adverse effect on the endometrium (Deligdisch, 2000). The increased oestrogenic stimulation that has been observed with tamoxifen's action on the lower genital tract may be beneficial, especially for those suffering from an adverse response following the administration of CC.

It was postulated that, by administration of TMX, it might be possible to mimic the action of CC in stimulation of ovarian follicles but to avoid the adverse effects of CC on the endometrium. In the present study, a prospective non-randomized trial was employed in a selected group of patients for intrauterine insemination (IUI) who failed to develop an adequate endometrial thickness during follicular monitoring in a previous ovulatory cycle. The aim was to evaluate the efficacy of treatment with TMX or CC in combination with alternate-day human menopausal gonadotrophin (HMG) in these patients. The potential positive effect of TMX on the endometrium, and consequently early miscarriage and ongoing pregnancy rate, was investigated in these patients.

Materials and methods

Patient selection

The study was performed between January 2002 and December 2006. All patients with infertility for at least 1 year completed a standard infertility investigation. In addition to a thorough medical history and physical examination, pelvic ultrasonography was performed to detect anatomical uterine or adnexal abnormalities. Patients with any uterine or adnexal pathology were excluded. A hysterosalpingogram was performed to verify tubal patency and patients with abnormal hysterosalpingograms were excluded from the study. A semen analysis was performed on all male partners and the World Health Organization (1999) criteria were used to confirm normality. According to previously published data, which showed a significantly decreased pregnancy rate in IUI cycles (Huang *et al.*, 1996), male partners with a total motile sperm count of less than 5×10^6, ml were excluded. Other exclusion criteria included female age over 38 years, presence of polycystic ovarian syndrome (diagnosis based on the presence of two out of three according to the Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004): oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries), previous history of adnexal or uterine surgery, or any other contraindication to ovulation induction. All the study couples had undergone at least one to three cycles of follicular monitoring with timed intercourse before undergoing ovulation induction and IUI. All those patients who failed to develop an endometrial thickness of at least 8 mm during follicular monitoring were recruited into this study. They were counselled regarding the novel use of TMX to avoid the adverse effect on the endometrium. Patients were fully informed regarding the indication, rational, mechanism of action and the possible side effects of the drugs. They were not randomized and the choice of receiving TMX or not was left to the patient. All couples underwent a maximum of three cycles of IUI treatment.

Standard ovulation stimulation and cycle monitoring protocol

Under patients' informed consent, all patients enrolled in this study were allocated into two treatment groups. After a spontaneous or progesterone-induced episode of withdrawal bleeding, all patients underwent baseline ultrasonography to confirm the absence of ovarian cysts before the drugs were given. Ovarian stimulation was performed with TMX (Nolvadex, Zeneca Pharmaceuticals, Wilmington, DE, USA) 40 mg per day from day 3 of the menstrual cycle for 7 days (Group A), or CC (Clomid; Merrell Pharmaceuticals Inc., Kansas City, KS, USA) 100 mg per day from day 3 for 5 days (Group B), in combination with 150 IU of HMG (Pergonal; Serono, Geneva, Switzerland) on cycle days 4, 6, 8 and 10 for four doses. Transvaginal ultrasonography was performed on days 11 or 12.

At each ultrasonographic scan, the internal diameter of each visible follicle was measured in two planes and the average diameter was calculated. In addition, the endometrial thickness, defined as the maximum distance between the echogenic interfaces of the myometrium, was measured in the mid-sagittal plane from the outer edge of the endometrial–myometrial interface to the outer edge in the widest part of the endometrium. The sonographers involved in this trial were blind to the kind of treatment.

Depending on the size of the recruited follicles, all patients either stopped stimulation and received a single dose of 10,000 IU human chorionic gonadotrophin (HCG; Pregnyl; NY Organon, Oss, The Netherlands) injection when at least one leading follicle was greater than 20 mm, or continued HMG injection on alternate days until at least one dominant follicle was greater than 20 mm.

IUI was performed 24–36 h after HCG injection. All patients received luteal phase support with 200 mg of vaginally administered micronized progesterone (Utrogestan; Laboratoires Piette International S.A., Brussels, Belgium) daily starting from the day following insemination for at least 14 days.

Three patients in Group A and two in Group B were excluded from the study due to poor response to follicular stimulation. There were 61 patients in Group A (corresponding to 81 cycles) and 70 in Group B (corresponding to 82 cycles) that completed the study and were allowed for further analysis.

Definition of pregnancy

The pregnancy rate was calculated based on a positive urine pregnancy test or a serum βHCG concentrations greater than 50 mIU, ml. Any pregnancy that resulted in a loss (spontaneous abortion, missed abortion, ectopic pregnancy, etc.) was considered to be an abnormal pregnancy. Ongoing pregnancies were gestations that reached 20 weeks or more.

Statistical analysis

Statistical analysis was carried out using Statistics Package for Social Sciences software (SPSS Inc., Chicago, IL, USA). Student's *t*-test, chi-squared test and Fisher's exact test were used as appropriate. A *P*-value of less than 0.05 was considered statistically significant.

Results

Background patient characteristics

The two groups had similar demographic characteristics, in terms of mean age of the female patients, duration of infertility, infertility status (primary or secondary) and the percentage of various diagnostic aetiological categories including ovulatory dysfunction, male factor, endometriosis, or unexplained infertility (**Table 1**).

Cycle characteristics

The cycle characteristics of follicular stimulation in the two treatment groups are shown in **Table 2**. There were no significant differences between the groups in sperm concentration, sperm motility and the percentage of sperm with normal morphology per insemination. CC in combination with alternate-day HMG resulted in a decrease in the duration of stimulation (12.49 ± 1.22 versus 13.49 ± 1.79 days) and amount of HMG ampoules (9.03 ± 1.61 versus 9.85 ± 1.78) required to achieve follicular maturation. The number of follicles that were 14 mm or larger on the day of HCG administration was significantly higher in the CC-treated group (4.2 ± 1.5 versus 3.2 ± 1.3) (all P < 0.001).

Endometrial thickness

The endometrial thickness was estimated on the day of HCG administration. There was a significant increase in endometrial thickness (6.7 ± 1.3 and 10.8 ± 2.3 mm) ($P < 0.001$) in TMX-treated patients. Only four cycles in this group (4/81, 4.9%), in contrast to most cycles in CC-treated group (71/82, 86.6%), failed to develop an endometrium thicker than 8 mm ($P < 0.001$).

Pregnancy outcome

The difference in the pregnancy rates per treatment cycle between Group A (26/81, 32.1%) and Group B (13/82, 15.9%) was statistically significant ($P = 0.015$). As for miscarriage, significantly more early pregnancy losses occurred in Group B (8/13, 61.5%) than in Group A (2/26, 7.7%) ($P = 0.001$).

Table 1. Characteristics of patients undergoing ovulation induction and intrauterine insemination.

Variables	*Group A (TMX)*	*Group B (CC)*
No. of cycles	81	82
Age (years)[a,b]	31.6 ± 3.23	31.1 ± 4.01
Years of infertility[a,b]	3.57 ± 2.45	2.95 ± 1.94
Infertility status[c]		
Primary	52 (64.2)	47 (57.3)
Secondary	29 (35.8)	35 (42.7)
Causes of infertility[b]		
Ovulatory	15 (18.5)	9 (11.0)
Male factor	10 (12.3)	12 (14.6)
Endometriosis	11 (13.6)	10 (12.2)
Unexplained	45 (55.6)	51 (62.2)

Values are number (percentage) unless otherwise stated. There were no statistically significant differences between the two groups; CC = clomiphene citrate; TMX = tamoxifen.
[a]Values are mean ± SD.
[b,c]Student's *t*-test or chi-squared test respectively.

Table 2. Comparison of cycles treated with tamoxifen (TMX) or clomiphene citrate (CC) protocols.

Variables	*Group A (TMX)*	*Group B (CC)*	P-*value*
No. of cycles[a]	81	82	
HMG ampoules[a]	9.85 ± 1.78	9.03 ± 1.61	0.007
Days of HCG injection[a]	13.49 ± 1.79	12.49 ± 1.22	0.002
Endometrial thickness[a] (mm)	10.8 ± 2.3	6.7 ± 1.3	<0.001
No. of endometria <8 mm on HCG day[b] (%)	4 (4.9)	71 (86.6)	<0.001
No. of follicles ≥14 mm on HCG day[a]	3.2 ± 1.3	4.2 ± 1.5	<0.001
Sperm parameters			
Sperm concentration (10^6, ml)[a]	66.6 ± 52.0	58.8 ± 38.1	NS
Motility (%)[a]	60.0 ± 18.2	62.8 ± 17.5	NS
Normal morphology (%)[a]	61.0 ± 16.1	64.0 ± 11.7	NS
No. of pregnancies per cycle (%)[a]	26/81 (32.1)	13/82 (15.9)	0.015
No. of early pregnancy loss (%)[c]	2/26 (7.7)	8/13 (61.5)	0.001
No. of ectopic pregnancies (%)[c]	1/26 (3.8)	0 (0)	NS
No. of ongoing pregnancies (%)[c]	23/81 (28.4)	5/82 (6.1)	<0.001

Values are mean ± SD unless otherwise stated; HCG = human chorionic gonadotrophin; HMG = human menopausal gonadotrophin; NS = not statistically significant.
[a,b,c]Student's *t*-test, chi-squared and Fisher's exact tests respectively.

The ongoing pregnancy rate was also significantly higher in the TMX-treated group than in the clomiphene-treated group (23/81, 28.4% versus 5/82, 6.1%; $P < 0.001$).

Discussion

An association of various cycle characteristics and treatment outcome has been evaluated since the introduction of assisted reproduction techniques. Endometrial thickness is one of the key factors in determining the likelihood of success in assisted reproduction (Check *et al.*, 1991, 1993; Dickey *et al.*, 1992; Noyes *et al.*, 1995; Rinaldi *et al.*, 1996; Yuval *et al.*, 1999; De Geyter *et al.*, 2000; Bassil, 2001; Schild *et al.*, 2001). Possibly because of the multiple confounding factors involved in assisted reproduction, conflicting reports exist concerning the possible relationship between endometrial thickness and treatment outcome (Check *et al.*, 1991; Noyes *et al.*, 1995; De Geyter *et al.*, 2000; Zhang *et al.*, 2005). Although the establishment of a successful pregnancy with a thin endometrium (no more than 4 mm) has been reported (Sundstrom, 1998), a minimal endometrial thickness of 8 mm or more as prerequisite for implantation, although controversial, is widely accepted (Basir *et al.*, 2002; McWilliams and Frattarelli, 2007). Ultrasound observation of the endometrium prior to HCG administration in IUI cycles has provided important clues as to why conception fails to occur in spite of good follicular development in some clomiphene cycles. Furthermore, preclinical (biochemical) abortions markedly increase in patients whose endometrial thickness was less than 8 mm on the day of HCG administration (Dickey *et al.*, 1993b,c).

Previously recommended methods of increasing endometrial thickness on the day of HCG administration include starting clomiphene earlier in the cycle (Wu and Winkel, 1989), adding oestrogen concomitantly during CC treatment (Yagel *et al.*, 1992; Gerli *et al.*, 2000), low-dose aspirin supplements (Weckstein *et al.*, 1997; Check *et al.*, 1998), use of an aromatase inhibitor such as letrozole (Mitwally and Casper, 2001; Healey *et al.*, 2003), use of sildenafil to increase endometrial blood flow (Sher and Fisch, 2000) and delaying the administration of HCG (Dickey *et al.*, 1993c). However, the real value of these managements remains controversial and thus warrants further investigation. Moreover, Novartis Pharmaceuticals has formally advised healthcare professionals not to use letrozole (Femara; Novartis Pharmaceuticals, East Hanover, NJ, USA) in premenopausal women, specifically for ovulation induction in the treatment of infertility (Fontana and Leclerc, 2005). A recent study compared the efficacy of tamoxifen with that of clomiphene citrate for ovulation induction in anovulatory women (Boostanfar *et al.*, 2001). The overall ovulation and pregnancy rates were similar in both groups. The fact that tamoxifen may be superior to clomiphene citrate because it does not appear to have an adverse effect on the endometrium (Deligdisch, 2000) has not been tested in the subgroup of patients with thin endometrium, who may most benefit from TMX instead of CC as an ovulation induction agent.

In this study, a prospective, non-randomized trial was undertaken to compare the efficacy of concomitant treatment with TMX or CC in combination with alternate-day HMG in patients who failed to develop an adequate endometrial thickness in a previous ovulation induction cycle. We exploited TMX's dual action as an ovarian stimulating agent and an oestrogenic stimulation effect on the endometrium for these patients. Although previous studies (Gerhard and Runnebaum, 1979; Ruiz-Velasco *et al.*, 1979; Messinis and Nillius, 1982; Boostanfar *et al.*, 2001; Steiner *et al.*, 2005) revealed that ovulation and pregnancy rate are both comparable when low, medium and high doses of TMX (20, 40 and 60 mg) and CC (50, 100, 150 mg) are used

in anovulatory patients, no studies had evaluated the efficacy of either TMX or CC for ovulation induction (Tourgeman, 2003). In this study, treatment with CC in combination with alternate-day HMG resulted in a decrease in days and amount of gonadotrophin required to achieve follicular maturation. The number of follicles that were 14 mm or larger on the day of HCG administration was also significantly higher in the CC group ($P < 0.001$). This signified that, although comparable in ovulation induction, CC might be more effective than TMX as an adjunct to alternate-day HMG in ovulation induction cycles. Nevertheless, the differences are small and may have only marginal clinical significance. However, there was a significant increase in endometrial thickness in TMX-treated patients ($P < 0.001$). As previous reports failed to demonstrate a statistically significant relationship between days of ovarian stimulation and thickness of the endometrium (Zhang *et al.*, 2005), the increase in endometrial thickness in this study may be attributable to the fact that TMX therapy improves endometrial thickness directly from its oestrogenic effect on the endometrium and not from more stimulated follicles 14 mm or larger and consequently higher serum oestradiol concentration.

It is known that it is a huge error to assume that good follicle development is related with a genetically normal egg, so the pregnancy outcome between the two groups was also compared, including early miscarriage and ongoing pregnancy rate, which is most important for a successful infertility treatment. There are only limited and conflicting data concerning spontaneous abortion and the use of TMX. Recently, Wu (1997) reported lower miscarriage rates in patients with luteal-phase dysfunction treated with TMX as compared with CC treatment. Whereas, Ruiz-Velasco *et al.* (1979) reported a higher spontaneous abortion rate in their cohort of TMX-treated patients. In the current study, only those patients with a past history of thin endometrium were recruited. There were eight (8/13, 61.5%) early pregnancy losses in the CC-treated patients but only two (2/26, 7.7%) in the TMX group. The finding was consistent with previous studies that showed biochemical pregnancies were more frequent with thinner endometrium (Dickey *et al.*, 1992). It was speculated that the significant discrepancy between studies might be attributed to an improved endometrial environment for embryo implantation, which resulted from the different treatment modalities. In this subgroup of patients, TMX could effectively increase mean endometrial thickness, as in this study, and improve luteal-phase dysfunction, as in the study by Ruiz-Velasco *et al.* (1979). Consequently, early pregnancy losses could be decreased. Only four cycles in the TMX group (4.9%), in contrast to most cycles in 4/81 CC group (71/82, 86.6%), failed to develop an endometrium thicker than 8 mm. In addition to the difference in pregnancy rate per treatment cycle, there were much more miscarriages in CC-treated patients and thus considerably higher ongoing pregnancy rate in TMX-treated group.

It should be noted that this was not a randomized study. The choice of receiving TMX or CC was left to the patients. Although analysis of the patients' characteristics revealed no significant difference among the two treatment groups (**Table 1**), the possibility of selection bias and its consequent influence on the results does exist. Another drawback of this clinical trial is the lack of serum oestradiol monitoring during ovarian stimulation. As the initial site and mode of action of TMX, like CC, involve occupying oestradiol-binding sites on the hypothalamic–pituitary axis and preventing the negative feedback effect of oestradiol, the endocrine profiles of TMX-induced ovulatory cycles is analogous to that of CC (Tajima and Fukushima, 1983). Although there is extensive literature regarding methods for monitoring ovarian response during ovarian stimulation, previous studies have demonstrated that ultrasound imaging of the follicle and endometrial growth is sufficient for the majority of cases (Lass, 2003) and measurement of serum oestradiol is mostly used to avoid complications, particularly ovarian hyperstimulation syndrome (Wramsby *et al.*, 1987). The endocrine profiles were not routinely measured in our IUI patients as the ovulation protocol in this study was simplified and employed only low-dose alternate-day gonadotrophin in patients with normal ovarian reserve.

Regarding the safety of TMX use in women attempting pregnancy, concerns have been raised mainly on animal studies (Sadek and Bell, 1996; Halakivi-Clarke *et al.*, 2000) and a few case reports (Cullins *et al.*, 1994; Tewari *et al.*, 1997). A cause–effect relationship cannot be established. There has been one published case report of a male fetus exposed to tamoxifen during an entire gestation (Isaacs *et al.*, 2001). Even though the birth was premature, no congenital anomalies were noted. Moreover, when used for the purpose of ovarian stimulation, the drug is discontinued prior to ovulation or oocyte retrieval. In this study, no untoward adverse effect on the endometrium occurred with TMX treatment. Nevertheless, the possibility of an increased risk of genital cancer, especially endometrial cancer, following exposure to CC and TMX (Brinton, 2007), and also the relationship with adenomyosis in mouse models following TMX (Bayar *et al.*, 2006), still warrants further investigation.

As far as is known, this is the first prospective study comparing the effects of administering TMX or CC in combination with alternate-day HMG for ovarian stimulation in a subgroup of patients with prior documented thin endometrium. In the current trial, TMX-treated patients required longer stimulation and used more gonadotrophin, but recruited less mature follicles than CC-treated patients. These results suggest that TMX may not be a first-line treatment for ovulation induction cycles in patients with adequate endometrium. Nevertheless, this study has demonstrated a significantly increased endometrial thickness ($P < 0.001$) and pregnancy rate ($P = 0.015$), decreased early miscarriage ($P = 0.001$) and thus improved ongoing pregnancy rate ($P < 0.001$) in TMX-treated patients. These findings suggest that TMX is a promising alternative to CC for ovarian stimulation in the subgroup of patients who failed to develop an adequate endometrial thickness in a previous ovulation induction cycle.

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