

A Prospective, Randomized, Comparative Clinical Study of the efficacy of Letrozole and Clomiphene Citrate as adjuvants to Follicle-Stimulating hormone in Superovulation

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Abstract

Objective: To compare the efficacy of the aromatase inhibitor letrozole and clomiphene citrate (CC) as adjuvants to follicle-stimulating hormone (FSH) in superovulation.

Materials & Methods: A total of 120 patients with unexplained or mild male factor infertility were randomized to receive either letrozole or CC as adjuvants to FSH. From day 3 to 7 of the cycle 2.5 mg/d letrozole or 100 mg/d CC were administered followed by 75 IU/d FSH starting on day 7 until the day of human chorionic gonadotropin (hCG). Ovulation was triggered with 10,000 IU of hCG when the leading follicle (s) reached 18 mm in diameter. A single intrauterine insemination (IUI) was performed 36 h later. Ovarian (estradiol (E2) levels and number of follicles) and endometrial (endometrial thickness) response and pregnancy outcome were the main measures.

Results: The number of mature preovulatory follicles (1.9 ± 0.7 vs. 2.2 ± 0.5 , $P < 0.0001$) and the peak E2 level (791 ± 163 vs. 1137 ± 192 pg/ml, $P < 0.0001$) were significantly higher in the CC group than in the letrozole group. A significantly higher endometrial thickness was observed on the day of hCG in the letrozole group (9.1 ± 1.2 vs. 7.7 ± 1.4 mm, $P < 0.0001$). There was no significant difference between groups in clinical pregnancy rates (38.4% in the letrozole and 31.3% in the CC groups).

Conclusions: The aromatase inhibitor letrozole appears to be a good alternative to CC in superovulation therapy.

Key Words: Aromatase inhibitor, letrozole, clomiphene citrate, FSH, superovulation.

Introduction

Typically, patients with unexplained or mild male factor infertility are offered superovulation combined with intrauterine insemination (IUI) as first-line therapy to enhance fecundity(1-3).

The effectiveness of clomiphene citrate (CC), a non-steroidal agent that has both estrogenic and antiestrogenic effects, in inducing ovulation is well established(4). In anovulatory women, the use of CC is widely accepted as the first-line therapy because of its low cost and easy administration(5). Its use is associated with a high ovulation rate of 60% - 80%, but with a lower pregnancy rate of about 50%(4,5). However, several disadvantages have been described, including potential negative effects on the cervical mucus(5,6), uterine blood flow(7), the endometrium(8), embryo development(9), and overall pregnancy outcome(4,5,10).

Gonadotropin preparations, either urinary or recombinant, have been used to stimulate ovulation in women who are resistant to CC as well as to stimulate the production of several mature follicles in conjunction with assisted reproduction technologies(11). Unfortunately, multiple gestation, ovarian hyperstimulation syndrome (OHSS), inconvenience, and higher treatment cost are drawbacks of these compounds(12).

Recently, it has been suggested that a new group of highly selective aromatase inhibitors, including letrozole; that suppress estrogen biosynthesis may successfully substitute for CC in superovulation regimens(13-15). Aromatase inhibitors have a reduced half-life (about 2 days) compared with CC (>2 weeks) which exerts a central estrogen-depletion effect of long duration(16). Letrozole increases endogenous gonadotropin secretion, but unlike CC, it does not lead to estrogen receptor (ER) depletion(13,14). It could therefore decrease the requirement for gonadotropins without adverse effects on peripheral tissues such as the endometrium(17,18).

The objective of the present study was to compare the efficacies of the aromatase inhibitor letrozole and CC as adjuvants to gonadotropin stimulation in superovulation combined with IUI therapy in couples with unexplained or mild male factor infertility.

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Material and methods

This prospective randomized clinical study included couples with unexplained or mild male factor infertility treated between June 2002 and May 2005 in the Department of Obstetrics and Gynecology, Menofiya University and its outpatient clinic. All patients underwent standard infertility investigations including early follicular phase transvaginal ultrasonography (TV/US) and measurement of serum FSH, TSH, and prolactin, confirmation of tubal patency by hysterosalpingography and pelvis normality by laparoscopy and semen analysis(19).

Inclusion criteria were female age <35y, >1 year infertility, patent fallopian tubes, a normal uterine cavity, a basal day 3 serum FSH level <12 mIU/mL, and the presence of at least 10 million rapidly motile sperm/mL (mild male factor). The local Ethics Committee approved the study. Patients were counseled individually about the study and protocols by a resident coordinator. Patients who elected to participate gave their written informed consent before participation.

All couples underwent a maximum of 3 cycles of treatment. Patients were randomized using a computer-generated random table into 2 groups:

1. The letrozole group (59 patients, 147 cycles) who received 2.5 mg/day letrozole (Femara, Novartis) from days 3 to 7 of the cycle.
2. The CC group (61 patients, 159 cycles) who received 100mg/day CC (Clomid, Serono) from days 3 to 7 of the cycle. All physicians were blinded to allocation.

Protocol

After either letrozole or CC, all patients received 75 IU/day FSH (Fostimon, IBSA) starting on day 7 (sequential manner) until the day of hCG administration. All patients underwent baseline TV/US in the early follicular phase to confirm absence of ovarian cysts which were defined as any sonolucent structure with a mean diameter > 15 mm(20).

Subsequent US scans were performed on day 9 of the cycle and then daily after the mean diameter of the largest follicle reached 16 mm. At each US 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 endometrial-myometrial junctions, was measured in the plane through the central longitudinal axis of the uterus(21). Ovulation was triggered with 10,000 IU of hCG (Choriomon, IBSA) when the leading follicle(s) reached 18 mm in diameter. Serum estradiol (E2) levels were measured on day 3 and the day of hCG administration.

A single IUI was performed 36 h after hCG administration by using a Labotect ET catheter (Labotect GmbH, Göttingen). Semen has been processed by wash/centrifugation in Earle's salt solution (Biochrom AG, Berlin) and suspensions of motile spermatozoa were prepared in a final volume of 0.5 mL. The luteal phase was supplemented with vaginal micronized progesterone (400mg/day, Prontogest pessaries, IBSA). Serum β -hCG was measured 14 days later. A pregnancy was established by visualization of a gestational sac by 6 weeks gestation using TV/US.

Statistical Analysis

For statistical analysis, a commercially available statistical package SPSS version 13 (SPSS, Chicago, IL) was used. We evaluated the total number and size of the follicles, endometrial thickness and type, the number of gonadotropin ampoules and the dose,

mean E2 level, pregnancy rate (chemical and clinical), and miscarriage rate. When the assumption of normality was met, mean differences between the two groups were analysed using a Student's t-test. To evaluate differences between proportions (e.g., pregnancy rates) a X2 test was used. Results are expressed as mean + SD unless otherwise indicated. P values below 0.05 were considered as statistically significant

Results

A total of 120 infertile couples (59 in letrozole and 61 in CC groups) completed the treatment cycles (147 in letrozole and 159 in CC groups). Demographic characteristics showed no significant differences between groups (Table 1). The number of follicles >10 mm on cycle day 9 and the number of follicles >16 mm on day of hCG were significantly higher in the CC group than in the letrozole group (Table 2). The total dose of FSH and the peak E2 level (on the day of hCG) were significantly higher in the CC group than in the letrozole group. The day of hCG was significantly earlier and, the endometrial thickness was significantly higher in the letrozole group than in the CC group (Table 2). There was no significant difference between groups in pregnancy rates (38.4% in the letrozole and 31.3% in the CC groups). In contrast, the abortion rate was significantly higher in the CC group than in the letrozole group (Table 2).

Table (1): Demographic characteristics of patients treated with letrozole + FSH and CC + FSH

Variable	Letrozole + FSH (n=59)	CC + FSH (n=61)	P-Value
Age (ys)	29.7 + 3.5	28.5 + 3.7	0.14+
Weight (Kg)	67.5 + 5.2	68.2 + 6.1	0.58+
Height (m)	1.64 + 0.3	1.61 + 0.4	0.71+
BMI (Kg/m2)	26.4 + 3.1	27.3 + 4.1	0.27+
Duration of infertility (ys)	3.6 + 0.6	3.4 + 0.7	0.18+
Day 3 FSH (mIU/mL)	6.8 + 1.7	7.2 + 1.5	0.27+
Day 3 E2 (pg/mL)	51.3 + 3.9	49.7 + 4.2	0.08+
Unexplained infertility (n)	48	52	0.63+
Mild male factor infertility (n)	11	9	

+ = Not significant BMI= Body mass index

Table (2): Ovarian-endometrial response and pregnancy outcome

Variable	Letrozole + FSH (n=147)	CC + FSH (n=159)	P-Value
No. of follicles >10 mm on cycle day 9	2.7 + 1.2	3.1 + 1.4	0.008**
No. of follicles >16 mm on day of hCG	1.9 + 0.7	2.2 + 0.5	<0.0001**
Total dose of FSH (IU)	357 + 51	371 + 46	0.01*
Peak E2 on day of hCG (pg/mL)	791 + 163	1137 + 192	<0.0001**
Day of hCG	11.8 + 0.9	12.2 + 0.7	<0.0001**
Endometrial thickness on day of hCG (mm)	9.1 + 1.2	7.7 + 1.4	<0.0001**
Chemical preg- nancy rate per cycle	29/147 (19.7%)	27/159 (17%)	0.56+
Chemical preg- nancy rate per couple	29/59 (49.2%)	27/61 (44.3%)	0.7+
Clinical preg- nancy rate per cycle	23/147 (15.6%)	19/159 (11.9%)	0.4+
Cumulative clini- cal pregnancy rate per couple	23/59 (38.4%)	19/61 (31.3%)	0.44+
Abortion rate	3/23 (13%)	8/19 (42.1%)	0.04*
Multiple preg- nancy	1/23 (4%)	3/19 (15.8%)	0.3+

+ = Not significant * = Significant ** = Highly significant

Discussion

Superovulation combined with IUI has been used to enhance fecundity for couples with unexplained or mild male factor infertility(1-3). Because of its antiestrogenic action, the use of CC has been associated with adverse effects mainly on the quality of the cervical mucus and on the endometrial development(22). Higher doses and prolonged use of CC may also aggravate these effects(23,24). Nevertheless, gonadotropin therapy is associated with a significant cost, inconvenience, and discomfort to the patient due to the need for injections, higher risk for multiple pregnancy and increased risk of OHSS(25). Recently, aromatase inhibitors have been used in ovarian stimulation protocols(13,14,26). By blocking the conversion of androgens to estrogens (E), they eliminate rapidly the circulating E2, thus releasing the hypothalamic/pituitary axis from negative feedback. As a result, there is an increase in the production and release of FSH, which is readily available to stimulate follicular growth. Furthermore, acute E withdrawal leads to increased peripheral activin production which further stimulates pituitary FSH production and secretion(27). Androgen accumulation within the follicular microenvironment up-regulates FSH receptor expression(28) as well as ovarian insulin-like growth factor 1 (IGF-1) levels, both of which act synergistically to augment FSH action and promote follicular development(29,30).

In our study, the mean number of mature follicles (>16mm) on the day of hCG was significantly higher in the CC group than in

the letrozole group. This is in agreement with Mitwally and associates (2005)(18) who found CC treatment to be consistently associated with development of more ovarian follicles than with letrozole and Fatemi et al.(31) in their pilot study which demonstrated lower E levels and fewer follicles in the letrozole group. In contrast, Barroso et al. (2006)(32) found similar number of mature follicles on day of hCG in CC and letrozole groups.

The total dose as well as the cost of FSH required was significantly higher and the day of hCG was significantly later in the CC group than in the letrozole group. In a prospective nonrandomized study, Mitwally and Casper(17) compared letrozole +FSH, CC+FSH, and FSH alone in women with unexplained infertility undergoing superovulation and IUI. The authors concluded that similar to CC, letrozole reduced the required FSH dose for superovulation without the undesirable antiestrogenic effects sometimes observed with CC. The same authors compared letrozole +FSH and FSH alone and demonstrated improved ovarian response in the combination group as evidenced by a reduced dose of gonadotropins as well as a higher number of mature follicles(14).

The estrogen levels in women on aromatase inhibitors were found to be 2-3 times lower than those reported in CC cycles, however, endometrial thickness was greater in the aromatase inhibitor cycles(33). In our study, despite significantly lower E2 levels in the letrozole group, endometrial thickness was significantly higher in the letrozole group than in the CC group. This is in accord with the findings of Fisher et al.(34) who compared the effects of CC and letrozole on normal ovulatory women and the findings of Mitwally and Casper(17) who compared letrozole +FSH, CC + FSH, and FSH alone in women with unexplained infertility. Other investigators reported similar endometrial thickness in women treated with letrozole or CC(35).

In numerous studies, the endometrial thickness after ovarian stimulation has been correlated to the chance of conception(36,37). Most investigators agree that an endometrial thickness of at least 6 mm is necessary for successful implantation(36). In contrast to CC, aromatase inhibitors do not bind to ERs, therefore their use is not associated with ER depletion. Furthermore, it has been shown that elimination of E2 from the circulation leads to up-regulation of ERs in the endometrium(38). It has been speculated that E deprivation during the use of aromatase inhibitors may lead to a subsequent increase in endometrial sensitivity to E, which accelerates the endometrial proliferation and development and improves blood flow(39). In support to this notion, Cortinez et al.(40) concluded that letrozole induced high midluteal progesterone, leading to both a normal endometrial histology and development of pinopodes, considered to be relevant markers of endometrial receptivity.

We couldn't detect any significant difference in pregnancy rate per cycle as well as in the cumulative pregnancy rate per couple between letrozole and CC groups. This is consistent with the findings of Barroso et al.(32) and Al-Fozan et al.(35). However, the miscarriage rate in our study was significantly higher in the CC group than in the letrozole group. This is in accord with the findings of several investigators(35,41) and may have been due to the different mechanisms of action of letrozole and CC(35). We postulate that this might be due to the relatively short half-life of letrozole, which allowed complete endometrial recovery before implantation.

In conclusion, the present study provided further evidence for beneficial effects of the use of the aromatase inhibitor letrozole in combination with FSH in couples with unexplained and mild male factor infertility undergoing superovulation/IUI therapy. Benefits observed in this study were the achievement of a lower number of mature follicles, a lower dose of FSH required, a higher endometrial thickness as well as a similar pregnancy rate and a lower miscarriage rate compared with CC plus FSH. However, further prospective and randomized studies are needed to establish a potential beneficial effect on pregnancy outcome.

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