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Aromatase inhibition reduces gonadotrophin dose required for controlled ovarian stimulation in women with unexplained infertility

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BACKGROUND: Adding clomiphene citrate (CC) to FSH for controlled ovarian stimulation (COS) decreases FSH dose required for optimum stimulation. However, because of its anti-estrogenic effects, CC may be associated with lower pregnancy rates offsetting the FSH-dose reduction benefit. Previously, we reported the success of aromatase inhibition in inducing ovulation without antiestrogenic effects. METHODS: A prospective pilot study that included women with unexplained infertility undergoing COS and intrauterine insemination. Thirty-six women received the aromatase inhibitor letrozole + FSH, 18 women received CC + FSH and 56 women received FSH only. Each woman received one treatment regimen in one treatment cycle. All patients were given recombinant or highly purified FSH (50–150 IU/day) starting on day 3 to 7 until day of hCG. RESULTS: The FSH dose needed was significantly lower in letrozole + FSH and CC + FSH groups compared with FSH-only without a difference in number of follicles >1.8 cm. Pregnancy rate was 19.1% in the letrozole + FSH group, 10.5% in the CC + FSH group and 18.7% in the FSH-only group. Both pregnancy rate and endometrial thickness were significantly lower in CC + FSH group compared with the other two groups. Estradiol (E_2) levels were significantly lower in the letrozole + FSH group compared with the other two groups. CONCLUSIONS: Similar to CC, aromatase inhibition with letrozole reduces FSH dose required for COS without the undesirable antiestrogenic effects sometimes seen with CC.

Key words: aromatase inhibitor/clomiphene citrate/controlled ovarian stimulation/letrozole/unexplained infertility

Introduction

Gonadotrophin therapy is the mainstay of most forms of infertility treatment and adds considerably to the cost of the assisted reproduction therapies. Controlled ovarian stimulation (COS) with gonadotrophin injection and intrauterine insemination (IUI) is used alone or in combination for the management of unexplained infertility, male factor infertility, and other cases of infertility in which the female partner has open Fallopian tubes and the male partner has motile sperm (Guzick *et al*., 1999).

A systematic review of randomized studies to evaluate the effectiveness of IUI demonstrated that pregnancy rates were significantly higher in women who received gonadotrophins, either FSH or hMG, compared with those who did not undergo COS (Hughes, 1997) prior to IUI. The reported pregnancy rates per cycle have usually varied between 8 and 22% (Sunde *et al*., 1988; Dodson and Haney, 1991; Peterson *et al*., 1994; Brzechffa *et al*., 1998; Cohlen *et al*., 1998).

The rationale for COS in women with unexplained infertility, who by definition have regular ovulatory menstrual cycles, is to enhance the likelihood of pregnancy by increasing the number of oocytes available for fertilization and to overcome a possible subtle defect in ovulatory function not uncovered by conventional testing (Fisch *et al*., 1989). IUI, by increasing the density of motile sperm available to these oocytes, might further increase the monthly probability of pregnancy (Guzick *et al*., 1998).

Clomiphene citrate (CC) has been used in the treatment of anovulatory infertility since 1962. By depleting the estrogen receptors, CC acts as an anti-estrogen 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; Kousta *et al*., 1997). Recently, sequential

Table I. Patients' characteristics

	Letrozole + FSH (n = 36)	FSH alone (n = 56)	CC + FSH (n = 18)
Age (years)	34.8 ± 4.2 (25–41)	34.8 ± 4.5 (24–44)	33 ± 4.8 (23–41)
Duration of infertility (years)	3.3 ± 1.3 (2–6)	2.7 ± 1 (1–6)	2.6 ± 1.2 (1–5)
No. of prior treatment cycles	4.3 ± 1.8 (1–6)	2.3 ± 1.7 (1–7)	2.1 ± 1.2 (1–5)

Data presented as mean ± SD (range).
CC = clomiphene citrate.

CC and gonadotrophin (hMG or FSH) therapy has become an increasingly utilized method of COS for patients who fail CC treatment (Kemmann and Jones, 1983; Rose, 1992; Dickey *et al*., 1993b; Lu *et al*., 1996). The value of adding CC during COS is to decrease the FSH dose required for optimum stimulation. However, CC use is associated with lower pregnancy rates because of its peripheral antiestrogenic effects offsetting the FSH dose reduction benefit.

Aromatase is a cytochrome *P*-450 haemoprotein-containing enzyme complex that catalyses the rate-limiting step in the production of estrogens, i.e. the conversion of androstenedione and testosterone into estrogens (Cole and Robinson, 1990; Akhtar *et al*., 1993). The aromatase enzyme is a good target for selective inhibition because estrogen production is a terminal step in the biosynthetic sequence.

Recently, a group of highly selective aromatase inhibitors (AI), including letrozole and anastrozole, has been approved for use in post-menopausal women with breast cancer to suppress estrogen production. These AI have a relatively short half-life (~48 h) compared with CC, and therefore would be eliminated from the body rapidly (Sioufi *et al*. 1997a;b). In addition, since no estrogen receptor down-regulation occurs, no adverse effects on estrogen target tissues, as observed in CC-treated cycles, would be expected.

We hypothesized that it may be possible to mimic the action of CC, without depletion of estrogen receptors, by administration of an AI in the early part of the menstrual cycle. This use of an AI would result in release of the hypothalamic–pituitary axis from estrogenic negative feedback, thereby increasing gonadotrophin secretion and resulting in stimulation of ovarian follicles.

In prior reports, we showed that aromatase inhibition is successful in inducing and augmenting ovulation without antiestrogenic effects (Mitwally and Casper, 2000a;b;c; 2001). The objective of this study was to test the hypothesis that the use of the aromatase inhibitor, letrozole, in conjunction with FSH for COS, would decrease the dose of gonadotrophins required for COS similar to CC with FSH when compared with FSH only as a control.

Materials and methods

We obtained approval from the Research Ethics Board of The University of Toronto and Mount Sinai Hospital for the use of the aromatase inhibitor, letrozole, for ovarian stimulation. The study was conducted in the Reproductive Biology Units of Toronto General Hospital and Mount Sinai Hospital, and at the Toronto Center for Advanced Reproductive Technology. These clinics are academic tertiary referral centres affiliated with the Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Toronto, Canada. Patients were enrolled in the study from January 2000 to March 2001.

This was a non-randomized prospective study, which included 110 ovulatory women with unexplained infertility or mild male factor infertility undergoing COS and IUI. None of the patients had polycystic ovarian syndrome or oligo-anovulation. Unexplained infertility was diagnosed by exclusion of known factors of infertility. Ovulation was confirmed with follicular monitoring by transvaginal sonography (TVS) and serial measurements of serum estradiol (E_2) and LH hormone levels during a natural (no treatment cycle) and/or mid-luteal progesterone >15 nmol/l associated with regular menstrual cycle. Tubal patency was confirmed by hysterosalpingography and/or pelvic laparoscopy and male factor infertility was excluded by semen parameters meeting the World Health Organization (1999) criteria. All the study couples had ⩾1 year of infertility, and had undergone at least one to three cycles of follicular monitoring with timed intercourse before undergoing COS and IUI with partner's sperm.

The two standard protocols which are usually applied for COS in our study centres included the use of FSH either alone or in conjunction with CC. The addition of CC and the choice of the type and dose of FSH were usually decided according to the preference of the primary treating physician at the units. The treatment protocol was decided during a consultation visit prior to starting the treatment cycle. The choice was based on the clinical profile of the patient including age, weight, and duration of infertility as well as prior response to FSH and or CC.

Patients were counselled regarding the novel use of aromatase inhibitors to enhance ovarian response to FSH stimulation during COS. The experimental nature of the use of an aromatase inhibitor for ovulation induction was discussed. Thirty-six patients volunteered to use the aromatase inhibitor after the preliminary nature of the study was described to them and were included in the first study group. They received the aromatase inhibitor, letrozole (Femara®; Novartis, USA), 2.5 mg/day from day 3 to day 7 of the menstrual cycle, plus FSH injection [50–150 IU/day starting on day 7 until the day of hCG (10 000 IU)]. Eighteen women in the second study group received CC (Serophene®; Serono, Canada) 100 mg from day 5 to day 9 of the menstrual cycle plus FSH injection [50–150 IU/day starting on day 5 until the day of hCG (10 000 IU)]. FSH injection only [50–225 IU/day starting on day 3 until the day of hCG (10 000 IU)] was given to 56 women constituting the control group for the first two study groups. Each patient received one treatment regimen in one treatment cycle only. All patients received recombinant FSH (Puregon®; Organon, Canada; or Gonal-F®; Serono) or highly purified FSH (Fertinorm®; Serono).

Because of the experimental nature of the use of an aromatase inhibitor for augmentation of ovulation, the patients were not randomized for this preliminary clinical trial and the choice of receiving an aromatase inhibitor was exclusively left to the patient. However, at the end of the study period, analysis of the patients'

Table II. Features of all treatment cycles: letrozole + FSH group versus FSH group

	Letrozole + FSH (n = 36)	FSH only (n = 56)	P-value
Total FSH dose/cycle (IU)	465 ± 309 (150–1500)	1114 ± 393 (525–2025)	< 0.001
Day of hCG administration	12.5 ± 1.9 (9–19)	11.4 ± 1.4 (8–15)	NS
Follicles >18 mm	3 ± 1.2 (1–6)	2.7 ± 1.5 (1–6)	NS
Endometrial thickness (cm)	0.91 ± 0.2 (0.7–1.2)	1 ± 0.2 (0.7–1.3)	NS
Estradiol on day of hCG administration (pmol/l)	1377 ± 184 (386–2910)	2697 ± 1441 (487–6620)	< 0.001
Estradiol/mature follicle (>18 mm) (pmol/l)	530 ± 341 (113–1385)	1164 ± 668 (324–3431)	< 0.001
LH on day of hCG administration (IU/ml)	18 ± 14 (1–95)	11.2 ± 9.2 (1.4–51)	NS
Clinical pregnancy rate (%)	22.2	21.4	NS

NS = not significant.

Table III. Features of all treatment cycles: CC + FSH group versus FSH group

	CC + FSH (n = 18)	FSH only (n = 56)	P-value
Total FSH dose/cycle (IU)	619 ± 432 (200–2250)	1114 ± 393 (525–2025)	< 0.001
Day of hCG administration	12.3 ± 2.3 (8–18)	11.4 ± 1.4 (8–15)	NS
Follicles >18 mm	3.2 ± 1.3 (2–5)	2.7 ± 1.5 (1–6)	NS
Endometrial thickness (cm)	0.8 ± 0.2 (0.5–1)	1 ± 0.2 (0.7–1.3)	< 0.01
Estradiol on day of hCG administration (pmol/l)	3623 ± 1432 (1250–6782)	2697 ± 1441 (487–6620)	NS
Estradiol/mature follicle (>18 mm) (pmol/l)	1085 ± 497 (417–2279)	1164 ± 668 (324–1431)	NS
LH on day of hCG administration (IU/ml)	9.8 ± 7.2 (2.6–26)	11.2 ± 9.2 (1.4–51)	NS
Clinical pregnancy rate (%)	11.1	21.4	< 0.05

CC = clomiphene citrate; NS = not significant.

characteristics revealed no significant difference among the two study groups and the control group in age, duration of infertility, or number of prior IUI cycles, as shown in Table I.

The development of the ovarian follicles was monitored by both transvaginal ultrasound measurement of the mean follicular diameter as well as serial assays of estradiol and LH levels every 1 to 3 days during the follicular phase. The patient monitoring was performed, depending on the menstrual cycle start date, by one of five physicians on call for 1 week rotations. The dose and duration of FSH treatment were adjusted during the monitoring of the follicular development according to the patient's response including the number of the growing follicles and estradiol levels. The COS goal was to achieve a total of three mature ovarian follicles with a mean diameter of >18 mm on the day of hCG stimulation. hCG (Profasi®; Serono; or Pregnyl®; Organon) was given as a single s.c. injection of 10 000 IU to trigger ovulation when the mean diameter of at least two ovarian follicles was >18 mm.

IUI was performed 36 h after hCG administration if no endogenous LH surge occurred. If an endogenous LH surge occurred on the day of hCG administration, IUI was done on the following day. An LH surge was defined as an increase in LH level >100% over the mean of the preceding 2 days. IUI was performed by the same two infertility nurses for all patients.

Pregnancy was diagnosed by quantitative βhCG 2 weeks after the insemination. Clinical pregnancy was confirmed by observing fetal cardiac pulsation 4 weeks after positive pregnancy test by TVS.

Statistical analysis

The various outcome measures are expressed as mean ± SD. The following statistical tests were used where appropriate to analyse the various data among the three groups (two study groups and one control group). Analysis of variance, group t-test or Student's t-test, χ^2-test and Bonferroni t-test were used to compare data. $P < 0.05$ was considered statistically significant. The statistical tests were performed with SigmaStat for Windows Version 1.0 software (SigmaStat Software HighEdit Professional Copyright® 1993, MicroHelp Inc. and HeilerSoftware GmbH, USA).

Results

The total FSH dose/cycle was statistically significantly lower ($P < 0.05$) in both study groups (letrozole + FSH group and CC + FSH group) when compared with the control group (FSH only) but not between the two study groups when compared with each other (Table II, Table III, Table IV). There was no difference in number of follicles with a mean diameter >1.8 cm, LH level or day of hCG among the three patient groups. The pregnancy rate was 22.2% in the letrozole + FSH group, 11.1% in the CC + FSH group and 18.7% in the FSH only group. The pregnancy rate was significantly lower in the CC + FSH group when compared with the other two groups ($P < 0.05$).

E_2 levels throughout the follicular phase (Figure 1), total E_2 on the day of hCG administration, and E_2 per mature follicle in the letrozole + FSH group were significantly ($P < 0.01$) lower compared with the other two groups. Despite these significantly lower E_2 levels in the letrozole + FSH group, the endometrial thickness was not statistically different from the endometrial thickness in the FSH-only group (Table II).

Table IV. Features of all treatment cycles: letrozole + FSH group versus CC + FSH group

	Letrozole + FSH (n = 36)	CC + FSH (n = 18)	P-value
Total FSH dose/cycle (IU)	465 ± 309 (150–1500)	619 ± 432 (200–2250)	NS
Day of hCG administration	12.5 ± 1.9 (9–19)	12.3 ± 2.3 (8–18)	NS
Follicles >18 mm	3 ± 1.2 (1–6)	3.2 ± 1.3 (2–5)	NS
Endometrial thickness (cm)	0.91 ± 0.2 (0.7–1.2)	0.8 ± 0.2 (0.5–1)	< 0.05
Estradiol on day of hCG administration (pmol/l)	1377 ± 184 (386–2910)	3623 ± 1432 (1250–6782)	< 0.001
Estradiol/mature follicle (>18 mm) (pmol/l)	530 ± 341 (113–1385)	1085 ± 497 (417–2279)	< 0.001
LH on day of hCG administration (IU/ml)	18 ± 14 (1–95)	9.8 ± 7.2 (2.6–26)	< 0.05
Clinical pregnancy rate (%)	22.2	11.1	< 0.05

Data presented as mean ± SD (range).
CC = clomiphene citrate; NS = not significant.

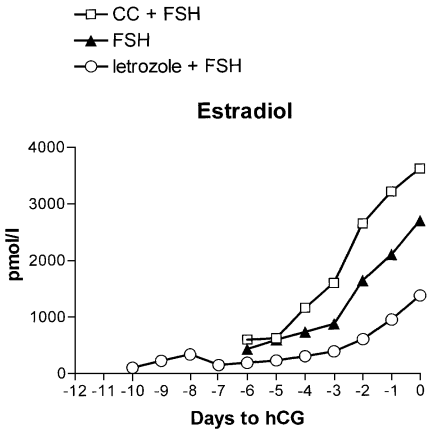

Figure 1. Serum estradiol (E_2) concentrations in pmol/l in the follicular phase of cycles stimulated with clomiphene citrate (CC) and FSH (n = 18), FSH alone (n = 91), or letrozole + FSH (n = 42). Day 0 is the day of hCG administration. The three curves are significantly ($P < 0.01$) different from each other.

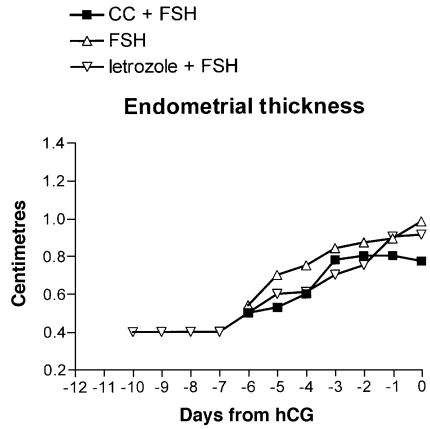

Figure 2. Cross-sectional endometrial thickness in centimetres measured by transvaginal ultrasound in the follicular phase of cycles stimulated with clomiphene citrate (CC) and FSH (n = 18), FSH alone (n = 91), or letrozole + FSH (n = 42). Day 0 is the day of hCG administration. Endometrial thickness in FSH-only cycle is significantly ($P < 0.05$) greater than CC + FSH cycles at all time points, and greater than letrozole + FSH cycles from day –6 to day –2, inclusive. Endometrial thickness in letrozole + FSH cycles and the FSH-only cycles was similar on the day of hCG (day 0) and the day prior to hCG (day –1).

In the CC + FSH group and in the letrozole + FSH group, the endometrial thickness was significantly ($P < 0.05$) lower than the FSH-only group throughout most of the follicular phase (Figure 2). However, by the end of the follicular phase (day –1 and day of hCG), the endometrial thickness in the letrozole + FSH group was the same as the FSH-only group (Figure 1 and Table II). In contrast, CC treatment was associated with significantly lower endometrial thickness despite significantly higher levels of estrogen, demonstrating the antiestrogenic effect associated with CC treatment. There was no difference among the three patient groups regarding the frequency of LH surges on the day of hCG administration.

Table V, Table VI and Table VII show the various characteristics of the treatment cycles in which an endogenous LH surge occurred on the day of hCG administration. It is interesting that there was no difference in the endometrial thickness on day of hCG day or in the pregnancy rate per cycle among the three patient groups. Compared with the other patients, the mean level of LH was statistically significantly greater in the letrozole + FSH group.

Table VIII, Table IX and Table X show the various characteristics of the treatment cycles in which no endogenous LH surge occurred on the day of hCG administration. The same pattern of differences in the cycle characteristics was observed as in Table II (all treatment cycles).

Discussion

In this study, co-treatment with the aromatase inhibitor letrozole significantly reduced the FSH dose required during COS, similar to co-treatment with CC. The beneficial effect of letrozole was not associated with the antiestrogenic effects seen with CC treatment as demonstrated by the significantly lower endometrial thickness noted with CC treatment, the significantly higher E_2 level and the significantly lower CC + FSH pregnancy rate.

The reduced FSH dose required for COS associated with aromatase inhibition may be due to a central and/or a

Table V. Features of treatment cycles with endogenous LH surge: letrozole + FSH group versus FSH group

	Letrozole + FSH (n = 36)	FSH only (n = 56)	P-value
Total FSH dose/cycle (IU)	409 ± 242 (150–1100)	1214 ± 289 (675–1800)	< 0.001
Day of hCG administration	12.7 ± 1.9 (11–19)	11.4 ± 1.5 (8–15)	< 0.05
Follicles >18 mm	2.9 ± 1.1 (1–5)	2.6 ± 1.5 (2–6)	NS
Endometrial thickness (cm)	0.9 ± 0.2 (0.7–1.2)	1 ± 0.2 (0.7–1.3)	NS
Estradiol on day of hCG administration (pmol/l)	1540 ± 877 (400–2910)	3213 ± 1483 (1912–6620)	< 0.001
Estradiol/mature follicle (>18 mm) (pmol/l)	600 ± 372 (171–1385)	1514 ± 990 (423–2870)	< 0.001
LH on day of hCG administration (IU/ml)	31.1 ± 29 (5.3–95)	17.3 ± 9.3 (9.6–51)	< 0.01
Clinical pregnancy rate (%)	22.2	17.9	NS

Data presented as mean ± SD (range).
NS = not significant.

Table VI. Features of treatment cycles with endogenous LH surge: CC + FSH group versus FSH group

	CC + FSH (n = 18)	FSH only (n = 56)	P-value
Total FSH dose/cycle (IU)	629 ± 196 (300–600)	1214 ± 289 (675–1800)	< 0.05
Day of hCG administration	12.6 ± 3.5 (8–18)	11.4 ± 1.5 (8–15)	NS
Follicles >18 mm	3 ± 1.2 (2–5)	2.6 ± 1.5 (2–6)	NS
Endometrial thickness (cm)	0.9 ± 0.2 (0.5–1.2)	1 ± 0.2 (0.7–1.3)	NS
Estradiol on day of hCG administration (pmol/l)	3562 ± 1701 (1250–6120)	3213 ± 1483 (1912–6620)	NS
Estradiol/mature follicle (>18 mm) (pmol/l)	1193 ± 612 (417–2279)	1514 ± 990 (423–2870)	NS
LH on day of hCG administration (IU/ml)	17.8 ± 5.2 (11.9–26)	17.3 ± 9.3 (9.6–51)	NS
Clinical pregnancy rate (%)	33.3	17.9	NS

Data presented as mean ± SD (range).
NS = not significant.

Table VII. Features of treatment cycles with endogenous LH surge: letrozole + FSH group versus CC + FSH group

	Letrozole + FSH (n = 36)	CC + FSH (n = 18)	P-value
Total FSH dose/cycle (IU)	409 ± 242 (150–1100)	629 ± 196 (300–600)	NS
Day of hCG administration	12.7 ± 1.9 (11–19)	12.6 ± 3.5 (8–18)	NS
Follicles >18 mm	2.9 ± 1.1 (1–5)	3 ± 1.2 (2–5)	NS
Endometrial thickness (cm)	0.9 ± 0.2 (0.7–1.2)	0.9 ± 0.2 (0.5–1.2)	NS
Estradiol on day of hCG administration (pmol/l)	1540 ± 877 (400–2910)	3562 ± 1701 (1250–6120)	< 0.001
Estradiol/mature follicle (>18 mm) (pmol/l)	600 ± 372 (171–1385)	1193 ± 612 (417–2279)	< 0.001
LH on day of hCG administration (IU/ml)	31.1 ± 29 (5.3–95)	17.8 ± 5.2 (11.9–26)	< 0.01
Clinical pregnancy rate (%)	22.2	33.3	NS

Data presented as mean ± SD (range).
CC = clomiphene citrate; NS = non-significant.

peripheral mechanism of action. Centrally, inhibition of estrogen synthesis by an aromatase inhibitor may release the estrogenic negative feedback on the hypothalamus and/or pituitary resulting in an increase in endogenous gonadotrophin secretion leading to enhancement of ovarian follicular development. Peripherally, at the ovarian level, inhibition of the conversion of androgens into estrogens by aromatase inhibition may lead to temporary accumulation of the androgens. Androgens were found to increase follicular sensitivity to FSH through amplification of the FSH receptor gene expression either directly or through other mediators such as the insulin-like growth factor system (Giudice, 1992; Adashi, 1993; Vendola *et al*., 1998; 1999; Weil *et al*., 1999). Other mechanisms yet to be determined may also be working at the

Table VIII. Features of treatment cycles without endogenous LH surge: letrozole + FSH group versus FSH group

	Letrozole + FSH (n = 36)	FSH only (n = 56)	P-value
Total FSH dose/cycle (IU)	521 ± 356 (200–1500)	1024 ± 475 (525–2025)	< 0.001
Day of hCG administration	12.3 ± 1.9 (9–18)	11.4 ± 1.4 (9–15)	NS
Follicles >18 mm	3.1 ± 1.3 (2–6)	2.8 ± 1.5 (2–6)	NS
Endometrial thickness (cm)	0.9 ± 0.2 (0.7–1.2)	1 ± 0.2 (0.7–1.3)	NS
Estradiol on day of hCG administration (pmol/l)	1214 ± 710 (386–3420)	2337 ± 1238 (664–5277)	< 0.001
Estradiol/mature follicle (>18 mm) (pmol/l)	460 ± 291 (113–1231)	975 ± 508 (324–2200)	< 0.001
LH on day of hCG administration (IU/ml)	4.9 ± 2.2 (1–8.6)	5.3 ± 2.9 (1.4–14)	NS
Clinical pregnancy rate (%)	22.2	25	NS

Data presented as mean ± SD (range).
NS = not significant.

Table IX. Features of treatment cycles without endogenous LH surge: CC + FSH group versus FSH group

	CC + FSH (n = 36)	FSH only (n = 56)	P-value
Total FSH dose/cycle (IU)	615 ± 510 (200–2250)	1024 ± 475 (525–2025)	< 0.05
Day of hCG administration	12.1 ± 1.1 (10–14)	11.4 ± 1.4 (9–15)	NS
Follicles >18 mm	3.3 ± 1.1 (2–5)	2.8 ± 1.5 (2–6)	NS
Endometrial thickness (cm)	0.7 ± 0.2 (0.5–1.1)	1 ± 0.2 (0.7–1.3)	< 0.01
Estradiol on day of hCG administration (pmol/l)	3657 ± 1256 (2157–6782)	2337 ± 1238 (664–5277)	NS
Estradiol/mature follicle (>18 mm) (pmol/l)	10256 ± 407 (540–1771)	975 ± 508 (324–2200)	NS
LH on day of hCG administration (IU/ml)	5.3 ± 3.1 (2.6–13.4)	5.3 ± 2.9 (1.4–14)	NS
Clinical pregnancy rate (%)	No pregnancies	25	

Data presented as mean ± SD (range).
CC = clomiphene citrate; NS = non-significant.

Table X. Features of treatment cycles without endogenous LH surge: letrozole + FSH group versus CC + FSH group

	Letrozole + FSH (n = 36)	CC + FSH (n = 36)	P-value
Total FSH dose/cycle (IU)	521 ± 356 (200–1500)	615 ± 510 (200–2250)	NS
Day of hCG administration	12.3 ± 1.9 (9–18)	12.1 ± 1.1 (10–14)	NS
Follicles >18 mm	3.1 ± 1.3 (2–6)	3.3 ± 1.1 (2–5)	NS
Endometrial thickness (cm)	0.9 ± 0.2 (0.7–1.2)	0.7 ± 0.2 (0.5–1.1)	< 0.01
Estradiol on day of hCG administration (pmol/l)	1214 ± 710 (386–3420)	3657 ± 1256 (2157–6782)	< 0.001
Estradiol/mature follicle (>18 mm) (pmol/l)	460 ± 291 (113–1231)	10 256 ± 407 (540–1771)	< 0.001
LH on day of hCG administration (IU/ml)	4.9 ± 2.2 (1–8.6)	5.3 ± 3.1 (2.6–13.4)	NS
Clinical pregnancy rate (%)	22.2	No pregnancies	

Data presented as mean ± SD (range).
CC = clomiphene citrate; NS = not significant.

peripheral level and need further study to improve our understanding of follicular development in both health and disease.

Co-treatment with CC during COS has been proposed as a means to reduce the cost of gonadotrophin treatment (Dickey *et al*., 1993b). However, there is a well-known discrepancy between the high ovulation rates after CC treatment and a relatively low pregnancy rate. The lower pregnancy rates may be associated with anti-estrogenic effects of CC on the endometrium (Gonen and Casper, 1990; Yeko *et al*., 1992;

Bonhoff *et al*., 1993; Massai *et al*., 1993; Wolman *et al*., 1994; Hosie and Murphy, 1995); and on the cervical mucus (Acharya *et al*., 1993; Asaad *et al*., 1993; Gelety and Buyalos, 1993; Massai *et al*., 1993). A decrease in uterine blood flow (Hsu *et al*., 1995) and impairment of placental protein 14 synthesis (Johnson *et al*., 1993) in addition to the increased subclinical pregnancy loss (Shoham *et al*., 1990; Bateman *et al*., 1992; Saunders *et al*., 1992) have also been suggested as contributing to lower pregnancy rates with CC treatment. Moreover, detrimental effects of CC on tubal transport (Whitelaw *et al*., 1970), and on the oocyte (Wramsby *et al*., 1987) and embryo (Schmidt *et al*., 1985; 1996; Laufer *et al*., 1983; London *et al*., 2000) have been suggested.

Whatever the mechanism(s) behind the lower pregnancy rate with CC treatment, the accumulation of CC in the body due to its long half-life (a few weeks) (Mikkelson *et al*., 1986) allows the occurrence of these deleterious effects. Due to the much shorter half-life, in addition to the absence of any antiestrogenic effects, we believe that use of the new aromatase inhibitors (third generation) would be associated with no deleterious effects on the final stages of follicular development and oocyte maturation or the early developing embryo. With a half-life of ~45 h (Sioufi *et al*., 1997a;b), administration of one of these new aromatase inhibitors in the early follicular phase should result in drug levels in the body that are extremely low or absent during the peri-ovulatory and luteal phases of the cycle.

Because measuring the endometrial thickness is relatively simple, non-invasive and routinely applied during follicular monitoring, we considered it as a clinically applicable method to monitor the antiestrogenic effects on the endometrium. Dickey and Holtkamp (1996) reported that in patients stimulated with CC for IUI, no pregnancy was observed when the endometrial thickness was 6 mm on the day of hCG administration, while all preclinical abortions occurred when endometrial thickness was 6–8 mm. In our present study, the endometrial thickness during CC treatment was significantly lower than the FSH-only and the letrozole + FSH groups. This finding is consistent with the persistent antiestrogenic effect of CC on endometrial thickness despite the high E_2 levels associated with multiple follicular development. To overcome the anti-estrogenic effects of CC, various methods have been suggested, generally without success. These include starting CC early on day 2 or 3 of the cycle (Dickey and Holtkamp, 1996; Triwitayakorn *et al*., 2002), adding ethinyl estradiol in the follicular phase (Yagel *et al*., 1992) and delaying administration of hCG (Dickey *et al*., 1993a). The administration of other anti-estrogenic drugs was also tried without significant benefits (Boostanfar *et al*., 2001).

Although the pregnancy rate in the CC + FSH group was statistically significantly lower when compared with the other two groups, we believe it is difficult to draw definitive conclusions from the present data regarding pregnancy rates. It is risky to compare outcomes in patients who were not randomized to the various treatment regimens. However, in the absence of any statistically significant difference between the patient groups in characteristics that might affect the achievement of pregnancy, we conclude that the pregnancy rate with aromatase inhibition is at the least acceptable while achieving the advantage of reducing the FSH dose needed for COS.

When the treatment cycles in which an endogenous LH surge occurred on the day of hCG administration were analysed separately (Tables V–VII), endometrial thickness in the CC + FSH group was found to be significantly better. Moreover, the only two pregnancies achieved during CC + FSH treatment occurred in cycles with an endogenous LH surge. The occurrence of an endogenous LH surge might indicate release of the hypothalamus and/or pituitary from the anti-estrogenic effect of CC allowing rising estrogen to exert its positive feedback and triggering the LH surge. This might also explain the favourable treatment outcome in terms of achieving pregnancy. Unfortunately, during CC treatment it is not possible to predict which patient would clear CC fast enough to allow the hypothalamus, pituitary and peripheral genital tissues to escape the antiestrogenic effect of CC. In a recent study, we found a better outcome in terms of achievement of pregnancy associated with the occurrence of endogenous LH surge compared with when hCG was given to trigger ovulation in the absence of an LH surge. This favourable outcome was most significantly seen with CC treatment (Mitwally *et al*., 2002).

In analysing the cycles with an endogenous LH surge (Tables V–VII), the mean LH level on the day of hCG administration was statistically significantly higher in the letrozole + FSH group when compared with the other two groups. Moreover, we found that the endogenous LH surge occurred significantly earlier in the FSH only group when compared with the letrozole + FSH group. We believe that the higher LH levels attained with letrozole treatment may indicate a more physiological LH surge due to the faster clearance of letrozole and the more physiological estrogen levels. The earlier occurrence of the LH surge during FSH only when compared with letrozole + FSH may reflect a premature LH surge due to the supraphysiological estrogen levels obtained during FSH stimulation.

The day of hCG administration was found to be later in the CC + FSH group. This might be due to the fact that, in CC + FSH treatment, patients started to receive treatment on day 5 of the menstrual cycle while in the other two treatment groups, treatment was started 2 days earlier (on day 3 of the menstrual cycle).

Gonadotrophins are used alone or in combination with CC to stimulate the growth and maturation of multiple oocytes. However, there is evidence from sharing of oocytes between a donor undergoing COS and a non-stimulated recipient that COS has a negative impact on implantation, independent of oocyte quality (Check *et al*., 1995). COS has also been found to be associated with unfavourable obstetric outcome (Tanbo, 1995; Maman *et al*., 1998). It is possible that supraphysiological levels of estrogen, attained during ovarian stimulation, may explain the adverse effects of ovarian stimulation on the outcome of infertility treatment (Paulson *et al*., 1990b; Hadi *et al*., 1994; Simón *et al*., 1995). Although the actual mechanism(s) of a possible adverse effect of high levels of estrogen on reproductive outcome are unknown, speculations include deleterious effects of estrogen on the endometrium

(Garcia *et al*., 1984; Forman *et al*., 1988; Kolb *et al*., 1997), the embryo (Pellicer *et al*., 1989; Paulson *et al*., 1990a; Ertzeid *et al*., 1992; Warner *et al*., 1998), the coagulation system (Kim *et al*., 1981; Lox *et al*., 1995), and the oviduct (Van der Auwera *et al*., 1999).

One approach to improve treatment outcome has been to reduce the intensity of ovarian stimulation and subsequent estrogen levels. This approach includes minimal stimulation cycles or natural cycle IVF, and reducing the FSH dose (step-down protocol) to improve endometrial receptivity (Simón *et al*., 1998). However, these measures have the drawback of reducing the number of mature follicles, which is the main objective of ovarian stimulation. In our study, letrozole use was associated with significantly lower E_2 levels when compared with the other stimulation protocols (FSH-only and CC + FSH), while resulting in the same number of mature follicles. Reducing estrogen synthesis by aromatase inhibition may therefore be a promising alternative to achieve a reduction in the serum concentration of estrogen in the peri-ovulatory and peri-implantation period, while still allowing multiple ovulation or retrieval of multiple oocytes in assisted reproductive treatment. Moreover, reducing the FSH dose needed for appropriate ovarian stimulation, as demonstrated in the present study, is an additional benefit. We have recently discussed the future avenues of applying aromatase inhibitors for infertility management (Mitwally and Casper, 2002b) including the potential benefit for women with poor response to FSH ovarian stimulation as we reported in a preliminary series of poor responders (Mitwally and Casper, 2002a).

A significant drawback in our present clinical trial is the non-randomized design of the study. However, due to the novel use of the aromatase inhibitors for ovarian stimulation, our institutional research board would not have approved a randomized study without a preliminary observational pilot trial. We believe that the present observational trial was mandatory to precede any definitive randomized studies in order to test for the feasibility of the idea of using aromatase inhibitors to reduce FSH dose needed for COS. We believe the positive results of the present trial should encourage us and others to proceed with more definitive prospective randomized clinical trials to prove or disprove our findings.

In summary, the results of the present study suggest that the concomitant use of the aromatase inhibitor, letrozole, during COS results in a reduction of the dose of FSH required to achieve a mean of three mature follicles prior to IUI without the deleterious peripheral anti-estrogenic effects often observed with CC. In addition, the low physiological levels of estrogen in the letrozole + FSH group may contribute to an improvement in pregnancy rates compared with the CC + FSH study group.

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