# Aromatase Inhibition Reduces the Dose of Gonadotropin Required for Controlled Ovarian Hyperstimulation

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# Aromatase Inhibition Reduces the Dose of Gonadotropin Required for Controlled Ovarian Hyperstimulation

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**OBJECTIVE:** *To compare the use of the aromatase inhibitor, letrozole, in conjunction with follicle-stimulating hormone (FSH) injection, and FSH alone for controlled ovarian hyperstimulation (COH) in patients with polycystic ovarian syndrome (PCOS) or ovulatory infertility.*

**METHODS:** *This nonrandomized study included two study groups: 26 patients with PCOS and 63 with ovulatory infertility (unexplained infertility [41 patients], male factor infertility [17 patients], and endometriosis [5 patients]), who received letrozole in addition to FSH; and two control groups: 46 PCOS patients and 308 with ovulatory infertility (unexplained infertility [250 patients], male factor infertility [42 patients], and endometriosis [16 patients], who received FSH only. All patients had intrauterine insemination (IUI). Main outcome measures included dose of FSH used per cycle, number of preovulatory follicles greater than 16 mm in diameter, cancellation rate, and pregnancy rate.*

**RESULTS:** *The FSH dose required for ovarian stimulation was significantly lower when letrozole was used in both study groups compared to the control groups without a significant difference in number of follicles greater than 16 mm. IUI cancellation rate was significantly lower with letrozole treatment in PCOS patients. In women with PCOS, clinical pregnancy rate per completed IUI cycle was 26.5% in the letrozole plus FSH group versus 18.5% in the FSH-only group. In ovulatory infertility patients, the pregnancy rate was similar in both study and control groups (11%).*

**CONCLUSION:** *We believe that inhibition of estrogen synthesis by aromatase inhibition will release the estrogenic negative feedback, resulting in an increase in endogenous FSH secretion. Moreover, by inhibiting conversion of androgens into estrogens, accumulating androgens may increase follicular sensitivity to FSH. Such a protocol has the potential to lower FSH treatment cost and may improve response for low responders who require high FSH doses during ovarian stimulation. (J Soc Gynecol Investig 2004;11: 406–15)* 

**KEY WORDS:** Aromatase inhibitors, letrozole, polycystic ovarian syndrome, infertility, ovarian stimulation.

Infertility is a worldwide problem that has been estimated to affect up to 6% of the population in North America, 5.4% in Europe, 3% in the Middle East, 10.1% in Africa, 4.8% in Asia and Oceania, 3.1% in Latin America, and 6.5% in the Caribbean.[1] Although background prevalence rates now appear to be reasonably stable, there is evidence of an increase in the rate of referrals for medical help.[2]

In about 25% of infertile couples, no definite cause will be found, even after complete investigation. These couples are said to have unexplained infertility. In another 25% of patients, infertility is due to anovulation, secondary to polycystic ovarian syndrome (PCOS) in most of the patients.[1–6] In the management of both unexplained infertility and chronic anovulation, ovarian stimulation is used either alone or in conjunction with assisted reproductive technologies such as intrauterine insemination (IUI) or in vitro fertilization and embryo transfer (IVF-ET).[7–14]

A systematic review of randomized studies to evaluate the effectiveness of IUI demonstrated that pregnancy rates were significantly higher in women who received gonadotropins, compared with those who did not undergo ovarian stimulation[15] prior to IUI. Reported pregnancy rates per cycle varied between 8% and 22%.[16–20] The rationale for ovarian stimulation in women with ovulatory infertility, eg, unexplained infertility and endometriosis-related infertility, who by definition have regular ovulatory menstrual cycles, is to enhance the likelihood of pregnancy by increasing the number of eggs available for fertilization.[21] IUI, by increasing the density of motile sperm available to these eggs, might further increase the

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**Table 1.** Patients' Characteristics

| Infertility group | Age (y) | | | Duration of infertility (y) | | | No. of prior treatment cycles | | |
|---|---|---|---|---|---|---|---|---|---|
| | FSH | Letrozole + FSH | *P* | FSH | Letrozole + FSH | *P* | FSH | Letrozole + FSH | *P* |
| PCOS | 32.8 ± 3.7 | 32.8 ± 3.7 | .5 | 3.1 ± 1.1 | 3.6 ± 0.5 | .1 | 3.3 ± 2.4 | 4.2 ± 2.6 | .02 |
| Ovulatory | 35.1 ± 4.1 | 35 ± 4.2 | .4 | 2.9 ± 2.2 | 3.3 ± 1.7 | .3 | 2.9 ± 2 | 3.9 ± 2.3 | .001 |
| Unexplained | 35.3 ± 4.2 | 35.8 ± 4.3 | .3 | 3.1 ± 2.4 | 3.2 ± 1.8 | .5 | 2.75 ± 1.3 | 4.2 ± 2.5 | .001 |
| Male | 34.7 ± 3.6 | 33.5 ± 3.7 | .1 | 2.4 ± 1 | 3.1 ± 1.5 | .2 | 3.3 ± 1.8 | 4.3 ± 1.7 | .05 |
| Endometriosis | 34.3 ± 3.7 | 3.5 ± 2.7 | .3 | 3 ± 1 | 4 ± 1.4 | .3 | 3.7 ± 2.2 | 4.8 ± 1.8 | .04 |

Data presented as means ± SD. $P < .05$ is considered statistically significant.

monthly probability of pregnancy, particularly in male factor infertility.[22]

Anovulatory women with PCOS who wish to become pregnant have traditionally been treated with the antiestrogen clomiphene citrate (CC), with successful ovulation in approximately 70–85% of patients, although only 33–45% conceive.[23,24] In CC failures, ie, failure to ovulate or failure to achieve pregnancy, gonadotropins have been used as a second-line treatment to induce ovulation. However, gonadotropin treatment is associated with higher risk of severe ovarian hyperstimulation syndrome (OHSS), especially in women with PCOS, and therefore requires intensive monitoring.[25] In addition, the use of gonadotropins is associated with several other drawbacks, including high-order multiple gestation, parenteral administration, and increased cost compared to oral ovulation induction agents.

Recently, we reported the success of using an aromatase inhibitor for induction of ovulation in ovulatory women with PCOS and augmentation of ovulation in women with ovulatory infertility.[26] In this study, we investigated the combination of the aromatase inhibitor, letrozole, with FSH to reduce the dose of FSH needed to achieve optimum ovarian stimulation in women with PCOS or ovulatory infertility.

## MATERIALS AND METHODS

We obtained approval from the Institutional Research Board of The University of Toronto and Mount Sinai Hospital for the use of an aromatase inhibitor for ovarian stimulation. Each patient provided informed consent before enrollment into the study. The study was conducted in two tertiary referral academic centers: the Reproductive Biology Unit of Mount Sinai Hospital and the Toronto Center for Advanced Reproductive Technology. These clinics are 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 July 2001.

### Enrollment of Patients

Patients with PCOS or ovulatory infertility (unexplained infertility, male factor infertility, and endometriosis-related infertility) presenting for ovarian stimulation and IUI were offered the option of enrollment into the study of the aromatase inhibitor, letrozole, in conjunction with FSH injections to reduce the dose of FSH required for optimum ovarian stimulation. Patients were thoroughly counseled regarding the experimental nature of the new indication (the aromatase inhibitor) and the hypothesis of using aromatase inhibitors to enhance ovarian response to FSH stimulation.

Patients who opted to participate in the study received the letrozole at a dose of 2.5 mg daily from cycle day 3 to 7, in addition to FSH injections that started on day 7 of the menstrual cycle. This was a nonrandomized study, which included two study groups that received the aromatase inhibitor in addition to FSH injections and two control groups that received FSH injections only. In women with PCOS who did not have a spontaneous menstrual cycle, withdrawal bleeding was induced by administering medroxyprogesterone acetate 10-mg tablets every day for 10 days. Due to the experimental nature of the use of an aromatase inhibitor for ovarian stimulation, the patients were not randomized for this 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' characteristics revealed no significant difference among the two study groups and the control groups in age or duration of infertility. However, the number of prior ovarian stimulation cycles was significantly higher in the study subjects receiving letrozole. All of the study couples had been infertile for at least 1 year and had undergone at least one to three cycles of follicular monitoring with timed intercourse before undergoing ovarian stimulation and IUI with their partner's spermatozoa (Table 1). However, this does not correct the nonrandomized design of this trial and a randomized design would result in a better homogenous study and control groups.

The two study groups included 28 patients with PCOS who started 53 treatment cycles and 63 patients with ovulatory infertility (41 with unexplained infertility, 17 with male factor infertility, and 5 with endometriosis-related infertility), who started 81 treatment cycles. The control groups included 46 patients with PCOS who started 130 treatment cycles and 308 patients with ovulatory infertility who started 544 treatment cycles (250 with unexplained infertility, 42 with male factor infertility, and 16 with endometriosis-related infertility). Diagnosis of PCOS was made according to the National Institutes of Health consensus criteria.[27] The diagnosis of unexplained infertility was made by exclusion of known factors of infertility. Ovulation was confirmed with follicular monitoring by transvaginal ultrasonography (TVS) and serial measurements of serum estradiol ($E_2$) and luteinizing hormone (LH) levels during a natural (no treatment) cycle and/or mid-

**Table 2.** Number of Started and Completed Cycles According to Treatment and Infertility Diagnosis

| | FSH | | | | | Letrozole + FSH | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Completed IUI | | Cancelled IUI | | | Completed IUI | | Cancelled IUI | |
| Infertility group | Started cycles (n) | No. | % | No. | % | Started cycles (n) | No. | % | No. | % |
| All patients | 674 | 572 | 85* | 102 | 15* | 134 | 124 | 92* | 10 | 8* |
| PCOS | 130 | 81 | 62* | 49 | 38* | 53 | 49 | 92* | 4 | 8* |
| Ovulatory infertility | 544 | 491 | 90 | 53 | 10 | 81 | 75 | 93 | 6 | 7 |
| Unexplained infertility | 446 | 405 | 91 | 41 | 9 | 56 | 50 | 89 | 6 | 11 |
| Male factor infertility | 74 | 64 | 86 | 10 | 14 | 19 | 19 | 100 | 0 | 0 |
| Endometriosis | 24 | 22 | 92 | 2 | 8 | 6 | 6 | 100 | 0 | 0 |

* Statistically significant ($P < .05$).

luteal progesterone greater than 15 nM associated with regular menstrual cycles. Tubal patency was confirmed by hysterosalpingography and or pelvic laparoscopy, and male factor infertility was excluded by semen parameters meeting the World Health Organization criteria. Endometriosis was diagnosed by laparoscopy.

## Stimulation Protocols

The choice of the type and dose of FSH was decided according to the preference of the primary treating physician at the units. The treatment protocol was determined during a consultation visit prior to starting the treatment cycle. The choice was mainly based on the clinical profile of the patient, including age, weight, and duration of infertility, as well as prior response to FSH.

In the study centers, the usual FSH-only stimulation protocol includes starting FSH injections on the third day of the menstrual cycle beginning with a dose from 50–150 IU/d. The FSH dose is then adjusted according to the patient's response to achieve about two or three mature follicles (>16 mm) on the day of human chorionic gonadotropin (hCG) administration.

When the aromatase inhibitor, letrozole (Femara; Novartis, East Hanover, NJ), was used, it was given at a dose of 2.5 mg/d from day 3 to 7 of the menstrual cycle, followed by FSH injection starting at a dose of 50–150 IU/d beginning on day 7 until the day of hCG. The dose of FSH was adjusted according to patient response to achieve two or three mature follicles (>16 mm) on the day of hCG administration.

We empirically chose the regimen of aromatase inhibitor administration applied in this study. Shorter or longer regimens may be equally or even more effective. However, we believed that starting the aromatase inhibitor before day 3 might be too early as estrogen levels could be too low to exert significant negative feedback on endogenous gonadotropin production. On the other hand, extending the administration of aromatase inhibitor beyond day 7 would not allow enough time for endometrial development to reach its normal preovulatory thickness. In addition, earlier cessation of treatment would allow clearance of the aromatase inhibitor before the period of implantation and organogenesis. In our preliminary studies, we found the dose of 2.5 mg of letrozole to be effective in significantly lowering $E_2$ levels and inducing ovulation. However, again, this dose was based on convenience and other doses may be more effective. Unfortunately, all of the available data about the pharmacokinetics of aromatase inhibitors are based on studies conducted in postmenopausal women.

All patients received recombinant (Gonal-F, Serono, Oakville, Canada; or Puregon, Organon, Scarborough, Canada) or highly purified FSH (Fertinorm; Serono). There was no significant difference between the study and control groups in the use of recombinant or highly purified FSH. hCG (Profasi, Serono; or Pregnyl, Organon) was given as a single injection of 10,000 IU to trigger ovulation when the mean diameter of at least two ovarian follicles was greater than 16 mm.

## Cycle Monitoring

The development of the ovarian follicles was monitored by both TVS measurement of the mean follicular diameter and serial assays of $E_2$ 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 $E_2$ levels. The goal was to achieve a total of three mature ovarian follicles with a mean diameter of 18 mm on the day of hCG stimulation.

IUI was performed 40 hours after hCG administration if no endogenous LH surge occurred. If an endogenous LH surge was detected on the day of hCG administration, IUI was performed on the following 2 days. An LH surge was defined as an increase in LH level greater than 100% over the mean of the preceding 2 days. The same two infertility nurses performed the intrauterine inseminations in 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 were expressed as means ± SD. Student's $t$ test, chi-square test, and Bonferroni $t$ test were used where appropriate to analyze the various data among the two

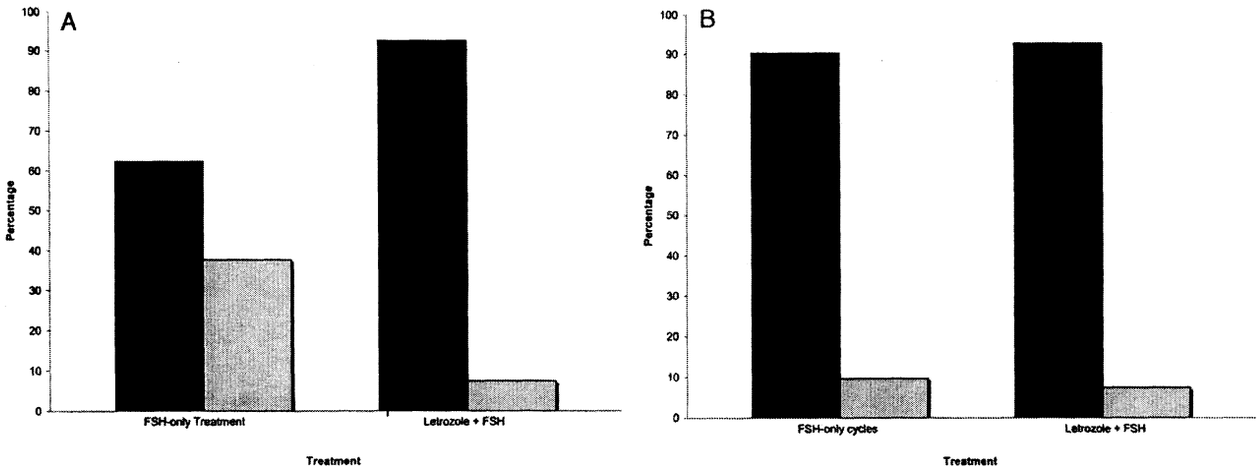


**Figure 1.** Percentage of completed and cancelled IUI cycles out of the total started cycles in women with A) PCOS and B) ovulatory infertility. In women with PCOS, the IUI cancellation rate was significantly lower with letrozole plus FSH treatment compared to FSH-only treatment. In women with ovulatory infertility, the rate was also lower with letrozole plus FSH treatment, but the difference was not statistically significant. *Black bars,* completed IUI cycles; *gray bars,* cancelled IUI cycles.

study groups and each equivalent control group, *P* values less than .05 were considered statistically significant. The statistical tests were performed with SigmaStat for Windows Version 1.0 software (SigmaStat Software HighEdit Professional, MicroHelp Inc and HeilerSoftware GmbH, San Rafael, CA).

## RESULTS

Although the study was not randomized, there was no statistically significant difference between the study and control groups as regards age or duration of infertility. However, patients in the study groups had significantly more prior failed treatment cycles (Table 1). This is not surprising because frustration associated with repeated failed cycles would be expected to encourage patients to try new experimental treatments, and would bias the results against the letrozole plus FSH treatment.

Table 2 shows the number of started treatment cycles and the number of completed and cancelled IUI cycles according to the infertility diagnosis and treatment modality. Overall, there were fewer IUI cancelled cycles with letrozole treatment when compared to FSH-only treatment (Figure 1). The difference was statistically significant among all started cycles and in the PCOS patients.

The two major reasons for IUI cancellations included the two extremes of response to ovarian stimulation, ie, achievement of too many mature follicles (more than six follicles) or failure to achieve more than one follicle. Patients with an inadequate response failed to develop more than one follicle greater than 16 mm, whereas patients with an excessive response had several mature follicles (more than six follicles >16 mm) that would markedly increase the risk for high-order multiple pregnancy. In patients with an inadequate response, IUI was cancelled and replaced with timed intercourse. In patients with an excessive response, IUI was converted to IVF. There were other reasons for IUI cancellation in a few cycles, including anovulation in some women with PCOS, presence of ovarian cysts, and failure to obtain a semen sample adequate for insemination. Table 3 lists the various reasons for IUI cancellations according to the infertility diagnosis and treatment group.

Table 4 shows the number of patients and number of treatment cycles in each of the study and control groups.

**Table 3.** Reasons for Cancellation of IUI

| Reason for cancellation | Infertility group | FSH 102 (15%) cancelled cycles | | Letrozole + FSH 10 (8%) cancelled cycles | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| Conversion to IVF | PCOS | 9 | 18.4 | 0 | 0 |
| | Ovulatory infertility | 42 | 79.2 | 4 | 66.7 |
| Inadequate response | PCOS | 28 | 57.1 | 3 | 75.0 |
| | Ovulatory infertility | 3 | 5.7 | 0 | 0.0 |
| Others | PCOS | 12 | 24.5 | 1 | 25.0 |
| | Ovulatory infertility | 8 | 15.1 | 2 | 33.3 |
| No. of cancelled cycles | PCOS | 49 | 18.4 | 4 | 0 |
| | Ovulatory infertility | 53 | 79.2 | 6 | 66.7 |

**Table 4.** Number of Patients and Number of Completed Treatment Cycles

| | FSH | | | | | | Letrozole + FSH | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ovulatory infertility | | | | | | Ovulatory infertility | | |
| | All | PCOS | Ovulatory infertility | a | b | c | All | PCOS | Ovulatory infertility | a | b | c |
| No. of patients | 354 | 46 | 308 | 250 | 42 | 16 | 89 | 26 | 63 | 41 | 17 | 5 |
| No. of treatment cycles | 572 | 81 | 491 | 405 | 64 | 22 | 124 | 49 | 75 | 50 | 19 | 6 |

a = unexplained infertility; b = male infertility; c = endometriosis-related infertility.

Tables 5 and 6 show the various characteristics of the treatment cycles (letrozole plus FSH versus FSH only) in women with PCOS and ovulatory infertility, respectively. Tables 5 and 6 include data for all completed treatment cycles, as well as for the first completed treatment cycles only. The findings were the same when we analyzed all treatment cycles or single cycle for each patient (the first cycles). The amount of FSH required for COH was significantly lower with letrozole treatment in both PCOS and ovulatory infertility groups without a significant difference in the number of follicles greater than 16 mm. Although $E_2$ levels ($E_2$ on the day of hCG administration and $E_2$ per follicle > 16 mm) were significantly lower with letrozole treatment, there was no significant difference in the thickness of the endometrium on the day of hCG administration between the letrozole plus FSH and FSH-only cycles. The day of hCG administration tended to be later with letrozole treatment cycles. However, this difference was not statistically different.

In ovulatory women, the pregnancy rate per completed IUI cycle was similar (11%) in FSH-only and letrozole plus FSH cycles. In women with PCOS, the pregnancy rate per completed IUI cycle was 26.5% in the letrozole plus FSH group and 18.5% in the FSH-only group. The difference was not significantly different.

Figure 2 shows the pattern of $E_2$ and LH changes in PCOS and ovulatory infertility patients with FSH-only (Figure 2A) and letrozole plus FSH (Figure 2B) along the follicular phase. In FSH-only treatment cycles, $E_2$ levels steadily increased throughout the follicular phase with significant escalation after the midfollicular phase. Towards the end of the follicular phase, $E_2$ levels were much higher in ovulatory infertility patients than in patients with PCOS, in whom the $E_2$ rise reached a plateau on the day before hCG administration.

In letrozole plus FSH cycles, $E_2$ levels decreased during the first third of the follicular phase coinciding with letrozole administration, then began to increase slowly throughout the second third of the follicular phase, coinciding with letrozole clearance from the body and concomitant FSH administration. Interestingly, the difference between the maximum $E_2$ levels attained on the day of hCG administration was not significant between ovulatory infertility patients and patients with PCOS as there was blunting of the $E_2$ levels in PCOS patients on the day before hCG administration.

FSH-only treatment was associated with significantly lower LH levels in both ovulatory infertility patients and patients with PCOS when compared with letrozole plus FSH treatment (Figure 2A versus B). This difference was marked during the first two thirds of the follicular phase and on the day before and the day of hCG administration.

With letrozole treatment (Figure 2B), we observed both a negative feedback effect of $E_2$ levels on LH production in the early follicular phase and a negative feedback effect of $E_2$ in the

**Table 5.** Characteristics of All Completed Treatment Cycles and First Completed Treatment Cycles in Women With PCOS

| | FSH | Letrozole + FSH | *P* |
|---|---|---|---|
| All completed treatment cycles | | | |
| Total FSH dose (U/cycle) | 1268 ± 592 | 587 ± 389 | .001 |
| No. of follicles ≥16 mm on hCG day | 2.5 ± 1.8 | 2.7 ± 1.6 | .27 |
| Day of hCG administration | 13.9 ± 3 | 14.5 ± 2.9 | .17 |
| $E_2$ on day of hCG administration (pM) | 1951 ± 1713 | 1243 ± 941 | .001 |
| $E_2$/follicle ≥16 mm (pM) | 868 ± 406 | 477 ± 216 | .001 |
| LH in cycles with LH endogenous surge (U/mL) | 18.5 ± 16.6 | 29.9 ± 18.6 | .04 |
| Endometrium thickness on day of hCG administration (mm) | 9.1 ± 2.1 | 9.4 ± 1.5 | .3 |
| Clinical pregnancy rate/cycle | 18.5% | 26.5% | .12 |
| First completed treatment cycles | | | |
| Total FSH dose (U/cycle) | 1309 ± 557 | 677 ± 454 | .001 |
| No. of follicles ≥16 mm on hCG day | 3.1 ± 2.1 | 2.5 ± 1.5 | .27 |
| Day of hCG administration | 13.7 ± 3 | 14.75 ± 2.4 | .09 |
| $E_2$ on day of hCG administration (pM) | 1930 ± 1666 | 1057 ± 525 | .001 |
| $E_2$/follicle ≥16 mm (pM) | 860 ± 410 | 534 ± 238 | .001 |
| LH in cycles with LH endogenous surge (U/mL) | 17.5 ± 12 | 30.8 ± 20.1 | .04 |
| Endometrium thickness on day of hCG administration (mm) | 8.9 ± 2.2 | 9.6 ± 0.1 | .09 |
| Clinical pregnancy rate/cycle | 23.9% | 34.6% | .08 |

Data presented as mean ± SD

**Table 6.** Characteristics of All Completed Treatment Cycles and First Completed Treatment Cycles in Women With Ovulatory Infertility

| | FSH | Letrozole + FSH | P |
|---|---|---|---|
| All completed treatment cycles | | | |
| Total FSH dose (U/cycle) | 1350 ± 677 | 497 ± 542 | .001 |
| No. of follicles ≥16 mm on hCG day | 3.2 ± 1.8 | 2.97 ± 1.3 | .13 |
| Day of hCG administration | 11.4 ± 2.3 | 11.95 ± 1.6 | .06 |
| $E_2$ on day of hCG administration (pM) | 2719 ± 1590 | 1369 ± 813.5 | .001 |
| $E_2$/follicle ≥16 mm (pM) | 1038 ± 670 | 542 ± 469 | .001 |
| LH in cycles with LH endogenous surge (U/mL) | 13.4 ± 10.5 | 19.8 ± 22.1 | .04 |
| Endometrium thickness on day of hCG administration (mm) | 9.6 ± 1.95 | 9.3 ± 1.8 | .1 |
| Clinical pregnancy rate/cycle | 11% | 11% | NS |
| First completed treatment cycle | | | |
| Total FSH dose (U/cycle) | 1312 ± 676 | 497 ± 586 | .001 |
| No. of follicles ≥16 mm on hCG day | 3.2 ± 1.8 | 2.94 ± 1.3 | .19 |
| Day of hCG administration | 11.4 ± 1.8 | 11.9 ± 1.6 | .07 |
| $E_2$ on day of hCG administration (pM) | 2851 ± 1687 | 1321 ± 803 | .001 |
| $E_2$/follicle ≥16 mm (pM) | 1123 ± 755 | 547 ± 511 | .001 |
| LH in cycles with LH endogenous surge (U/mL) | 13.3 ± 10.2 | 20 ± 23 | .05 |
| Endometrium thickness on day of hCG administration (mm) | 9.7 ± 2 | 9.2 ± 1.7 | .6 |
| Clinical pregnancy rate/cycle | 13.6% | 12.7% | .21 |

Data presented as mean ± SD.
NS = not significant.

last third of the follicular phase. Of interest, higher $E_2$ levels were required to exert negative feedback on LH production in women with PCOS.

In both FSH-only and letrozole plus FSH treatment cycles, positive feedback of $E_2$ on LH production and the start of LH surge occurred about 3 days before the day of hCG administration. In FSH-only cycles, the rise in LH levels leading to the endogenous LH surge was blunted for 2 days before the significant escalation on the last day before hCG was administered. However, in letrozole plus FSH cycles, LH levels started to rise significantly 2 days before the day of hCG administration.

## DISCUSSION

In the present study, we found that the addition of an aromatase inhibitor during controlled ovarian hyporstimulation (COH) and IUI significantly reduced the dose of FSH required for optimum ovarian stimulation. There was a significant reduction in IUI cancellations, especially in women with PCOS. Compared to FSH-only treatment, a similar number of ovarian follicles greater than 16 mm were obtained with less than 50% of the FSH dose when an aromatase inhibitor was added. These benefits were achieved along with a similar or better clinical pregnancy rate compared to FSH alone in ovulatory infertility and PCOS patients, respectively.

Stimulating endogenous gonadotropin production to allow the use of lower doses of exogenous FSH for COH-IUI has typically been used with CC in conjunction with exogenous FSH.[28–30] Unfortunately, the successful reduction in FSH dose required for optimum COH with the addition of CC was not associated with promising pregnancy rates, possibly due to the antiestrogenic effects of CC particularly at the level of the endometrium[31–33] and cervix,[34–36] as well as other possible targets.[37–40]

Applying FSH alone for COH is associated with issues such as the risk of life-threatening OHSS, as well as high-order multiple gestation in some patients. Therefore, the use of FSH for COH requires intensive monitoring. Other important concerns with FSH treatment include the high cost of the medication and the need for parenteral administration. Most of the above-mentioned drawbacks and concerns are dependent on the amount of FSH administered for COH, and the risk increases with higher doses.

Another important concern in COH-IUI cycles is the fairly high cancellation rate. Besides its economic impact, IUI cycle cancellation is usually associated with significant frustration and disappointment both in the infertile couple and in health care providers. The two major reasons for IUI cancellations include over-response with formation of several mature follicles and poor response with formation of an inadequate number of mature follicles. In the first case, completion of the IUI cycle is associated with the risk of high-order multiple gestation and its potential disastrous outcome both on the maternal and fetal sides. In the case of poor response, completion of the IUI cycle is associated with unacceptably low pregnancy rates and significant economic loss due to the cost of processing semen and insemination.

For these reasons, low-dose FSH stimulation protocols have been recommended for COH-IUI cycles.[41,42] However, although this approach seems to be logical, in practice there is a high rate of inadequate response in some patients and disappointingly low pregnancy rates together with high cancellation rates.[43–45] Other protocols to increase pregnancy rates with COH and IUI include increasing the dose of FSH[46]; using cotreatment with $E_2$,[47] growth hormone,[48] or birth control pills;[49] and using gonadotropin-releasing hormone (GnRH) agonists in different protocols.[50] Unfortunately, all of these strategies have met with only limited success and no single method has proved to be significantly superior. Moreover, the increased complexity and cost of such approaches have pre-

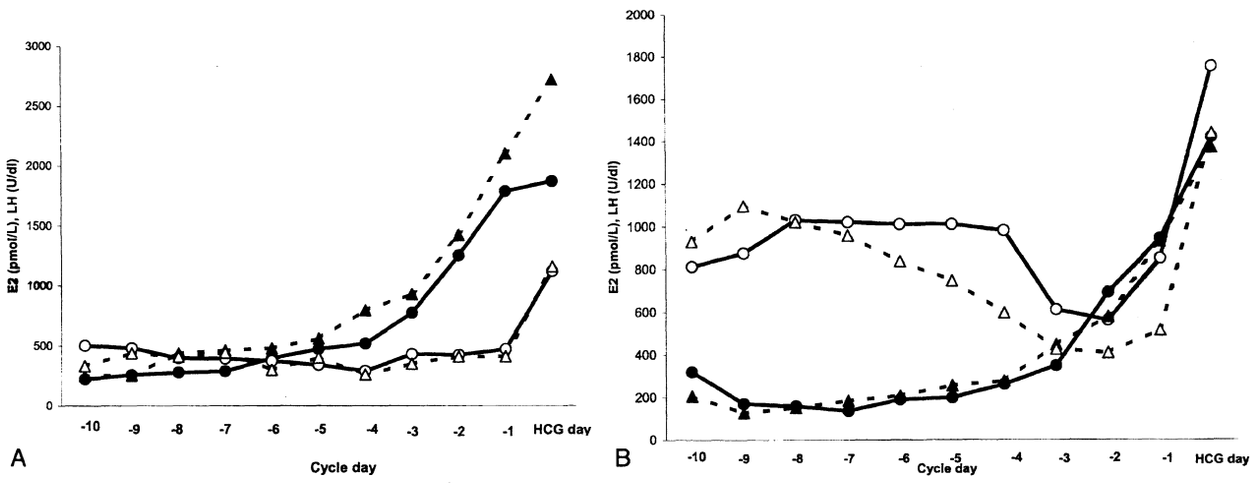


**Figure 2.** Relationship between $E_2$ and LH levels along the follicular phase in women with PCOS and ovulatory infertility who received A) FSH-only and B) letrozole plus FSH treatment. Data include levels from completed IUI cycles. Letrozole plus FSH treatment cycles were associated with significantly lower $E_2$ levels, and LH levels were higher; the levels of both $E_2$ and LH seemed to be within more physiologic ranges when compared to FSH-only cycles. *Closed circles,* $E_2$ in PCOS patients; *open circles,* LH in PCOS patients; *closed triangles,* $E_2$ in ovulatory patients; *open triangles,* LH in ovulatory patients.

vented their wide application in patients undergoing COH-IUI, except in low responders.

In the late 1990s, we explored the hypothesis that it might be possible to mimic the action of CC, without depletion of estrogen receptors (ER), by administration of an aromatase inhibitor in the early part of the menstrual cycle. We hypothesized that the result of blocking estrogen production from all sources by inhibiting aromatization would release the hypothalamic/pituitary axis from estrogenic negative feedback, thereby increasing endogenous gonadotropin secretion and resulting in stimulation of ovarian follicles. The selective non-steroidal aromatase inhibitors have a relatively short half-life (~40 hours) compared to CC, and would be ideal for this purpose because they are eliminated from the body rapidly.[51,52] In addition, we expected no adverse effects on estrogen target tissues, since no estrogen receptor down- regulation occurs in contrast to the estrogen receptor depletion observed in CC-treated cycles.

In women with PCOS, relative oversuppression of FSH may be the result of excessive androgen produced from the ovary being converted to estrogen by aromatization in the brain. The aromatase inhibitors suppress estrogen production in both the ovaries and the brain. In the case of PCOS, therefore, aromatase inhibitors should result in a robust increase in FSH release and subsequent follicle stimulation and ovulation. We speculate that the actual FSH release is likely blunted by the high levels of circulating inhibin found in PCOS patients[53–55] that would not be affected by aromatase inhibition. In addition, because aromatase inhibition does not antagonize ER in the brain, the initiation of follicle growth leads to increasing concentrations of both $E_2$ and inhibin.[56,57] This would result in a normal secondary feedback loop that limits FSH response to aromatase inhibition, thereby avoiding the risk of high multiple ovulation and OHSS.

We also believe that aromatase inhibitors also act locally in the ovary to increase follicular sensitivity to FSH. This may result from accumulation of intraovarian androgens, because conversion of androgen substrate to estrogen is blocked by aromatase inhibition. Recent data support a stimulatory role for androgens in early follicular growth in primates.[58] Testosterone was found to augment follicular FSH receptor expression in primates, suggesting that androgens promote follicular growth and estrogen biosynthesis indirectly by amplifying FSH effects.[59,60] In addition, androgen accumulation in the follicle may stimulate insulin-like growth factor I (IGF-I), along with other endocrine and paracrine factors, which may synergize with FSH to promote folliculogenesis.[61–64] It is likely that women with PCOS already have a relative aromatase deficiency in the ovary leading to increased intra-ovarian androgens.[65,66] The increased androgen levels likely lead to the multiple small follicles responsible for the polycystic morphology of the ovaries in these women. The androgens, as described above, may also increase FSH receptors, making these PCOS ovaries exquisitely sensitive to an increase in FSH either through exogenous administration of gonadotropins (hence the high risk of OHSS) or through endogenous increases in FSH as a result of decreased central estrogen feedback induced by aromatase inhibition. In the latter case, we postulate that a relatively small rise in FSH, because of a normal inhibin/estrogen feedback loop as described above, leads to single or low multiple follicle development, thus avoiding the occurrence of OHSS.

In FSH-only treatment cycles, $E_2$ levels were observed to rise steadily during the follicular phase with a significant escalation in the late-follicular phase. On the other hand, in letrozole plus FSH cycles, $E_2$ levels decreased during the early follicular phase and then began to increase slowly during the mid-follicular phase to reach a maximum level on the day of

hCG administration. The $E_2$ level on the day of hCG was significantly lower than the levels attained in FSH-only treatment cycles. We believe that the more physiologic $E_2$ levels during the follicular phase of the COH cycles would be associated with fewer adverse effects on the development of the endometrium than FSH-only ovarian stimulation and supraphysiologic $E_2$ levels. This could be a factor in improving the implantation rate.

There is evidence from sharing of oocytes between a donor undergoing ovarian stimulation and a nonstimulated recipient that ovarian stimulation has a negative impact on implantation, independent of oocyte quality.[67] Ovarian stimulation has also been found to be associated with unfavorable obstetric outcome.[68,69] It is possible that supraphysiologic levels of estrogen, attained during ovarian stimulation, may explain the adverse effects of ovarian stimulation on the outcome of infertility treatment.[70–72] Although the actual mechanism(s) of a possible adverse effect of high levels of estrogen on reproductive outcome is unknown, speculations include deleterious effects of estrogen on the endometrium,[73–75] the embryo,[71,76–78] the coagulation system,[79,80] and the oviduct.[81] The significantly lower $E_2$ levels associated with aromatase inhibition during ovarian stimulation could provide a protective mechanism against the possible deleterious effects of supraphysiologic $E_2$ levels.

When considering the clinical application of aromatase inhibitors in infertility management, the potential direct effect of these agents on the developing oocyte, and fertilization and embryogenesis is an important concern. The short half-life of the aromatase inhibitors and limited administration to the early part of the follicular phase allow the rapid clearance of the medications before the stage of fertilization and embryogenesis. In addition, the absence of accumulation of the aromatase inhibitors or any of their metabolites would tend to impart a safety factor for ovarian stimulation. We have reported preliminary data on pregnancy outcome after the use of aromatase inhibitors for ovarian stimulation supporting the safety of these medications for this indication.[82]

We believe that the use of aromatase inhibitors in conjunction with FSH for COH-IUI is associated with several advantages. This includes the reduced risk of severe OHSS and reduced rate of multiple pregnancy, as well as the significant reduction in the cost of treatment associated with high pregnancy rates. These benefits are achieved with an inexpensive, orally administered medication with a short half-life and few adverse effects. However, the results of this observational study need to be confirmed by randomized clinical trials that would test the hypothesis that the addition of an aromatase inhibitor to gonadotropin stimulation will be beneficial.

## REFERENCES


1. Farley TMM, Belsey FH. The prevalence and etiology of infertility. In: Biological components of fertility. Proceedings of the African Population Conference, Dakar, Senegal, Vol 1. Laege, Belgium: International Union for the Scientific Study of Population, 1988:2.1.15–2.1.30.
2. Templeton A. Infertility-epidemiology, aetiology and effective management. Health Bull (Edinb) 1995;53:294–8.
3. Hull M, Glazener C, Kelly NJ, et al. Population study of causes, treatment, and outcome of infertility. BMJ 1985;291:1693–7.
4. Templeton A, Fraser C, Thompson B. The epidemiology of infertility in Aberdeen. BMJ 1990;301:148–52.
5. Snick HKA, Snick TS, Evers JLH, Collins JA. The spontaneous pregnancy prognosis in untreated subfertile couples: The Walcheren primary care study. Hum Reprod 1997;12:1582–8.
6. Cahill DJ, Wardle PG. Management of infertility BMJ 2002;325:28–32.
7. Franks S: Polycystic ovary syndrome. N Engl J Med 1995;333:853–61.
8. Guzick DS, Carson SA, Coutifaris C, et al. Efficacy of superovulation and intrauterine insemination in the treatment of infertility. N Engl J Med 1999;340:177–83.
9. Melis GB, Paoletti AM, Strigini F, et al. Pharmacologic induction of multiple follicular development improves the success rate of artificial insemination with husband's semen in couples with male-related or unexplained infertility. Fertil Steril 1987; 47:441–5.
10. Dodson WC, Whitesides DB, Hughes CL, et al. Superovulation with intrauterine insemination in the treatment of infertility: A possible alternative to gamete intrafallopian transferand in vitro fertilization. Fertil Steril 1987;48:441–5.
11. Serhal PF, Katz M, Little V, Woronowski H. Unexplained infertility: The value of Pergonal superovulation combined with intrauterine insemination. Fertil Steril 1988;49:602–6.
12. Fioretti P, Paoletti AM, Strigini F, et al. Induction of multiple follicular development as a therapy for unexplained or male-related infertility. Gynecol Endocrinol 1989;3:45–53.
13. Melis GB, Strigini F, Mais V, et al. Critical reappraisal of the clinical effectiveness of different methods of assisted fertilization. J Endocrinol Invest 1990;13:263–74.
14. Kemmann E, Bohrer M, Shelden R, et al. Active ovulation management increases the monthly probability of pregnancy occurrence in ovulatory women who receive intrauterineinsemination. Fertil Steril 1987;48:916–20.
15. Hughes EG. The effectiveness of ovulation induction and intrauterine insemination in the treatment of persistent infertility: A meta-analysis. Hum Reprod 1997;12:1865–72.
16. Sunde A, Kahn JA, Molne K. Intrauterine insemination: A European collaborative report. Hum Reprod 1988;2:69–73.
17. Dodson WC, Haney AF. Controlled ovarian hyperstimulation and intrauterine insemination for treatment of infertility. Fertil Steril 1991;55:457–67.
18. Peterson CM, Hatasaka HH, Jones KP, et al. Ovulation induction with gonadotrophins and intrauterine insemination compared with in vitro fertilization and no therapy: A prospective, nonrandomized, cohort study and meta-analysis. Fertil Steril 1994;62:535–44.
19. Brzechffa PR, Daneshmand S, Buyalos RP. Sequential clomiphene citrate and human menopausal gonadotrophin with intrauterine insemination: The effect of patient age on clinical outcome. Hum Reprod 1998;13:2110–4.
20. Cohlen BJ, te Velde ER, van Kooij RJ, et al. Controlled ovarian hyperstimulation and intrauterine insemination for treating male subfertility: A controlled study. Hum Reprod 1998;13:1553–8.
21. Fisch P, Casper RF, Brown SE, et al. Unexplained infertility: Evaluation of treatment with clomiphene citrate, human chorionic gonadotrophin or in vitro fertilization. Fertil Steril 1989;51:828–33.
22. Guzick DS, Sullivan MW, Adamson GD, et al. Efficacy of treatment for unexplained infertility. Fertil Steril 1998;70;207–13.
23. The ESHRE Capri Workshop: Female infertility: Treatment options for complicated cases. Hum Reprod 1997;12:1191–6.

24. The ESHRE Capri Workshop: Infertility revisited: The state of the art today and tomorrow. Hum Reprod 1996;11:1779–807.
25. Fluker MR, Urman B, Mackinnon M, et al. Exogenous gonadotropin therapy in World Health Organization groups I and II ovulatory disorders. Obstet Gynecol 1994;83:189–96.
26. Mitwally MFM, Casper RF. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. Fertil Steril 2001;75:305–9.
27. Zawadzki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: Towards a rational approach. In: Dunaif A, Givens JR, Haseltine F, Merriam GR, eds. Polycystic ovary syndrome. Boston: Blackwell, 1992:377–84.
28. Dickey RP, Olar TT, Taylor SN, et al. Sequential clomiphene citrate and human menopausal gonadotrophin for ovulation induction: Comparison to clomiphene citrate alone and human menopausal gonadotrophin alone. Hum Reprod 1993;8:56–9.
29. Kemman E, Jones JR. Sequential clomiphene citrate-menotropin therapy for induction or enhancement of ovulation. Fertil Steril 1983;39:772–9.
30. Rose BI. A conservative, low-cost superovulation regimen. Int J Fertil 1992;37:339–42.
31. Gonen Y, Casper RF. Sonographic determination of an adverse effect of clomiphene citrate on endometrial growth. Hum Reprod 1990;5:670–4.
32. Nelson LM, Hershlag A, Kurl RS, Hall JL, Stillman RJ. Clomiphene citrate directly impairs endometrial receptivity in the mouse. Fertil Steril 1990;53:727–31.
33. Li TC, Warren MA, Murphy C, Sargeant S, Cooke ID. A prospective, randomised, cross- over study comparing the effects of clomiphene citrate and cyclofenil on endometrial morphology in the luteal phase of normal fertile women. Br J Obstet Gynaecol 1992;99:1008–13.
34. Randall JM, Templeton A. Cervical mucus score and in vitro sperm mucus interaction in spontaneous and clomiphene citrate cycles. Fertil Steril 1991;56:465–8.
35. Gysler M, March C, Mishell DJ Jr, Bailey EJ. A decade's experience with an individualized clomiphene treatment regimen including its effects on the postcoital test. Fertil Steril 1982;37:161–7.
36. Gelety TJ, Buyalos RP. The effect of clomiphene citrate and menopausal gonadotropins on cervical mucus in ovulatory cycles. Fertil Steril 1993;60:471–6.
37. Hsu CC, Kuo HC, Wang ST, Huang KE. Interference with uterine blood flow by clomiphene citrate in women with unexplained infertility. Obstet Gynecol 1995;86:917–21.
38. Oktay K, Berkowitz P, Berkus M, Schenken RS, Brzyski RG. The re-incarnation of an old question—clomid effect on oocyte and embryo? Fertil Steril 2000;74:422–3.
39. Branigan EF, Estes MA. Minimal stimulation IVF using clomiphene citrate and oral contraceptive pill pretreatment for LH suppression. Fertil Steril 2000;73:587–90.
40. Zayed F. Outcome of stimulated in vitro fertilisation (SIVF) using clomiphene citrate and human menopausal gonadotropin in different infertility groups. Clin Exp Obstet Gynecol 1999;26:227–9.
41. Lu PY, Lee SH, Chen ALJ, Erickson LD, Atkinson EJ, Ory SJ. Minimal stimulation achieves pregnancy rates comparable to human menopausal gonadotropins in the treatment of infertility. Fertil Steril 1996;65:583–7.
42. Dhaliwal LK, Sialy RK, Gopalan S, Majumdar S. Minimal stimulation protocol for use with intrauterine insemination in the treatment of infertility. J Obstet Gynaecol Res 2002;28:295–9.
43. Houmard BS, Juang MP, Soules MR, Fujimoto VY. Factors influencing pregnancy rates with a combined clomiphene citrate/gonadotropin protocol for non-assisted reproductive technology fertility treatment. Fertil Steril 2002;77:384–6.
44. Tomlinson MJ, Amissah-Aruthur JB, Thompson KA, Kasraie JI, Bentick B. Prognostic indicators for intrauterine insemination (IUI): statistical model for IUI success. Hum Reprod 1996;11:1892–6.
45. Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L, Martikainen H. Intrauterine insemination treatment in subfertility: An analysis of factors affecting outcome. Hum Reprod 1999;14:698–703.
46. van Hooff MH, Alberda AT, Huisman GJ, Zeilmaker GH, Leerentveld RA. Doubling the human menopausal gonadotrophin dose in the course of an in-vitro fertilization treatment cycle in low responders: A randomized study. Hum Reprod 1993;8:369–73.
47. Russell IB. Pre-cycle estrogen treatment and poor responders. Assist Reprod Rev 1995;5:82–9.
48. Dor J, Seidman DS, Amudai E, Bider D, Levran D, Mashiach S. Adjuvant growthhormone therapy in poor responders to in-vitro fertilization: A prospective randomized placebo- controlled double-blind study. Hum Reprod 1995;10:40–3.
49. Gonen Y, Jacobsen W, Casper RF. Gonadotropin suppression with oral contraceptives before in-vitro fertilization. Fertil Steril 1990;53:282–7.
50. Howles CM, Macnamee MC, Edwards RG. Short term use of an LHRH agonist to treatpoor responders entering an in-vitro fertilization programme. Hum Reprod 1987;8:655–6.
51. Sioufi A, Gauducheau N, Pineau V, et al. Absolute bioavailability of letrozole in healthy post-menopausal women. Biopharm Drug Dispos 1997;18:779–89.
52. Sioufi A, Sandrenan N, Godbillon J, et al. Comparative bioavailability of letrozole under fed and fasting conditions is 12 healthy subjects after a 25 mg single oral administration. Biopharm Drug Dispos 1997;186:489–97.
53. Roberts VJ, Barth S, El-Roeiy A, Yen SSC. Expression of inhibin/activin system messenger ribonucleic acids and proteins in ovarian follicles from women with polycystic ovarian syndrome. J Clin Endocrinol Metab 1994;79:1434–9.
54. Yamoto M, Minami S, Nakano R. Immunohistochemical localization of inhibin subunits in polycystic ovary. J Clin Endocrinol Metab 1993;77:859–62.
55. Jaatinen T, Penttila T, Kaipia A, Ekfors T, Parvinen M, Toppari J. Expression of inhibin $\alpha$, $\beta_A$ and $\beta_B$ messenger ribonucleic acids in the normal human ovary and in polycystic ovarian syndrome. J Endocrinol 1994;143:127–37.
56. Anderson RA, Groome NP, Baird DT. Inhibin A and inhibin B in women with polycystic ovarian syndrome during treatment with FSH to induce mono-ovulation. Clin Endocrinol Oxf 1998;48:577–84.
57. Lockwood GM, Muttukrishna S, Groome NP, Matthews DR, Ledger WL. Mid-follicular phase pulses of inhibin B are absent in polycystic ovarian syndrome and are initiated by successful laparoscopic ovarian diathermy: A possible mechanism regulating emergence of the dominant follicle. J Clin Endocrinol Metab 1998;83:1730–5.
58. Weil SJ, Vendola K, Zhou J, et al Androgen receptor gene expression in the primate ovary: Cellular localization, regulation, and functional correlations. J Clin Endocrinol Metab 1998;837:2479–85.
59. Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. J Clin Endocrinol Metab 1999;848:2951–6.
60. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest 1998;10112:2622–9.
61. Vendola K, Zhou J, Wang J, Famuyiwa OA, Bievre M, Bondy CA. Androgens promote oocyte insulin-like growth factor I expression and initiation of follicle development in the primate ovary. Biol Reprod 1999;612:353–7.

62. Adashi E. Intraovarian regulation the proposed role of insulin-like growth factors. Ann NY Acad Sci 1993;687:10–2.
63. Giudice LC. Insulin-like growth factors and ovarian follicular development. Endocr Rev 1992;13:641–69.
64. Yen SSC, Laughlin GA, Morales AJ. Interface between extra-and intra-ovarian factors in polycystic ovary syndrome PCOS. Ann NY Acad Sci 1993;687:98–111.
65. Agarwal SK, Judd HL, Magoffin DA. A mechanism for the suppression of estrogen production in polycystic ovary syndrome. J Clin Endocrinol Metab 1996;8110:3686–91.
66. Jakimiuk AJ, Weitsman SR, Brzechffa PR, Magoffin DA. Aromatase mRNA expression in individual follicles from polycystic ovaries. Mol Hum Reprod 1998;41:1–8.
67. Laufer N, Pratt BM, DeCherney AH, et al. The in vivo and in vitro effects of clomiphene citrate on ovulation, fertilization, and development of cultured mouse oocytes. Am J Obstet Gynecol 1983;147:633–9.
68. Tanbo T, Dale PO, Lunde O, et al. Obstetric outcome in singleton pregnancies after assisted reproduction. Obstet Gynecol 1995;86:188–92.
69. Maman E, Lunenfeld E, Levy A, et al. Obstetric outcome of singleton pregnancies conceived by in vitro fertilization and ovulation induction compared with those conceived spontaneously. Fertil Steril 1998;70:240–5.
70. Hadi FH, Chantler E, Anderson E, et al. Ovulation induction and endometrial steroid receptors. Hum Reprod 1994;9: 2405–10.
71. Paulson RJ, Sauer MV, Lobo RA. Factors affecting embryo implantation after human in vitro fertilization: a hypothesis. Am J Obstet Gynecol 1990;163:2020–3.
72. Simón C, Cano F, Valbueña D, et al. Clinical evidence for a detrimental effect on uterine receptivity of high serum ooestradiol concentrations in high and normal responder patients. Hum Reprod 1995;10:2432–7.
73. Forman R, Fries N, Testart J, et al. Evidence for an adverse effect of elevated serum oestradiol concentration on embryo implantation. Fertil Steril 1988;49:118–2.
74. Garcia JE, Acosta AA, Hsiu JG, Jones HWJ. Advanced endometrial maturation after ovulation induction with human menopausal gonadotrophin/human chorionic gonadotrophin for in vitro fertilization. Fertil Steril 1984;41:31–5.
75. Kolb BA, Najmabadi S, Paulson RJ. Ultrastructural characteristics of the luteal phase endometrium in patients undergoing controlled ovarian stimulation. Fertil Steril 1997;67:625–30.
76. Pellicer A, Ruiz A, Castellvi RM, et al. Is the retrieval of high-numbers of oocytes desirable in patients treated with gonadotrophin-releasing hormone analogues (GnRHa) and gonadotrophins? Hum Reprod 1989;4:536–40.
77. Ertzeid G, Storeng R. Adverse effects of gonadotrophin treatment on pre- and postimplantation development in mice. J Reprod Fertil 1992;96:649–55.
78. Warner CM, Cao W, Exley GE, et al. Genetic regulation of egg and embryo survival. Hum Reprod 1998;13 Suppl 3:178–90.
79. Kim HC, Kemmann E, Shelden RM, et al. Response of blood coagulation parameters to elevated endogenous 17 beta-oestradiol levels induced by human menopausal gonadotrophins. Am J Obstet Gynecol 1981;140:807–10.
80. Lox C, Canez M, DeLeon F, et al. Hyperestrogenism induced by menotropins alone or in conjunction with luprolide acetate in in vitro fertilization cycles: The impact on hemostasis. Fertil Steril 1995;63:566–70.
81. Van der Auwera I, Pijnenborg R, Koninckx PR. The influence of in-vitro culture versus stimulated and untreated oviductal environment on mouse embryo development and implantation. Hum Reprod 1999;14:2570–4.
82. Mitwally MFM, Casper RF. Pregnancy outcome after the use of an aromatase inhibitor for ovarian stimulation. Fertil Steril 2002; 78(suppl 1):S277–8.