

Letrozole and gonadotropins versus luteal estradiol and gonadotropin-releasing hormone antagonist protocol in women with a prior low response to ovarian stimulation

Alyaa Elassar, M.D., M.Sc., Lawrence Engmann, M.D., John Nulsen, M.D., and Claudio Benadiva, M.D., H.C.L.D.

Center for Advanced Reproductive Services, University of Connecticut Health Center, Department of Obstetrics and Gynecology, University of Connecticut, Farmington, Connecticut

Objective: To compare in vitro fertilization outcomes after ovarian stimulation using letrozole/antagonist (LA) versus luteal-phase estradiol (E₂)/gonadotropin-releasing hormone (GnRH) antagonist (LPG) in poor responders.

Design: Retrospective study.

Setting: Academic center.

Patient(s): Ninety nine women with ≥ 2 prior cycles, at a starting dose of ≥ 300 IU gonadotropins, who yielded fewer than five oocytes or a prior cycle cancellation.

Intervention(s): In the LPG group ($n = 52$), transdermal E₂ initiated every other day starting day 10 after the luteinizing hormone (LH) surge; one day 11, patients began daily administration of antagonist for 3 consecutive days, with gonadotropins started on the second day of menstruation. In the LA group ($n = 47$), letrozole 5 mg/day initiated on the second day of spontaneous menstruation for 5 days then gonadotropins added on day 5; for both groups a flexible antagonist protocol was used.

Main Outcome Measure(s): Ongoing pregnancy, number of oocytes retrieved, and cancellation rate.

Result(s): The total dose of gonadotropins administered and E₂ levels on day of hCG administration were statistically significantly lower with the LA protocol. The number of oocytes retrieved (6.1 ± 3.0 vs. 7.9 ± 4.8), number of transferred embryos (2.2 ± 1.0 vs. 2.4 ± 1.4), and cancellation rate (55.3% vs. 36.5%) were similar in both groups. Ongoing pregnancy rates per transfer (40% vs. 21.2%) and per initiated cycle (19.1% vs. 13.5%) were similar in the LA and LPG groups, respectively.

Conclusion(s): Aromatase inhibitor regimens can be a feasible alternative to the LPG protocol in recurrent low ovarian response. (Fertil Steril® 2011;95:2330–4. ©2011 by American Society for Reproductive Medicine.)

Key Words: Controlled ovarian stimulation, GnRH antagonist, letrozole, luteal estradiol, poor response

The accurate identification and efficient management of poor responders remains one of the most enigmatic challenges in assisted reproductive technology (ART) (1). The lack of uniform definition of a low ovarian response causes difficulty in comparing treatment outcomes (2). Poor response to controlled ovarian hyperstimulation (COH) is associated with a low E₂ and follicular response to gonadotropins, and a reduced number of retrieved oocytes and available embryos for transfer. Poor responders are sometimes difficult to identify before COH and are generally resistant to a multitude of intervention strategies. Although tests for ovarian reserve have a reasonable capacity to predict poor response (3), they have a low ability to predict the occurrence of pregnancy; clearly, the actual ovarian response to stimulation remains the ultimate test (1).

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Reprint requests: Claudio Benadiva, M.D., H.C.L.D., Center for Advanced Reproductive Services, Dowling South Building, 263 Farmington Avenue, Farmington, CT 06030-6224 (E-mail: benadiva@up.uchc.edu).

Several protocols have been proposed to improve in vitro fertilization (IVF) outcomes in patients with low ovarian response. Although some of the suggested protocols may enhance the ovarian response, none have demonstrated a significant improvement in pregnancy rates.

Luteal-phase estradiol (E₂) and gonadotropin releasing-hormone (GnRH) antagonists have been used as adjuvants before gonadotropin stimulation in antagonist protocols (4, 5). This approach allows synchronization of early antral follicle growth in the luteal phase before COH with a subsequent increase in oocyte yield and possible improvement in pregnancy rates (4). Estrogen prevents the luteal follicle-stimulating hormone (FSH) rise and may also increase sensitivity to gonadotropins, while the GnRH antagonist induces luteolysis and prevents the FSH rise (6, 7). Luteal antagonist administration is associated with lower basal FSH and inhibin levels, and a reduction in the size and variability in the diameter of antral follicles (8).

The potential application of aromatase inhibitors (AIs) for ovarian stimulation was recognized by Mitwally et al. (9), who were also the first to suggest that AIs might improve ovarian

response (10). Aromatase inhibitors augment follicular growth by releasing the hypothalamus from the negative feedback effects of E₂ (central mechanism) and via the accumulation of intrafollicular androgen substrate, which may increase the expression of FSH receptors (peripheral mechanism) (9) and possibly other intraovarian factors (e.g., gonadotropin surge attenuating factor) (11). The central mechanism releases endogenous gonadotropins that are chemically different from recombinant FSH in terms of their carbohydrate moiety (12), and the peripheral mechanism may increase the response to gonadotropins. Few studies so far (13–17) have used AIs in low responders. Our study compared the letrozole/antagonist protocol (LA) to the luteal E₂/GnRH antagonist protocol (LPG) in women who had exhibited low ovarian response in prior IVF attempts.

MATERIALS AND METHODS

Patients

This was a retrospective cohort study where 99 low-responder patients were included between January 2009 and October 2010. Approval for the study was obtained from the University of Connecticut institutional review board. Patients included were younger than 42 year old, with two or more prior ovarian stimulation cycles at a starting dose of gonadotropins of 300 IU or more that had yielded fewer than five oocytes, or a prior cycle cancellation due to low follicular recruitment. Low follicular recruitment was defined as ≤ 3 follicles, ≥ 15 mm in diameter, after 10 days of stimulation. All patients included had had no prior ovarian surgery or exposure to chemotherapy or radiotherapy. The protocols used in prior failed cycles included GnRH agonist down-regulation, GnRH antagonist, and microdose leuprolide. Ninety nine patients were stimulated with a flexible antagonist protocol, either using a luteal E₂ patch and GnRH antagonist (LPG) or using early follicular letrozole with no luteal pretreatment (LA). Each patient contributed a single cycle to this study, so there was no crossover between groups. Assignment of the patients to either protocol was made at their physician's discretion.

Stimulation Protocols

In the LPG group (n = 52), transdermal E₂ (Vivelle Dot 0.1 mg; Novartis, Miami, FL) was initiated every other day starting on day 10 after the LH surge. On the 11th day, patients began daily administration of ganirelix acetate (Ganirelix; Organon Pharmaceuticals, Roseland, NJ), 0.25 mg SC for 3 consecutive days. Patients presented on day 2 of their ensuing menses for measurement of FSH, LH, and E₂ levels and a baseline ultrasound. The last E₂ patch was removed and ovarian stimulation was started using high-dose gonadotropins, an average of 450 IU of recombinant FSH (Gonal F; Serono, Rockland, MA) in combination with 150 IU of human menopausal gonadotropin (hMG, Menopur; Ferring Pharmaceuticals, Tarrytown, NY). Ganirelix was restarted when the lead follicle measured ≥ 13 mm or if E₂ >300 pg/mL was encountered, and was continued until the day of human chorionic gonadotropin (hCG) administration. We administered 5,000–10,000 IU SC of hCG when at least three lead follicles had attained or exceeded 17-mm mean diameter.

During the same time interval, 47 patients underwent ovarian stimulation using the letrozole protocol (LA). Letrozole (Femara; Novartis, East Hanover, NJ) at a dose of 5 mg/day was initiated on the second day of spontaneous menstruation and was continued for 5 days. On day 5, 450 IU of recombinant FSH and 150 IU of hMG were commenced. The criteria for starting ganirelix to prevent a premature LH surge were the same as in the LPG protocol. The criterion for hCG administration was the presence of at least two follicles ≥ 20 mm in diameter.

Transvaginal oocyte retrieval was performed approximately 35 hours after hCG administration. Oocyte insemination or intracytoplasmic sperm injection was performed as indicated, and all embryos were transferred on day 3. Luteal phase supplementation was achieved in both groups by administering 50 mg IM of progesterone daily starting the evening after oocyte retrieval and continuing until a negative pregnancy test or confirmed clinical pregnancy (presence of an intrauterine gestational sac with fetal cardiac activity).

TABLE 1

Letrozole/antagonist (LA) versus luteal-phase estradiol/gonadotropin-releasing hormone antagonist (LPG) in poor responders: demographic and clinical characteristics of study participants.

	LA (n = 47)	LPG (n = 52)	P value
Age (y)	39.1 $\pm$ 2.8	39.2 $\pm$ 0.2	.51
BMI (kg/m ²)	26.6 $\pm$ 7.0	26.7 $\pm$ 5.9	.90
Day-3 FSH (mIU/mL)	10.7 $\pm$ 5.0	9.5 $\pm$ 3.8	.27
Day-3 LH (mIU/mL)	4.9 $\pm$ 1.9	5.1 $\pm$ 2.2	.49
Day-3 E ₂ (pg/mL)	46.3 $\pm$ 18.6	40.9 $\pm$ 25.2	.22
History of FSH ≥ 12 (%)	34 (16/47)	17.3 (9/52)	.06
No. of prior IVF cycles	4.6 $\pm$ 1.8	3.3 $\pm$ 1.8	<.01
No. of prior canceled cycles	1.8 $\pm$ 1.2	1.1 $\pm$ 0.8	<.01

Note: Values are expressed as mean $\pm$ standard deviation or percentage rates (%). BMI = body mass index; E₂ = estradiol; FSH = follicle-stimulating hormone; LH = luteinizing hormone.

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The primary outcome measure was the ongoing pregnancy rate (>20 weeks' gestation) per started cycle. Secondary outcome measures were cancellation rate, number of oocytes retrieved and transferable embryos, and implantation and clinical pregnancy rates.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (release 17.0; SPSS Inc., Chicago, IL). Student's *t*-test was used for comparison of continuous variables, and chi-square or Fisher's exact test were used for comparison of proportions. *P* < .05 was considered statistically significant. Data were expressed as mean $\pm$ standard deviation.

RESULTS

The patients were comparable in terms of age, body mass index, and basal serum FSH, LH, and E₂ levels (Table 1). Forty-one patients in the LA group (87.2%) and 43 in the LPG group (82.7%) had at least one prior cycle cancellation due to poor follicular recruitment. Patients with no prior cycle cancellations had at least two preceding IVF cycles with retrieval of fewer than five oocytes and no pregnancies. The number of prior failed and canceled cycles was statistically significantly higher in the letrozole group (see Table 1). Of note, 36 patients in the LA group (76.6%) underwent a prior ovarian stimulation using the LPG protocol. As expected, the FSH levels in the treatment cycle were statistically significantly lower in LPG group. The total dose of gonadotropins administered (4,388 $\pm$ 1,703 vs. 6,193 $\pm$ 1,018 IU), and peak E₂ levels (675 $\pm$ 458 vs. 1,256 $\pm$ 799 pg/mL) were lower in the LA group (Table 2).

Twenty-six (55.3%) cycles in the LA group and 19 (36.5%) in the LPG group did not reach embryo transfer (ET) (*P* = .06) (see Table 2). Although there was no difference in the number of retrieved oocytes, the number of metaphase II oocytes (3.8 $\pm$ 2.4 vs. 6.6 $\pm$ 4.3) and the number of 2 pronuclei oocytes (3.0 $\pm$ 2.3 vs. 5.3 $\pm$ 4.1) were statistically significantly lower with the LA protocol (Table 3). The fertilization rate, number of embryos transferred, and implantation rate were similar between the groups.

There was also no statistically significant difference in the ongoing pregnancy rate per started cycle between the two groups: for LA,

TABLE 2

Letrozole/antagonist (LA) versus luteal-phase estradiol/gonadotropin-releasing hormone antagonist (LPG) in poor responders: COH response in study participants.

	LA (n = 47)	LPG (n = 52)	P value
FSH at start of COH	9.2 ± 2.8	4.07 ± 3.0	<.01
Stimulation days	11.1 ± 2.6	11.3 ± 3.3	.88
Total gonadotropins (IU)	4,388 ± 1,703	6,193 ± 1,018	<.01
Peak E ₂ (pg/mL)	675 ± 458	1256 ± 799	<.01
Cancellation rate (%)	55.3 (26/47)	36.5 (19/52)	.06
Canceled cycles due to poor ovarian response (%)	29.8 (14/47)	25 (13/52)	.6
Canceled ET after retrieval (%) ^a	10.6 (5/47)	7.7 (4/52)	.6
Canceled cycles due to premature LH surge (%)	14.9 (7/47)	3.8 (2/52)	.06

Note: Values are expressed as mean ± standard deviation or percentage rates (%). COH = controlled ovarian hyperstimulation; ET = embryo transfer; LH = luteinizing hormone.

^a Due to failed fertilization or arrest of embryo development.

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TABLE 3

Letrozole/antagonist (LA) versus luteal-phase estradiol/gonadotropin-releasing hormone antagonist (LPG) in poor responders: in vitro fertilization outcomes.

	LA (n = 47)	LPG (n = 52)	P value
No. of oocytes retrieved	6.1 ± 3.0	7.9 ± 4.8	.08
No. of mature oocytes	3.8 ± 2.4	6.6 ± 4.3	<.01
Maturation rate (%)	64	83	<.01
No. of 2PN oocytes	3.0 ± 2.3	5.3 ± 4.1	<.01
Fertilization rate	69	71	.9
No. of embryos transferred	2.2 ± 1.0	2.4 ± 1.4	.85
Implantation rate (%)	16.7	16.3	.96
Clinical pregnancy rate			
Per started cycle (%)	25.5 (12/47)	26.9 (14/52)	.88
Per ET (%)	50 (10/20)	42.4 (14/33)	.59
Ongoing pregnancy rate			
Per started cycle (%)	19.1 (9/47)	13.5 (7/52)	.44
Per ET (%)	40 (8/20)	21.2 (7/33)	.14
Pregnancy loss rate (%)	25 (3/12)	50 (7/14)	.18

Note: Values are expressed as mean ± standard deviation or percentage rates (%). ET = embryo transfer; 2PN = 2 pronuclei.

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19.1% (9 out of 47), versus for LPG, 13.5% (7 out of 52). Two patients in the LA group achieved pregnancy after intrauterine insemination. Three instances of multifetal gestation (two sets of twins and one set of triplets) were noted in the letrozole-treated patients.

We followed up with the patients in the LA group who did not achieve an ongoing pregnancy (n = 38); 31 were referred to our donor oocyte program or opted for no further treatment. Seven patients decided to attempt an additional IVF cycle, and most of them (six out of seven) were canceled.

DISCUSSION

Poor response to COH connotes a quantitative reduction of follicular response and thus a lower number of oocytes retrieved. It is a major concern in assisted reproduction, and the best treatment option remains controversial. In this study, we compared the use of letrozole (LA) with luteal E₂/GnRH antagonist (LPG) in women with known prior low response. We noted statistically significantly lower gonadotropins used and E₂ levels with the LA protocol. A trend toward higher cancellation rate was noted in the LA group, possibly due to inclusion of more severe poor responders. Of interest was the lower number of metaphase II oocytes in the LA group despite administering hCG when the lead follicle measured 20 mm. The mean number of oocytes retrieved, transferable embryos, and implantation rate were comparable, as was the clinical pregnancy rate. There was a trend toward a higher ongoing pregnancy rate per ET in the LA group accompanied by a lower miscarriage rate. This may be explained by a possible improvement in endometrial receptivity or oocyte quality in the LA group. The lack of a statistically significant difference in the ongoing pregnancy rates may also be attributed to a type II error as the study was not powered enough to detect a difference of 20% in the ongoing pregnancy rate per ET between the two groups.

The luteal synchronization of follicular growth to increase oocyte yield was initially proposed by deZiegler et al. (18). Dragisic et al. (4) reported that the use of luteal E₂/antagonist yielded a lower can-

cellation rate and a higher number of retrieved oocytes and embryos transferred. Our group (19) compared 45 patients who underwent the LPG protocol with 76 who were stimulated with microdose GnRH agonist. The number of oocytes, available embryos, and pregnancy rates were comparable in both groups.

Letrozole is a third-generation highly selective nonsteroidal AI approved for use in postmenopausal women with breast cancer (20). It inhibits the aromatase by competitively binding to the heme of the cytochrome P450 subunit of the enzyme, resulting in a blockade of androstenedione conversion to E₂ (21). The resulting increase in intraovarian androgens may have a profound effect on early follicle growth, and androgen-receptor gene expression may be up-regulated in preantral and antral follicles (22, 23). It has been shown in primate models that androgen treatment increases the number of preantral and small antral follicles as it stimulates theca and granulosa cell proliferation and inhibits apoptosis (24, 25). The accumulation of follicular androgens may increase the follicular sensitivity through amplification of FSH-receptor gene expression (26) or stimulate the insulin-like growth factor 1 (IGF-I) system (27), which may act in synergy with FSH to promote follicular steroidogenesis (28). Some reports have shown a significantly improved ovarian response after adding transdermal testosterone before FSH treatment (29, 30). Also, AIs have been introduced as alternative ovulation-induction agents either alone (31) or as an adjunct to gonadotropins (32) with no apparent adverse effect on endometrium as was noted with antiestrogen therapies (32, 33). Mitwally and Casper (10) also reported an improved response to FSH stimulation with letrozole cotreatment in poor responders undergoing COH. Furthermore, treatment with AIs together with recombinant FSH in IVF cycles was found to be effective in reducing the total dose of gonadotropins and consequently the cost of IVF (14, 15). The reduced serum E₂ concentrations achieved with letrozole may limit the negative effect of cumulative E₂ levels on oocyte quality and endometrial receptivity (9, 34) while maintaining an adequate follicular development and estrogen biosynthesis (26).

Garcia-Velasco et al. (13) evaluated the effect of adding letrozole to an antagonist–gonadotropin regimen in 147 patients with a previously canceled IVF cycle. Despite similar doses of gonadotropins, the number of oocytes retrieved (6.1 vs. 4.3) and implantation rate (25% vs. 9.4%) were statistically significantly higher with letrozole.

Ozmen et al. (14), evaluated 70 couples attempting a second IVF cycle with a history of poor ovarian response. The patients were evenly randomized to either 5 mg/day of letrozole plus a fixed high dose of recombinant FSH, or the same recombinant FSH dose only. A flexible antagonist regimen was used in both groups. The mean total dose of gonadotropins and the cancellation rate were statistically significantly lower in the letrozole group. The clinical pregnancy rate per ET was comparable (25.8% and 20%), but the cost of achieving a clinical pregnancy was lower in the letrozole group. Similarly, a lower dose of gonadotropins and terminal E₂ were reported earlier by Goswami et al. (15).

A letrozole/antagonist protocol was also compared with the microdose flare protocol (16). The MF protocol was found to yield a higher ongoing pregnancy rate (52% vs. 37%). Of note, the higher success rate reported in this study together with the broad definition of poor response, including patients with predicted rather than actual poor response, has hindered the applicability of these findings to the general population of true poor responders (14, 17).

A recent large retrospective study (17) favored the letrozole protocol in comparison with the MF protocol in terms of higher rates of fertilization (85% vs. 80%) and implantation (14.5% vs. 9.8%). The cancellation rate was higher in the letrozole group (54.3%) compared with the MF group (35.7%). The clinical PR per started cycle was, however, similar in both groups (11.2% for MF vs. 10.4% for letrozole).

To the best of our knowledge, our study has been the first to compare the LPG protocol with the LA protocol in poor responders. We used 5 mg daily of letrozole for 5 days starting on the second day of menstrual cycle rather than the 2.5 mg/day dose administered in most of other studies (13, 15–17). The primary studies done to test the intrinsic potency of letrozole showed that, at doses of 2.5 mg/day, estrogen is inhibited by 97% (35); however, these studies were performed on nonstimulated granulosa cells, so a higher dose of AI may be needed to achieve comparable attenuation of aromatization in the actively dividing granulosa cells. A study comparing the efficacy of 2.5 mg versus 5 mg of letrozole plus gonadotropins in women undergoing COH and intrauterine insemination found that women treated with 5 mg required a lower dose of FSH (36).

Gonadotropins were added in our study 3 days after initiation of letrozole. Most of the other studies have started gonadotropins simultaneously with letrozole. We started gonadotropins 3 days later to allow the release of endogenous gonadotropins before initiation of exogenous stimulation. Another difference in our study was the administration of hCG when two follicles had reached ≥ 20 mm (37) in the letrozole group. Nevertheless, the number of metaphase II (MII) oocytes was still statistically significantly lower, as similarly demonstrated by others (17). It is interesting that all prior studies used 17 or 18 mm diameter as a criterion for hCG trigger, with no reported increased proportion of immature oocytes. The risk of a premature LH surge was not addressed in most studies. In our study, there was a trend toward a higher incidence of premature LH surges (LH ≥ 10 mIU/mL) in the LA group compared with the LPG group (14.9% vs 3.8%). This suggests that an early start and possibly a higher dose of the antagonist should be considered when using letrozole (38). In a study of normal responders, the use of letrozole was associated with higher levels of median LH concentration (39). Similar to clomiphene citrate protocols, the risk of premature LH surge appears to be increased in AI protocols (38), and it tends to occur at lower E₂ levels (11). Ovaries with diminished ovarian reserve are more prone to a premature LH surge, presumably due to the reduced production of GnRH-attenuating factor (11).

The use of letrozole in a conception cycle raises some safety issues. Despite an initial abstract reporting an increased risk of congenital cardiac malformations (40), reassuring data (41, 42) have since emerged to indicate that there is no such increase in the overall rates of congenital malformations or chromosomal abnormalities among a large number of children born to women treated with letrozole. In our study, we discontinued letrozole on day 6, 1 week or more before ET (half-life 45 hours) (35).

We were not able to identify a subgroup of low responders who would benefit from adding an AI to the antagonist-based protocol. We showed reasonable IVF outcomes after using letrozole/gonadotropins for COH in this poor prognosis group of low responders, comparable with the use of the luteal E₂/GnRH antagonist protocol. It appears from this study that using letrozole may require optimization to avoid a premature LH surge and to increase the yield of mature oocytes. Because of the retrospective nature of our study, these findings should be confirmed by prospective randomized trials with adequate power to test the efficacy of AI-based protocols against other interventions in low responders.

REFERENCES

1. Klugman I, Rosenwaks Z. Differentiating clinical profiles: predicting good responders, poor responders, and hyperresponders. *Fertil Steril* 2001;76:1185–90.
2. Surrey E, Schoolcraft W. Evaluating strategies for improving ovarian response of the poor responder undergoing assisted reproductive techniques. *Fertil Steril* 2000;73:667–76.
3. Broekmans F, Kwee J, Hendriks D, Mol B, Lambalk C. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* 2006;12:685–718.
4. Dragisic KG, Davis OK, Fasoulitis SJ, Rosenwaks Z. Use of a luteal estradiol patch and a gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation for in vitro fertilization in poor responders. *Fertil Steril* 2005;84:1023–6.
5. Elassar A, Mann JS, Engmann L, Nulsen J, Benadiva C. Luteal phase estradiol and antagonist protocol for controlled ovarian stimulation before in vitro fertilization in poor responders. *Fertil Steril* 2011;95:324–6.
6. Fanchin R, Salomon L, Castelo-Branco A, Olivennes F, Frydman N, Frydman R. Luteal estradiol pre-treatment coordinates follicular growth during controlled ovarian hyperstimulation with GnRH antagonists. *Hum Reprod* 2003;18:2698–703.
7. Fanchin R, Castelo Branco A, Kadoch IJ, Hosny G, Bagirova M, Frydman R. Premenstrual administration of gonadotropin-releasing hormone antagonist coordinates early antral follicle sizes and sets up the basis for an innovative concept of controlled ovarian hyperstimulation. *Fertil Steril* 2004;81:1554–9.
8. Fanchin R, Cunha-Filho JS, Schonauer LM, Righini C, de Ziegler D, Frydman R. Luteal estradiol administration strengthens the relationship between day 3 follicle-stimulating hormone and inhibin B levels and ovarian follicular status. *Fertil Steril* 2003;79:585–9.
9. Mitwally MF, Casper RF, Diamond MP. The role of aromatase inhibitors in ameliorating deleterious effects of ovarian stimulation on outcome of infertility treatment. *Reprod Biol Endocrinol* 2005;4:3–54.
10. Mitwally MF, Casper RF. Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders. *Fertil Steril* 2002;77:776–80.
11. de Ziegler D, Mattenberger C, Schwarz C, Ibecheole V, Fournet N, Bianchi-Demicheli F. New tools for optimizing endometrial receptivity in controlled ovarian hyperstimulation: aromatase inhibitors and LH/(mini)hCG. *Ann NY Acad Sci* 2004;1034:262–77.

12. Ulloa-Aguirre A, Midgley AR, Beitins IZ, Padmanabhan V. Follicle-stimulating isohormones: characterisation and physiological relevance. *Endocr Rev* 1995;16:765–87.
13. Garcia-Velasco JA, Moreno L, Pacheco A, Guillén A, Duque L, Requena A, et al. The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study. *Fertil Steril* 2005;84:82–7.
14. Ozmen B, Sonmezer M, Atabekoglu CS, Olmus H. Use of aromatase inhibitors in poor-responder patients receiving GnRH antagonist protocols. *Reprod Biomed Online* 2009;19:478–85.
15. Goswami SK, Das T, Chattopadhyay R, Sawhney V, Kumar J, Chaudhury K, et al. A randomized single-blind controlled trial of letrozole as a low-cost IVF protocol in women with poor ovarian response: a preliminary report. *Hum Reprod* 2004;19:2031–5.
16. Schoolcraft WB, Surrey ES, Minjarez DA, Stevens JM, Gardner DK. Management of poor responders: can outcomes be improved with a novel gonadotropin-releasing hormone antagonist/letrozole protocol? *Fertil Steril* 2008;89:151–6.
17. Yarali H, Esinler I, Polat M, Bozdag G, Tiras B. Antagonist/letrozole protocol in poor ovarian responders for intracytoplasmic sperm injection: a comparative study with the microdose flare-up protocol. *Fertil Steril* 2009;92:231–5.
18. de Ziegler D, Jääskeläinen AS, Brioschi PA, Fanchin R, Bulletti C. Synchronization of endogenous and exogenous FSH stimuli in controlled ovarian hyperstimulation (COH). *Hum Reprod* 1998;13:561–4.
19. Weitzman VN, Engmann L, DiLuigi A, Maier D, Nulsen J, Benadiva C. Comparison of luteal estradiol patch and gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation versus microdose gonadotropin-releasing hormone agonist protocol for patients with a history of poor in vitro fertilization outcomes. *Fertil Steril* 2009;92:226–30.
20. Winer EP, Hudis C, Burstein HJ, Chlebowski RT, Ingle JN, Edge SB, et al. American Society of Clinical Oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for women with hormone receptor positive breast cancer. *J Clin Oncol* 2002;20:3317–27.
21. Akhtar M, Njar V, Wright JN. Mechanistic studies on aromatase and related C-C bond cleaving P-450 enzymes. *J Steroid Biochem Mol Biol* 1993;44:375–87.
22. Murray AA, Gosden RG, Allison V, Spears N. Effect of androgens on the development of mouse follicles growing in vitro. *J Reprod Fertil* 1998;113:27–33.
23. Hillier SG, Tetsuka M, Fraser HM. Location and developmental regulation of androgen receptor in primate ovary. *Hum Reprod* 1997;12:107–11.
24. 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;101:2622–9.
25. Weil SJ, Vendola KA, Zhou J, Adesanya OO, Wang J, Okafor J, et al. Androgen receptor gene expression in the primate ovary: cellular localization, regulation, and functional correlations. *J Clin Endocrinol Metab* 1998;83:2479–85.
26. Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *J Clin Endocrinol Metab* 1998;84:2951–6.
27. Vendola KA, Zhou J, Wang J, Bondy CA. Androgens promote the IGF-I and IGF-I receptor gene expression in the primate ovary. *Hum Reprod* 1999;14:2328–32.
28. Demeestere I, Gervy C, Centner J, Devreker F, Englert Y, Delbaere A. Effect of insulin-like growth factor-I during preantral follicular culture on steroidogenesis, in vitro oocyte maturation, and embryo development in mice. *Biol Reprod* 2004;70:1664–9.
29. Massin N, Cedrin-Durnerin I, Coussieu C, Galey-Fontaine J, Wolf JP, Hugues JN. Effects of transdermal testosterone application on the ovarian response to FSH in poor responders undergoing assisted reproduction technique—a prospective, randomized, double-blind study. *Hum Reprod* 2006;21:1204–11.
30. Balasch J, Fabregues F, Penarrubia J, Carmona F, Casamitjana R, Creus M, et al. Pretreatment with transdermal testosterone may improve ovarian response to gonadotrophins in poor-responder IVF patients with normal basal concentrations of FSH. *Hum Reprod* 2006;21:1884–93.
31. Mitwally MF, 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.
32. Mitwally MF, Casper RF. Aromatase inhibition reduces gonadotropin dose required for controlled ovarian stimulation in women with unexplained infertility. *Hum Reprod* 2003;18:1588–97.
33. Fisher SA, Reid RL, Van Vught DA. A randomized double-blind comparison of the effects of clomiphene citrate and the aromatase inhibitor letrozole on ovulatory function in normal women. *Fertil Steril* 2002;78:280–5.
34. Papanikolaou EG, Bourgain C, Kolibianakis E. Steroid receptor expression in late follicular phase endometrium in GnRH antagonist IVF cycles is already altered, indicating initiation of early luteal phase transformation in the absence of secretory changes. *Hum Reprod* 2005;20:1541–7.
35. Mitwally MF, Casper RF. Aromatase inhibitors in ovulation induction. *Semin Reprod Med* 2004;22:61–78.
36. Noriega-Portella L, Noriega-Hoces L, Delgado A, Rubio J, Gonzales-Castaneda C, Gonzales GF. Effect of letrozole at 2.5 mg or 5.0 mg/day on ovarian stimulation with gonadotropins in women undergoing intrauterine insemination. *Fertil Steril* 2008;9:1818–25.
37. Oktay K, Hourvitz A, Sahin G, Oktom O, Saftro B, Cil A, et al. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. *Clin Endocrinol Metab* 2006;91:3885–90.
38. Lee TH, Lin YH, Seow KM, Hwang JL, Tzeng CR, Yang YS. Effectiveness of cetrorelix for the prevention of premature luteinizing hormone surge during controlled ovarian stimulation using letrozole and gonadotropins: a randomized trial. *Fertil Steril* 2008;90:113–20.
39. Verpoest WM, Kolibianakis E, Papanikolaou E, Smits J, Van Steirteghem A, Devroey P. Aromatase inhibitors in ovarian stimulation for IVF/ICSI: a pilot study. *Reprod Biomed Online* 2006;13:166–72.
40. Biljan MM, Hemmings R, Brassard N. The outcome of 150 babies following the treatment with letrozole or letrozole and gonadotropins [abstract]. *Fertil Steril* 2005;84(Suppl 1):S95.
41. Tulandi T, Martin J, Al-Fadhli R, Kabli N, Forman R, Hitkari J, et al. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. *Fertil Steril* 2006;85:1761–5.
42. Forman R, Gill S, Moretti M, Tulandi T, Koren G, Casper R. Fetal safety of letrozole and clomiphene citrate for ovulation induction. *J Obstet Gynaecol Can* 2007;29:668–71.