A randomized single-blind controlled trial of letrozole as a low-cost IVF protocol in women with poor ovarian response: a preliminary report

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BACKGROUND: Use of letrozole, a selective inhibitor of aromatase, reduces the gonadotrophin dose required to induce follicular maturation. We evaluated whether incorporation of letrozole could be an effective low-cost IVF protocol for poor responders. METHODS: A randomized, controlled, single-blind trial was conducted in the Assisted Reproduction Unit, Institute of Reproductive Medicine, Kolkata, India. Thirty-eight women with a history of poor ovarian response to gonadotrophins were recruited. Thirteen women (Let-FSH group) received letrozole 2.5 mg daily from day 3–7, and recombinant FSH (rFSH) 75 IU/day on days 3 and 8; and 25 women (GnRH-ag-FSH group) underwent long GnRH agonist protocol and stimulated with rFSH (300–450 IU/day). Ovulation was triggered by 10 000 IU of HCG followed by IVF-embryo transfer. The main outcome measures were total dose of rFSH (IU/cycle), terminal estradiol (E2) (pg/ml), numbers of follicles, oocytes retrieved and transferable embryo, endometrial thickness (mm), and pregnancy rate. RESULTS: Compared with the GnRH-ag-FSH group (2865 ± 228 IU), the Let-FSH group (150 ± 0 IU) received a significantly (P < 0.001) lower total dose of FSH. Except for terminal E2, which was significantly higher (P < 0.001) in the GnRH-ag-FSH group (380 ± 46 pg/ml) than the Let-FSH group (227 ± 45 pg/ml), the treatment outcomes in all other respects, including pregnancy rate, were statistically comparable. CONCLUSIONS: Adjunctive use of letrozole may form an effective means of low-cost IVF protocol in poorly responding women.

Key words: aromatase inhibitor/letrozole/low-cost IVF/poor ovarian responders

Introduction
Fertility is known to decline significantly in women after the age of 35 years, and fecundity is almost completely lost after 45 years of age (Serhal and Craft, 1989; Sauer et al., 1990). Advanced female age is associated with poor hormonal and follicular response to controlled ovarian hyperstimulation (COH). The low ovarian response is often the result of diminished ovarian reserve that may be caused by advanced age, prior ovarian surgery, environmental and genetic factors (Toner et al., 1991), severe endometriosis (Wardle et al., 1985) or pelvic infections (Keay et al., 1998). In most of the patients, however, low ovarian response to FSH stimulation remains unexplained (Ben-Rafael et al., 1986). Satisfactory pregnancy rates in older women undergoing oocyte donation indicates that fertility wanes with age in women because of a decline in oocyte quality and ovarian aging, rather than uterine aging (Sauer et al., 1990; Navot et al., 1991). Increasing patient age is associated with poor ovarian response, as represented by smaller ovarian volume, lower antral follicle count and poor stromal vascularity (Kupesic et al., 2003). Various protocols of ovarian stimulation have been proposed for optimizing IVF results in the poorly responding women (Tarlatzis et al., 2003); however, good response to stimulation still remains a challenge. The most extensively employed strategy to improve follicular response in these so-called ‘poor responders’ involves the use of high doses of gonadotrophins. A number of randomized controlled trials (van-Hooff et al., 1993; Rombauts et al., 1998) and retrospective studies (Land et al., 1996) have evaluated the effectiveness of high-dose FSH. The results of these studies have shown it to be of little or no benefit, although the cost of treatment, which is chiefly ascribed to high dose of gonadotrophins, was very high.

Aromatase inhibition has recently been focused on as an effective means of ovulation induction in the management of infertility (Mitwally and Casper, 2002a; b; 2003a; b).
Letrozole, a highly selective, non-steroidal aromatase inhibitor, could successfully induce ovulation in women with polycystic ovary syndrome (PCOS) (Mitwally and Casper, 2000), including even those who were resistant to clomiphene citrate (Mitwally and Casper, 2001). Recent studies demonstrated that addition of letrozole improved ovarian response to FSH in poor responders and reduced gonadotrophin dose required for COH in women with unexplained infertility (Mitwally and Casper, 2002a; 2003a). Healey et al. (2003) demonstrated that addition of letrozole to gonadotrophins increased the number of pre-ovulatory follicles without having a negative impact on pregnancy rates. These reports prompted us to hypothesize that adjunctive use of letrozole in COH protocol would minimize the gonadotrophin dose, and consequently the cost, of an IVF treatment cycle. The present investigation is an endeavour to evaluate the potential of letrozole–gonadotrophin combination therapy as a low-cost stimulation protocol in older women with poor ovarian response.

Materials and methods

Patients and treatment

This pilot study was performed as a randomized, single-blind, controlled trial in the Assisted Reproduction Unit, Institute of Reproductive Medicine, Salt Lake City, Kolkata, India, between July 2002 and August 2003. Approval by the Research Ethics Board of the Institute was obtained for the use of letrozole for ovarian stimulation and subsequent induction of IVF. Forty-eight women over 35 years of age, who had failed one to three IVF attempts due to poor ovarian response to conventional long GnRH agonist stimulation protocol, were primarily selected for this study. There were one to three un-intervened cycles between the last IVF attempt and the current treatment cycle. All patients were evaluated for their basal (day 2) gonadotrophin levels and other endocrinopathy during the cycle preceding the index cycle. Cases with severe endometriosis ($n=4$), history of previous pelvic surgery ($n=3$) or baseline (day 2) FSH $\geq 12$ mIU/ml ($n=1$) were carefully identified and excluded (Figure 1). Two women refused to participate in this study. The remaining 38 women (age range 36–41 years) were evaluated in the present study. Thirteen women were randomly selected (see below) and treated with the proposed low-cost IVF protocol consisting of letrozole and recombinant FSH (rFSH) (Let-FSH group), while the remaining 25 subjects were treated with the conventional long down-regulated protocol with incremental dose of rFSH (300–450 IU) (GnRH-ag-FSH group). Allocation of participants in the two groups was intentionally made unequal with a lesser number of subjects treated with letrozole, as this was a relatively new drug in the field of infertility management. The randomization ratio of Let-FSH protocol to GnRH-ag-FSH protocol was taken to be 1:2. Enrolling and counselling the participants and obtaining informed consent from each of them before randomization were carried out as per the institution’s protocol.

Sequentially numbered sealed envelopes were prepared and provided by the study coordinator, according to random-number tables.

Figure 1. Revised template of the CONSORT diagram showing the flow of participants through each stage of a randomized trial.
A double-blind study protocol was not possible, as the drug delivery method in the two groups was different. However, single-blinding was achieved by keeping the person enrolling participants, study investigators, ultrasound technicians and clinicians unaware of the type of protocol used. Only the statisticians had access to the unblinded data.

The Let-FSH group of patients received letrozole (Letroz; Sun Pharmaceuticals, Mumbai, India) at a dose of 2.5 mg daily orally from day 3 to 7 of the menstrual cycle, and rFSH (Gonal-F; Serono, Aubonne, Switzerland) was administered subcutaneously at a dose of 75 IU/day on days 3 and 8 of the menstrual cycle. The patients in the GnRH-ag-FSH group received daily subcutaneous injection of 500 µg of a GnRH agonist, leuprolide acetate (Lupride4; Sun Pharmaceuticals) starting from the mid-luteal phase of the previous cycle and continuing for a period of 14 days, or until the onset of the next menstruation, whichever was earlier. If the patient did not start menstruating by day 14 of GnRH agonist treatment, estradiol (E2) and LH levels were assayed and analysed. E2 levels ≤10 pg/ml or LH ≤3 mIU/ml were considered the presumptive evidences of down-regulation. However, if the patient did not meet the above criteria, GnRH agonist was continued for a further period of 4 days at the same dose level. The patients not reaching the set criteria for down-regulation even after extended GnRH agonist therapy were excluded from the study. rFSH was administered subcutaneously to the down-regulated subjects at a dose of 300 IU/day, with subsequent adjustment of the dose according to the dose–response scheme.

All patients were monitored for ovarian follicular development by transvaginal ultrasonography. When the average diameter of the leading follicle(s) reached ≥18 mm, blood was drawn for the assessment of terminal E2 and they were administered 10 000 IU HCG (Profasi, Serono, Switzerland) subcutaneously as a single dose. Oocytes were retrieved by transvaginal ultrasonography ~34–36 h after HCG administration. The retrieved oocytes were inseminated with spermatozoa of the husband. Embryo transfer was performed 40–42 h following insemination at 4–6 cell cleavage stages. All patients received 600 mg micronized progesterone (Ulteragestan; Laboratories Besins International, Paris, France) intravaginally daily until a pregnancy test was performed, and if the test was positive, progesterone treatment was continued up to 12 gestational weeks. Clinical pregnancy was defined when an ultrasound scan, performed 5 weeks after embryo transfer, revealed the presence of a viable fetus.

**Immunooassay of hormones**

Serum levels of LH and FSH were measured by a two-site chemiluminescent sandwich immunoassay system (ACS:180; Bayer Diagnostics Corporation, Tarrytown, NY, USA). All samples were assayed in duplicate. The LH and FSH values were expressed in terms of the reference standards (WHO 2nd IS 94/632 and WHO 2nd IS 80/552, respectively). Assay sensitivity for FSH was 0.3 mIU/ml and for LH was 0.07 mIU/ml. E2 levels were assayed by fully automated enzyme-linked fluorescence assay system (Vidas; bioMérieux, Marcy l’Étoile, France). The minimum detection limit was 9 pg/ml. The intra- and inter-assay coefficients of variation were 3.46% and 4.82% for FSH, 4.4% and 5.6% for LH and 4.2% and 5.2% for E2, respectively.

The primary outcome measure was comparative evaluation of pregnancy outcome, while additional measures included total dose of FSH administered, the number of mature follicles, the levels of terminal E2, number of oocytes retrieved, endometrial thickness and transferable embryos.

**Table I. Clinical characteristics of subjects treated with Let-FSH and GnRH-ag-FSH protocols**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Body mass index (kg/m²)</th>
<th>Basal FSH (IU/l)</th>
<th>Basal LH (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Let-FSH (n = 13)</td>
<td>38.5 ± 1.7</td>
<td>26.2 ± 3.1</td>
<td>7.5 ± 2.1</td>
<td>4.1 ± 1.9</td>
</tr>
<tr>
<td>GnRH-ag-FSH (n = 25)</td>
<td>39.1 ± 1.1</td>
<td>26.8 ± 3.6</td>
<td>8.6 ± 2.2</td>
<td>5.5 ± 2.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*All patients were euthyroid and normoprolactinaemic.

**Results**

All patients were euthyroid and normoprolactinaemic, and both groups were comparable with respect to all baseline clinical characteristics (Table I). The composite results on treatment outcome are summarized in Table II. According to the set criteria, all patients achieved GnRH receptor down-regulation within the stipulated period of GnRH agonist therapy. Compared with the GnRH-ag-FSH group, the Let-FSH group received an ~20-fold lower (P < 0.001) total dose of FSH (GnRH-ag-FSH 2865 ± 228 IU/cycle versus Let-FSH 150 ± 0 IU/cycle), and had significantly (P < 0.001) decreased levels of terminal E2 (GnRH-ag-FSH 380 ± 46 pg/ml versus Let-FSH 227 ± 45 pg/ml). However, the two groups did not differ significantly with respect to the numbers of matured follicles (GnRH-ag-FSH 2.3 ± 0.8 versus Let-FSH 1.8 ± 0.8), the numbers of retrieved oocytes (GnRH-ag-FSH 2.1 ± 0.7 versus Let-FSH 1.6 ± 0.8) and numbers of transferable embryo (GnRH-ag-FSH 1.3 ± 0.5 versus Let-FSH 1.2 ± 0.4), and endometrial thickness (mm) (GnRH-ag-FSH 7.4 ± 0.4 versus Let-FSH 8.5 ± 0.4).

There was no case of drop-out from either of the groups. One stimulated cycle in each group was cancelled due to poor ovarian response (emergence of no dominant follicle). The pregnancy rate/stimulated cycle also remained statistically
indifferent (Let-FSH 23% versus GnRH-ag-FSH 24%). Treatment with letrozole was well tolerated, with no apparent side effects.

Discussion
The success of aromatase inhibition by letrozole in inducing ovulation in anovulatory women with PCOS (Mitwally and Casper, 2000) and augmenting ovulation in ovulatory women (Mitwally and Casper, 2001) has been reported previously. It has also been shown that when letrozole is used with FSH, a significant reduction occurs in the FSH dose needed for COH (Mitwally and Casper, 2001). The successful use of letrozole in increasing ovarian sensitivity to gonadotrophins and inducing superovulation in poor responders (Mitwally and Casper, 2002a) prompted us to evaluate the potential role of letrozole–FSH combination in achieving successful IVF outcome in women with poor ovarian response.

There are no consistent criteria to define ‘poor responders’. The definition is usually based on previous ovarian stimulation cycles, but the parameters vary between studies. In the present investigation, women over 35 years of age exhibiting emergence of fewer than two dominant follicles in response to conventional stimulation protocol were defined as ‘elderly’ and ‘poor responders’. There are reports that the poor responders respond better to ‘flare-up’ protocols than to standard ‘long’ luteal protocols (Scott and Navot, 1994; Toth et al., 1996). In our set-up, however, long protocol down-regulation is the method of choice, and irrespective of age and follicular reserve of the women, our experience with flare-up protocol is not encouraging. The result of the study group was therefore compared with those of the conventional long-term down-regulated protocol.

It is of particular importance to note that despite the use of ~20-fold lower dose of FSH in the Let-FSH group, which entailed significant reduction of the cost of treatment, the outcomes in all major respects including pregnancy rate were comparable between the groups.

The precise mechanism of the ovarian effects of letrozole is as yet unexplored; however, some of the earlier observations and propositions on the effects of letrozole can be extrapolated to formulate a hypothesis. During the reproductive years, estrogens are chiefly produced in the ovary under the stimulation of aromatase. As the menopausal stage approaches, there occurs a decline in ovarian estrogen production; however, the extragonadal sites, notably adipocytes, continue to contribute peripheral production of estrogens that may act locally as paracrine or even intracellular factors (Labrie et al., 1997; Simpson et al., 2000). Because of selective inhibition of aromatase, letrozole significantly inhibited the overall production of estrogens, which was reflected in the decreased levels of terminal E2 in the Let-FSH group. Consequent withdrawal of the negative feedback effects of estrogens may allow the pituitary to produce more endogenous FSH. Moreover, attenuated aromatization may secondarily lead to accumulation of follicular androgens, which may increase the follicular sensitivity through amplification of FSH receptor gene expression (Vendola et al., 1999; Weil et al., 1999) or stimulate insulin-like growth factor-I, which may act in synergy with FSH (Giudice, 1992; Palter et al., 2001). All these effects may have phenomenal importance in the letrozole-mediated promotion of follicular maturation. It may be significant in this context to emphasize that the absence of GnRH down-regulation in this proposed low-cost protocol may entail premature LH surge and luteinization, leading to cancellation of the index cycle. However, possibly due to the small sample size, this problem was not encountered in the present study.

This preliminary study, designed to evaluate the efficacy of Let-FSH as a low-cost IVF protocol, involved a small number of patients; however, the results have been encouraging. We have been successful in reaching our objective to modify an expensive conventional down-regulated IVF protocol into one in which the cost was reduced to a larger extent, without compromising the rate of success. A number of protocols of ovarian stimulation have been proposed for poorly responding women, but these have little or no proven benefit (Karande et al., 1990; van-Hooff et al., 1993; Land et al., 1996). Moreover, the cost of treatment, chiefly owing to the high cost of gonadotropins, is frequently prohibitive. Suggestions have therefore been made that natural cycle IVF, which may produce high-quality embryo(s) without a high cost involvement, may be considered for so-called elderly poor responders; however, likelihood of pregnancy has been reported to be low (Bar-Hava et al., 2000). The present study bears the promise that as an alternative to natural cycle IVF, letrozole may have future prospects as a cost-saving stimulation protocol for IVF in women with poor ovarian response. Nevertheless, larger randomized studies are needed to confirm these data. It must also be taken into consideration that the use of letrozole as a low-cost IVF protocol, though exciting, has to be evaluated further carefully, as letrozole and other aromatase inhibitors have not been extensively used in women of reproductive age. This has been the main reason for employing selectively elderly women for this pilot study.

Acknowledgements
We thank Ms Manisha Dam of IRM for technical assistance.

References


Submitted on January 19, 2004; accepted on May 18, 2004