Article

Use of aromatase inhibitors in poor-responder patients receiving GnRH antagonist protocols

Dr Batuhan Özmen completed his residency programme in obstetrics and gynaecology at University of Ankara in 2003, obtaining his certification in reproductive medicine and artificial reproductive techniques in 2005. In 2006, he worked in the Department of Obstetrics and Gynecology of the University of Schleswig-Holstein in Lübeck under the supervision of Professor Safaa Al-Hasani on laboratory applications in reproductive medicine. He is interested in cryopreservation and vitrification of human gametes, reproductive surgery and human sperm DNA damage. He is now working in the Department of Reproductive Endocrinology at the University of Ankara, Turkey.

Dr Batuhan Özmen

Batuhan Ozmen[1,2,4], Murat Sönmezer[1,2], Cem Somer Atabekoglu[1,2], Hülya Olmuş[3]
[1]Center for Research on Human Reproduction, School of Medicine, University of Ankara, Ankara, Turkey; [2]Department of Obstetrics and Gyne2;cology, School of Medicine, University of Ankara, Ankara, Turkey; [3]Department of Statistics, Faculty of Science and Art, University of Gazi, Ankara, Turkey
[4]Correspondence: e-mail: batuhanozmen@gmail.com

Abstract

The efficacy of aromatase inhibitors incorporated in the ovarian stimulation protocols of poor-responder patients undergoing intracytoplasmic sperm injection-embryo transfer cycles was investigated. A total of 70 poor-responder patients were randomized into two groups on day 3 of their menstrual cycle. In Group A, an aromatase inhibitor (letrozole, 5 mg/day) was administered along with a fixed dosage (450 IU/day) of recombinant FSH (rFSH), whereas Group B were treated with the same rFSH dosage alone. A flexible regimen of gonadotrophin-releasing hormone antagonist was administered in both groups. The mean total dose of rFSH (2980 ± 435 IU versus 3850 ± 580 IU, $P < 0.05$) and serum concentrations of oestradiol on the day of human chorionic gonadotrophin administration (1870 ± 159 pg/ml versus 2015 ± 175 pg/ml, $P < 0.05$) were significantly lower in Group A compared with Group B, respectively. The rate of cycle cancellation due to poor ovarian response was lower in Group A (8.6%) than in Group B (28.6%), ($P < 0.05$). The costs of achieving a clinical pregnancy were US$11560 and US$17584, and the clinical pregnancy rates per embryo transfer were 25.8% and 20%, in groups A and B, respectively. In conclusion, adjunctive letrozole administration seems to restore an IVF cycle by decreasing the rate of cycle cancellation and seems to reduce the cost by reducing the total gonadotrophin dosage.

Keywords: *aromatase inhibitor, cost, GnRH antagonist, ICSI, poor response*

Introduction

Poor ovarian response to standard ovulation induction protocols, which mainly reflects diminished ovarian reserve, still remains as a major challenge in assisted reproduction. The clinical outcomes of assisted reproduction were reported as negatively affected in cases with poor ovarian response; however, the definition of poor response is still controversial (Kailasam *et al.*, 2004; Muasher *et al.*, 2006). Increased cycle cancellation and increased gonadotrophin consumption are the main encountered problems in ovulation induction regimens of these patients. Therefore, in addition to low success and decreased conception rates, the cost of treatment also increases.

Gonadotrophin-releasing hormone (GnRH) antagonists, due to their reported advantages over GnRH agonists, have more recently been recommended in ovarian stimulation cycles of poor-responders (Fasouliotis *et al.*, 2003; Shapiro and Mitchell-Leef, 2003; D'Amato *et al.*, 2004). Aromatase inhibitors (AI), other recent ovulation induction agents, suppress serum oestradiol concentrations, which results in a subsequent marked increase in serum FSH and LH concentrations. Letrozole, an AI, was reported to be an effective agent both in ovulation induction and in ovarian stimulation without any negative effects on the endometrium (Mitwally and Casper, 2001, 2002, 2003; Fisher *et al.*, 2002; Mitwally *et al.*, 2005). Furthermore, treatment with AI together with recombinant FSH (rFSH) in IVF

cycles of poor-responders was found to be effective in reducing gonadotrophin consumption and consequently the cost of IVF compared with rFSH-only regimens in GnRH analogue down-regulated cycles (Goswami *et al.*, 2004). In consequence, this study aimed to investigate the efficacy of AI in poor-responders undergoing ovarian stimulation with an GnRH antagonist regimen.

Materials and methods

Subjects

A total of 70 infertile women who were planned to undergo their second intracytoplasmic sperm injection (ICSI) – embryo transfer cycle and with a history of poor ovarian response in their first ICSI – embryo transfer cycle were enrolled in the study. Written informed consent was taken from all subjects before initiation of the treatment cycle. All of these enrolled patients had their first ICSI – embryo transfer cycle or the ovarian stimulation scheme at least 6 months prior to the current study. Poor response was determined as having at least one of the following criteria regarding the outcomes of previous ICSI – embryo transfer and ovarian stimulation cycles: (i) cycle cancellation due to low oestradiol concentrations on day 6 of the cycle (<130 pg/ml) or on the day of human chorionic gonadotrophin (HCG) administration(<450 pg/ml); or (ii) less than four retrieved oocytes. Of the enrolled patients ($n = 70$), 17 patients (24%) met criterium i, whereas 53 patients (76%) met criterium ii in their preceding cycle. The details of cycle cancellation and ovarian stimulation protocols of the first ICSI – embryo transfer and ovarian stimulation cycles are given in **Table 1**. In almost all of the patients (94%, $n = 66$) at least one of the basal hormone serum concentrations, either FSH or oestradiol, were found to be greater than the cut-off values regarding diminished ovarian reserve measured on the day 2–3 of the preceding menstrual cycle (FSH > 8.5 mIU/ml and/or oestradiol >50 pg/ml). In general, ovarian stimulation was initiated with lower gonadotrophin doses (225–300 IU/day) as a step-up protocol and then increased to higher doses (450–600 IU/day) on day 3 or subsequent follow-up, if inadequate ovarian response was encountered (**Table 1**). None of the patients included in the study had endocrine diseases including polycystic ovarian syndrome and/or thyroid disorders, autoimmune, inflammatory or genetic abnormalities.

Methods of ovarian stimulation scheme, patient monitoring and follow-up

Participants were randomized on day 2–3 of the index menstrual cycle into two groups using sealed envelopes. Group A ($n = 35$) was given 5 mg letrozole (Femara, 2 × 2.5 mg/day; Novartis, Turkey) on days 3–7 along with a fixed dose (450 IU/day) of rFSH (Puregon, 300–600 IU; Organon, Turkey; Gonal-F, 300–600 IU; Merck Serono, Turkey), which was initiated on day 5 and was continued until the day of HCG administration. In Group B ($n = 35$), the same fixed dose of rFSH alone was initiated on day 3 and was continued until the HCG day. In both groups, a multiple-dose flexible GnRH antagonist protocol (Orgalutran, 0.25 μg/day s.c.; Organon or Cetrotide 0.25 μg/day s.c.; Merck-Serono) was initiated when the leading follicle was >13–14 mm and/or serum oestradiol concentration was >350 pg/ml (**Figure 1**). Intramuscular urinary HCG (Pregnyl, 10,000 IU; Organon) was given to trigger ovulation. Follicular development was monitored by transvaginal ultrasonography (4–8 MHz vaginal probe; Shimatzu, Logic 3500, South Korea), serum oestradiol and by LH concentrations in both groups. Oocyte retrieval was performed 35–36 h after HCG triggering under local anaesthesia with a double lumen oocyte retrieval needle. Embryo transfer was performed on day 3 after oocyte retrieval with a soft transfer catheter (Swemed; Vitrolife, Kungsbacka, Sweden) under the guidance of abdominal ultrasonography (Shimatzu). A trial soft catheter was used before the current transfer procedure and cervical mucus and/or blood contact was noted. The luteal phase was supported using natural micronised progesterone vaginal capsules (Progestan, 200 mg t.i.d; Koçak Pharmacy, Turkey), which was continued throughout the first 12 weeks of gestation. Pregnancy was confirmed by a positive serum HCG concentration

Table 1. Details of cycle cancellation and ovarian stimulation protocols in the preceding intracytoplasmic sperm injection (ICSI)/embryo transfer cycles.

Cycles	n *(%)*	*Protocols*			
		GnRH-ant	*GnRH-a long protocol*	*Fixed rFSH*	*Step-up rFSH*
Total	70 (100)	58 (83)	12 (17)	54 (77)	16 (23)
Total cycles completed	41 (59)	35	6	32	9
Total cycles cancelled	29 (41)	–	–	–	–
Cycles cancelled on the 6th day	6 (9)	6	–	6	–
Cycles cancelled on HCG day	11 (16)	5	6	4	7
Cycles cancelled after oocyte retrieval[a]	12 (17)	12	–	12	–

GnRH-a = gonadotrophin-releasing hormone agonist; GnRH-ant = GnRH antagonist; HCG = human chorionic gonadotrophin;rFSH = recombinant FSH.

[a] The preceding ICSI cycle was cancelled due to following reasons; no metaphase II oocytes retrieved, no fertilization or no embryonic development.

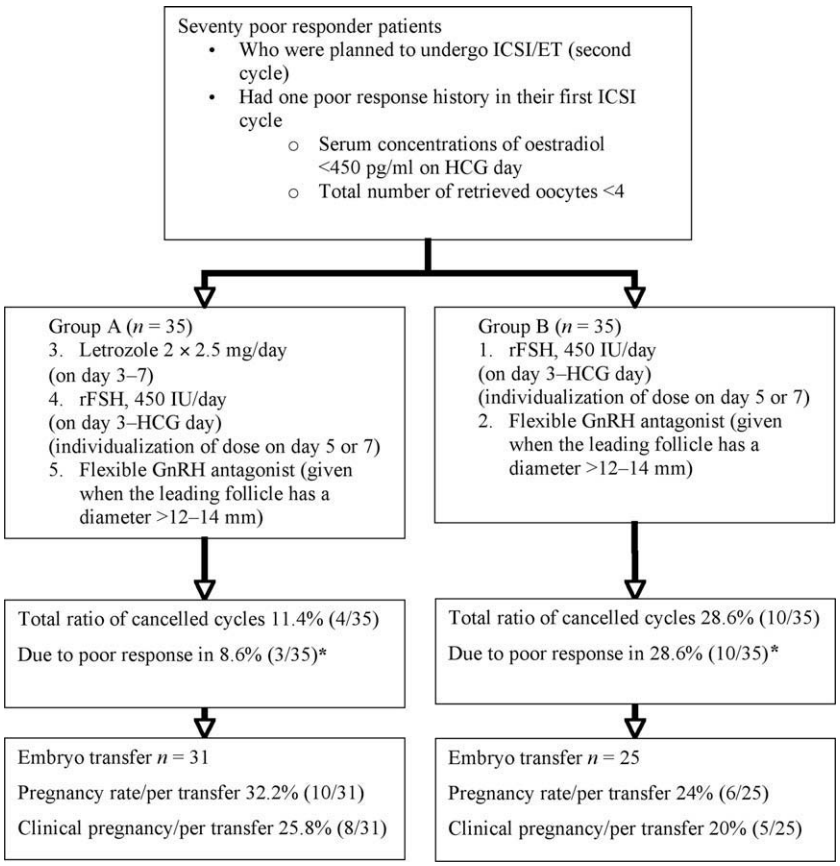

Figure 1. Flow-chart of patient participation in the study. * Statistical difference between groups A and B, $P < 0.05$.

>20 IU/ml measured on day 12 after embryo transfer, and clinical pregnancy was defined by observation of fetal heart beat using transvaginal ultrasonography on week 5–6 of gestation.

Criteria for cycle cancellations

Ovulation induction and ICSI–embryo transfer cycles were cancelled when one of the following criteria was found: (i) serum oestradiol concentrations <130 pg/ml on day 3 of gonadotrophin induction; (ii) <25% increase in serum oestradiol concentrations at 48 h intervals during ovarian stimulation; (iii) no follicular development and <50% increase in serum oestradiol at 48 intervals during follow-up; (iv) total fertilization failure; or (v) total arrest of embryonic development.

Immunoassay of hormones

Serum concentrations of LH and FSH were measured using two-site chemiluminescent sandwich immunoassay systems (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 second international standard 94/632 and WHO second international standard 80/552, respectively). Assay sensitivity for FSH was 0.3 mIU/ml and for LH was 0.07 mIU/ml. Oestradiol concentrations were assayed by fully automated enzyme-linked fluorescence assay system (Vidas; bioMérieux, Marcy l'Etoile, 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 oestradiol, respectively.

Main measurements and outcomes

The mean number of retrieved oocytes, mean number of mature oocytes (metaphase II; MII), mean number of follicles >14 mm and >17 mm on the day of HCG administration, mean serum oestradiol concentration on the day of HCG administration, mean cumulative gonadotrophin dosage, fertilization rates, mean number of 2 pronucleate (2PN) zygotes, mean number of day-2 embryos with more than four cells, mean number of day-3 embryos with more than seven cells, mean number of transferred embryos, mean number of transferred grade-A embryos, economic outcomes of the stimulation and implantation, pregnancy and clinical pregnancy rates were compared between two groups.

Statistics

Statistical comparisons were performed using Student's *t*-test, Mann–Whitney *U*-test, significance test for comparing two proportions (two-sided *z*-test) and Fisher's Exact test, as applicable. A *P* value of 0.05 was considered to be statistically significant.

Results

Details of infertility aetiologies in each group are given in **Table 2**. The mean age of participants and the mean basal hormone concentrations were not different among the groups. The mean number of follicles >14 mm or >17 mm on the day of HCG administration, mean number of retrieved oocytes and mean number of mature oocytes were similar in both groups (**Table 3**). In total, 145 and 107 MII oocytes were retrieved in groups A and B, respectively. However, the mean concentration of serum oestradiol on the day of HCG administration was found to be higher in Group B than in Group A (**Table 3**). The cumulative gonadotrophin dosage was also significantly lower in Group A than in Group B (2980 ± 435 IU versus 3850 ± 580 IU, $P < 0.05$), (**Table 3**). In Group A, total fertilization failure was encountered in only one patient, whereas in Group B, no total fertilization failure occurred. The fertilization rate was also similar among the two groups (**Table 4**). Particularly, in Group A, three patients had poor ovarian response and had the criteria of cycle cancellation due to inadequate increase in serum oestradiol with/without follicular growth that was defined in the criteria of cycle cancellation above, whereas in Group B, 10 patients met the same criteria (**Table 3**, $P < 0.05$). In total, 31 patients in Group A and 25 patients in Group B had embryo transfer after exclusion of the cancelled cycles. The mean number of 2PN zygotes, mean number of day-2 embryos with more than four cells, mean number of day-3 embryos with more than seven cells, mean number of transferred embryos, mean number of transferred Grade A embryos and the mean thickness of endometrium on the day of HCG administration were similar in both groups (**Table 3**). The rate of embryo transfer was also similar among the groups (**Table 4**). There were ten and six pregnancies, defined by positive serum HCG concentrations, in groups A and B, respectively. Of these pregnancies, eight clinical pregnancies in Group A and five clinical pregnancies in Group B were diagnosed by transvaginal ultrasound (**Figure 1**, **Table 4**). The pregnancy rate per cycle, pregnancy rate per embryo transfer and clinical pregnancy rate per embryo transfer showed a non-significant trend towards higher values in Group A compared with Group B (**Table 4**). Of the 12 patients who had less than foiur oocytes and experienced cycle cancellation in their preceding ovarian stimulation and/or ICSI–embryo transfer cycle, eight were in Group A and four were in the Group B. Of these 12 patients, two out of eight (25%) in Group A and three out of four patients (75%) in Group B again experienced cycle cancellation due to total fertilization failure in one case (in Group A) and poor ovarian response in four cases (one in Group A and three in Group B).

Economic analysis of ovarian stimulation cycles and ICSI–embryo transfer

All the costs are given both in new Turkish liras (YTL) and United States dollars wherein the exchange ratio was defined as US$0.83 for 1 YTL at the study time. After the investigation of the economic and commercial parameters of agents, cost of rFSH was calculated as 0.6866 YTL (US$0.57) per unit and total cost of the letrozole treatment per cycle (10 oral tablets) was calculated as 70 YTL (US$58). The means of the total required gonadotrophin per cycle cost were calculated as 2046 ± 298 YTL and 2643 ± 398 YTL (US$1698 $\pm$ 247 and US$2194 $\pm$ 330) in groups A and B, respectively. The difference of required dose of gonadotrophins between the two study arms was calculated as 870 IU, which represents a cost of 597 YTL (US$496). The cost of the difference of required gonadotrophin dose reached a median of 25.2% (22–29%) of the mean total cost of required gonadotrophin dose per cycle regarding the groups. The maximal total cost of a ovarian stimulation and ICSI–embryo transfer cycle was calculated to be 2116 YTL and 2643 YTL (US$1756 and US$2194), in groups A and B whereas the minimal cost was calculated to be 894 YTL and 824 YTL (US$742 and US$684), for the patients of groups A and B whose cycles were cancelled. Cost of a clinical pregnancy was calculated by the formula (sum of total costs of all patients)/(the number of diagnosed clinical pregnancies). Total costs per patient were calculated individually representing the sum of total costs of all administered induction agents during ovarian stimulation cycle and of clinical procedures such as ovarian stimulation cycle (cycle monitoring comprising folliculometry and laboratory), and ICSI–embryo transfer procedure (laboratory expenses). The costs of unused drugs and of cancelled clinical procedures were excluded. The costs of achieving a clinical pregnancy were found to be 13928 YTL and 21186 YTL (US$11560 and US$17584), in the letrozole co-treated group and rFSH-only group, respectively.

Discussion

Aromatase inhibitors have been introduced as an alternative ovulation induction agent either alone (Mitwally and Casper, 2001) or as an adjunctive ovulation induction agent along with gonadotrophins in ovarian stimulation (Mitwally and Casper, 2003). It was reported that transient inhibition of aromatase activity in early follicular phase (on days 5–9) with letrozole results in ovarian stimulation similar to clomiphene citrate with no apparent adverse effect on endometrium (Fisher *et al.*, 2002). Mitwally and Casper (2003) also supported that letrozole is effective in ovulation induction of anovulatory infertility and avoids the negative effects on the endometrium frequently seen with anti-oestrogen therapies. Moreover, they reported an improvement in ovarian response with letrozole (5 mg/day, given on days 3–7) adjunctive to gonadotrophins in ovarian stimulation and IUI protocol of poor-responders (Mitwally and Casper, 2002).

In a randomized study, Oktay *et al.* (2006) reported that ovarian stimulation with FSH plus letrozole along with GnRH antagonist appears to be a cost-effective alternative for fertility preservation in breast cancer patients with reduced oestrogen exposure and 44% reduction in gonadotrophin requirement in comparison to standard IVF. More recently Goswami *et al.* (2004) reported that co-treatment of letrozole with gonadotrophins significantly reduces gonadotrophin consumption and, consequently, the cost of the gonadotrophin stimulation. In this study, the adjunctive use of letrozole to rFSH stimulation was compared with rFSH-only stimulation along with down-regulation

Table 2. Patient characteristics and basal hormone concentrations in Groups A and B.

	Group A (n = 35)	*Group B (n = 35)*
Age (years)	38.2 ± 3.7 (34–39)	37.9 ± 3.4 (33–39)
Day-3 serum FSH (mIU/ml)	8.3 ± 4.5	7.6 ± 5.2
Day-3 serum LH (mIU/ml)	6.2 ± 2.6	5.8 ± 2.9
Day-3 serum oestradiol (pg/ml)	56.4 ± 34.5	52.7 ± 28.3
Day-3 serum progesterone (ng/ml)	1.1 ± 0.7	1.3 ± 0.4
Aetiology of infertility		
Male infertility	26 (9/35)	31 (11/35)
Ovulation disorders	17 (6/35)	14 (5/35)
Endometriosis	20 (7/35)	17 (6/35)
Tubo-peritoneal factor	17 (6/35)	11 (4/35)
Unexplained infertility	20 (7/35)	26 (9/35)

Values are mean ± SD (range) or percentage (number/total); Group A = letrozole with rFSH; Group B = rFSH alone. *P* values less than 0.05 were considered statistically significant. There were no statistically significant differences between the two groups.

Table 3. Cycle characteristics of Groups A and B.

	Group A (n = 35)	*Group B (n = 35)*	P *value*
Follicles >14 mm on HCG day	7.8 ± 3.4	7.6 ± 4.2	NS
Follicles >17 mm on HCG day	5.9 ± 1.4	5.4 ± 1.7	NS
Oestradiol concentration on HCG day (pg/ml)	1870 ± 159	2015 ± 175	<0.05
Total gonadotrophin consumption (IU)	2980 ± 435	3850 ± 580	<0.05
Cycle cancellation rate due to poor response (%)	8.6 (3/35)	28.6 (10/35)	<0.05
Total cycle cancellation rate (%)	11.4 (4/35)	28.6 (10/35)	NS
Retrieved oocytes	4.9 ± 1.6	4.8 ± 1.4	NS
Mature (MII) oocytes	4.7 ± 2.3	4.4 ± 1.8	NS
2PN embryos	4.2 ± 1.7	4 ± 0.8	NS
Day 2 embryos >4 cells	3.2 ± 1.1	3.3 ± 0.7	NS
Day-3 embryos >7 cells	2.1 ± 0.6	1.9 ± 0.7	NS
Mean no. of transferred embryos	1.9 ± 1.2	1.6 ± 1.3	NS
Transferred Grade A embryos[a]	1.2 ± 0.8	1.1 ± 0.7	NS
Thickness of endometrium on HCG day (mm)	9.3 ± 2.6	9.7 ± 3.2	NS

Values are mean ± SD or percentage (number); Group A = letrozole with rFSH; Group B = rFSH alone; HCG = human chorionic gonadotrophin; MII = metaphase II; NS = not statistically significant.

[a] Embryos were scored according to Steer *et al.* (1992).

Table 4. Intracytoplasmic sperm injection outcomes.

Success parameters	*Group A (n = 35)*	*Group B (n = 35)*
Fertilization (fertilized/total retrieved MII oocytes)	92.4 (134/145)	97.2 (104/107)
Embryo transfer	88.6 (31/35)	71.4 (25/35)
Pregnancy/per started cycle	28.6 (10/35)	17.1 (6/35)
Pregnancy/per embryo transfer	32.3 (10/31)	24.0 (6/25)
Clinical pregnancy/per embryo transfer	25.8 (8/31)	20.0 (5/25)

Values are percentage (number/total); Group A = letrozole with rFSH; Group B = rFSH alone; MII = metaphase II. There were no statistically significant differences between the two groups.

by conventional long luteal GnRH agonist regimen. Nevertheless, the authors did not mention if GnRH antagonist or GnRH agonists were used to prevent premature LH surge in the letrozole/rFSH group. Thus, although the results

favoured aromatase inhibitors, the methodological difference among the study groups might have affected the results. Moreover, high LH concentrations during letrozole administration along with lower oestradiol, higher testosterone and androstenedione concentrations in the follicular phase of patients who were stimulated with letrozole/rFSH plus GnRH antagonist has been demonstrated previously (Verpoest *et al.*, 2006).

On the contrary, in 2008, adjunctive usage of letrozole to gonadotrophins with GnRH antagonist, which is similar to the current study protocol, was found to be less efficient than microdose GnRH analogue regimen in respect of ongoing pregnancy rates (37% versus 52%) (Schoolcraft *et al.*, 2008). Nonetheless, the randomization of this study was 2:1 favouring microdose GnRH analogue regimen; the only statistically significant findings regarding clinical outcome being the ongoing pregnancy rate. Besides, even in ovarian stimulation cycles wherein letrozole was not administered, a recent randomized study clearly indicated the superiority of flexible GnRH antagonist plus FSH regimen over microdose flare-up GnRH analogue regimen in poor-responders (Lainas *et al.*, 2008).

Higher endometrial thickness and mean number of retrieved oocytes were reported previously (Verpoest *et al.*, 2006). This study notably indicated significant increases both in pregnancy and implantation rates such as doubling of rates without having negative anti-oestrogenic effects. Letrozole has been reported to lead to both a normal endometrial histology and development of pinopodes, those considered to be relevant markers of endometrial receptivity, with similar oestradiol concentrations to spontaneous cycles and higher midluteal progesterone during moderate ovarian stimulation cycles of ovulatory infertile patients (Cortínez *et al.*, 2005).

More recently, a large retrospective study indicated an increase in rates of fertilization (85% versus 80%), implantation (14.5% versus 9.8%) and with at least one top-quality embryo transferred (55% versus 49.6%) by adjunctive use of letrozole (2.5 mg/day) along with GnRH antagonist protocol compared with microdose GnRH agonist flare-up protocol in ICSI–embryo transfer cycles of poor-responders (Yarali *et al.*, 2009). However, although the clinical pregnancy rate per embryo transfer was found to be higher in the letrozole/GnRH antagonist group (22.8%) than in the microdose agonist group (17.4%) in that study, the difference between rates did not reach statistical significance. In the current study, despite lower serum oestradiol concentrations on the day of HCG administration in patients receiving letrozole than those undergoing FSH-only stimulation, favourable pregnancy rates without any statistical signifcance were observed in the letrozole group than in the rFSH-only group, which is parallel to the previous studies. On the contrary, the current study failed to show enhanced implantation rates as well as improved embryo quality by adjunctive usage of letrozole. On the other hand, one can assume that aromatase inhibitors, by altering androgenic and oestrogenic precursors, might improve the intra-follicular microenvironment and lead a chance to produce more oocytes with enhanced quality. However, converse to this hypothesis, the mean number of retrieved MII oocytes and embryo quality produced from MII oocytes were quite similar in this study as well as in other previous studies with the exception of data indicated by a few studies. Besides, there were no differences observed both in mean number of 2PN zygotes and day-2 or -3 top-quality embryos and embryonic development among both study arms in the current study. Therefore, literature data supports that aromatase inhibitors might enhance the potential of implantation and improve the diminished follicular response in poor-responder ovarian stimulation cycles along with comparable clinical pregnancy rates in letrozole co-treated and in non-co-treated groups, but the definitive effect of adjunctive letrozole administration on embryo quality is still awaiting further evidence.

A recent study indicated that even adjunctive use of low-dose letrozole (2.5 mg/day) to gonadotrophins in ovarian stimulation regimens seems to be a good alternative to adjunction of clomiphene citrate to gonadotrophins (Barroso *et al.*, 2006). Particularly, letrozole alone was also reported to be equally effective compared with clomiphene citrate and gonadotrophins in ovarian stimulation cycles (Jee *et al.*, 2006). Letrozole and gonadotrophins together in older infertile women (>40 years) was reported to result in significantly modified cycle characteristics without any reduction in pregnancy rates, and therefore was recommended with potential benefit in IUI cycles in older women (Bedaiwy *et al.*, 2008). The most interesting finding of this study was the observation of lower cancellation rates in the letrozole group than in the FSH-only group. The current study also showed that lower cycle cancellation rates can obtained by the adjunctive use of letrozole to gonadotrophins in ovarian stimulation cycles of poor-responder patients undergoing ICSI–embryo transfer.

Nevertheless, the current study has some limitations and some further points that remain to be clarified. First, it has low power in similarity to recent studies (Goswami *et al.*, 2004; Bedaiwy *et al.*, 2006) on IVF/ICSI cycles of poor-responder patients due to enrolment problems with the participants and low number of eligible subjects. However, the main findings of the current study were in parallel to previous large studies (Yarali *et al.*, 2009; Bedaiwy *et al.*, 2006, 2008) in respect of pregnancy rates, gonadotrophin consumption and serum peak oestradiol concentrations in the letrozole co-treated group. Second, it can be assumed that the cancellation rate, which was in favour of the letrozole co-treated arm, might have interfered with the different sampling time of serum oestradiol concentrations between two study arms. However, in both groups, serum oestradiol concentrations were studied after 3 days of gonadotrophin stimulation. In addition, the letrozole/GnRH antagonist protocol is a novel one wherein letrozole should commenced 2 days prior to gonadotrophin initiation. As expected, due to suppression of aromatase enzyme, almost all of the previous studies (Mitwally and Casper, 2001, 2003; Goswami *et al.*, 2004; Bedaiwy *et al.*, 2006, 2008; Yarali *et al.*, 2009) reported lower serum oestradiol concentrations all through the stimulation period in patients who received letrozole than in those who were stimulated with FSH-only. Therefore, cancellation rates might have

significantly altered, if the serum oestradiol sampling were performed on day 3 of stimulation in the letrozole group due a to short and inadequate period of gonadotrophin stimulation.

In 2006, a cost-efficiency analysis of adjunction of letrozole to gonadotrophin was compared with gonadotrophin-only stimulation in 872 ovarian stimulation cycles among patients with variable infertility aetiologies and favourable effects of co-treatment with letrozole were again evidently demonstrated (Bedaiwy *et al.*, 2006). The cost was reported to be US$3249.42 in the letrozole co-treated group whereas it was US$6712.00 in the FSH-only group. In the current study, the cost of achieving a clinical pregnancy was also found to be significantly cheaper in the letrozole co-treated group than in rFSH-only group (13928 YTL and 21186 YTL, or US$11560 and US$17584, respectively). Moreover, the cost of the difference in required gonadotrophin dose (870 IU) reached a median difference of 25.2% (22–29%) of the mean total cost of required gonadotrophin dose per cycle in respect of current study groups. Therefore, regardless of the lack of a statistically significant difference in pregnancy and/or clinical pregnancy rates per transfer among the study groups, the current study shows the significance in cost-efficiency of letrozole co-treatment in poor-responders. However, the current study is a preliminary analysis. It may represent the basis for calculating sample size of a larger randomized controlled trial wherein economic outcomes should be chosen as the only primary end-point.

Aromatase inhibitors were reported to be related to favourable pregnancy outcomes regarding similar miscarriage and ectopic pregnancy rates in comparison to pregnant women who conceived with or without ovulation induction where clomiphene citrate along with gonadotrophins only were administrated in stimulated cycles (Mitwally *et al.*, 2005). Besides, Mitwally *et al.* (2005) indicated that letrozole was found to be associated with lower multiple-gestation rates in comparison to clomiphene citrate. Furthermore, the concerns about the possible increased risk in congenital abnormalities by aromatase inhibitors was enlightened previously by a large study which clearly indicated no additional increase in the incidence of minor and major congenital malformations in letrozole only and/or in letrozole co-treated ovulation induction and ovarian stimulation cycles (Tulandi *et al.*, 2006).

In conclusion, the current study confirmed that letrozole, as an adjuvant agent, has benefits in reducing the required dose of gonadotrophin and in improving the success of ovarian stimulation cycles by decreasing cycle cancellation rates due to diminished ovarian response in poor-responders. Therefore, letrozole–rFSH plus GnRH antagonist could be presumed to be a novel, good and cost-effective candidate protocol especially in poor-responder patients with potential of reducing treatment costs. Consequently, letrozole should not be underestimated in poor-responders who previously experienced high cycle cancellation rates in their preceding ovarian stimulation cycles. Nevertheless, a large randomized controlled trial is still required for a further substantial analysis of the cost-efficiency of co-treatment of letrozole in ovarian stimulation cycles.

References

Barroso G, Menocal G, Felix H *et al.* 2006 Comparison of the efficacy of the aromatase inhibitor letrozole and clomiphene citrate as adjuvants to recombinant follicle-stimulating hormone in controlled ovarian hyperstimulation: a prospective, randomized, blinded clinical trial. *Fertility and Sterility* **86**, 1428–1431.

Bedaiwy MA, Forman R, Mousa NA *et al.* 2006 Cost-effectiveness of aromatase inhibitor co-treatment for controlled ovarian stimulation. *Human Reproduction* **21**, 2838–2844.

Bedaiwy MA, Shokry M, Mousa N *et al.* 2008 Letrozole co-treatment in infertile women 40 years old and older receiving controlled ovarian stimulation and intrauterine insemination. *Fertility and Sterility* **91**, 2501–2507.

Cortínez A, De Carvalho I, Vantman D *et al.* 2005 Hormonal profile and endometrial morphology in letrozole-controlled ovarian hyperstimulation in ovulatory infertile patients. *Fertility and Sterility* **83**, 110–115.

D'Amato G, Caroppo E, Pasquadibisceglie A *et al.* 2004 A novel protocol of ovulation induction with delayed gonadotropin-releasing hormone antagonist administration combined with high-dose recombinant follicle-stimulating hormone and clomiphene citrate for poor responders and women over 35 years. *Fertility and Sterility* **81**, 1571.

Fasouliotis SJ, Laufer N, Sabbagh-Ehrlich S *et al.* 2003 Gonadotropin-releasing hormone (GnRH)-antagonist versus GnRH-agonist in ovarian stimulation of poor responders undergoing ovulation induction IVF. *Journal of Assisted Reproduction and Genetics* **20**, 455–459.

Fisher SA, Reid RL, Van Vugt DA *et al.* 2002 A randomized double-blind comparison of the effects of clomiphene citrate and the aromatase inhibitor letrozole on ovulatory function in normal women. *Fertility and Sterility* **78**, 280–285.

Goswami SK, Das T, Chattopadhyay R *et al.* 2004 A randomized single-blind controlled trial of letrozole as a low-cost IVF protocol in women with poor ovarian response: a preliminary report. *Human Reproduction* **19**, 2031–2035.

Jee BC, Ku SY, Suh CS *et al.* 2006 Use of letrozole versus clomiphene citrate combined with gonadotropins in intrauterine insemination cycles: a pilot study. *Fertility and Sterility* **85**, 1774–1777.

Kailasam C, Keay SD, Wilson P *et al.* 2004 Defining poor ovarian response during IVF cycles, in women aged <40 years, and its relationship with treatment outcome. *Human Reproduction* **19**, 1544–1547.

Lainas TG, Sfontouris IA, Papanikolaou EG *et al.* 2008 Flexible GnRH antagonist versus flare-up GnRH agonist protocol in poor responders treated by IVF: a randomized controlled trial. *Human Reproduction* **23**, 1355–1358.

Mitwally MF, Caspe RF 2002 Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders. *Fertility and Sterility* **77**, 776–780.

Mitwally MF, Casper RF 2001 Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. *Fertility and Sterility* **75**, 305–309.

Mitwally MF, Casper RF 2003 Aromatase inhibition reduces gonadotrophin dose required for controlled ovarian stimulation in women with unexplained infertility. *Human Reproduction* **18**, 1588–1597.

Mitwally MF, Biljan MM, Casper RF 2005 Pregnancy outcome after the use of an aromatase inhibitor for ovarian stimulation. *American Journal of Obstetrics and Gynecology* **192**, 381–386.

Muasher SJ, Abdallah RT, Hubayter ZR 2006 Optimal stimulation protocols for in-vitro fertilization. *Fertility and Sterility* **86**, 267–273.

Oktay K, Hourvitz A, Sahin G *et al.* 2006 Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. *Journal of Clinical Endocrinology and Metabolism* **91**, 3885–3890.

Schoolcraft WB, Surrey ES, Minjarez DA *et al.* 2008 Management of poor responders: can outcomes be improved with a novel gonadotropin-releasing hormone antagonist/letrozole protocol? *Fertility and Sterility* **89**, 151–156.

Shapiro DB, Mitchell-Leef D 2003 GnRH antagonist in in-vitro fertilization: where we are now. *Minerva Ginecologica* **55**, 373–388.

Steer R, Mills CJ, Tan SL *et al.* 1992 The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer program. *Human Reproduction* **1**, 117–119.

Tulandi T, Martin J, Al-Fadhli R *et al.* 2006 Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. *Fertility and Sterility* **85**, 1761–1765.

Verpoest WMJA, Kolibianakis E, Papanikolaou E *et al.* 2006 Aromatase inhibitors in ovarian stimulation for IVF/ICSI: a pilot study. *Reproductive BioMedicine Online* **13**, 166–172.

Yarali H, Esinler I, Polat M *et al.* 2009 Antagonist/letrozole protocol in poor ovarian responders for intracytoplasmic sperm injection: a comparative study with the microdose flare-up protocol. *Fertility and Sterility* **92**, 231–235.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 24 October 2008; refereed 12 December 2008; accepted 11 May 2009.