

Letrozole as an adjunct treatment in antagonist cycles that previously failed in poor responders

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ABSTRACT

Objectives: To investigate whether adding letrozole in the early follicular phase of a gonadotropin-releasing hormone (GnRH) antagonist (GA) stimulation cycle improves in vitro fertilization (IVF) outcomes in poor responder patients.

Material and methods: To be included in this study, patients had to have had at least one previous GA cycle and a subsequent GA cycle with added early follicular phase letrozole (LzGA). A total of 41 poor responder patients were identified based on the Bologna criteria.

Results: The LzGA group had a lower dosage of follicular stimulating hormone (FSH) ($p = 0.001$), the duration of stimulation days ($p = 0.015$) and the duration of GnRH antagonist stimulation days ($p = 0.033$) when compared with controls. Comprehensive analysis of the cycle characteristics showed that the number of oocytes retrieved, the number of MII oocytes retrieved, the number of fertilized oocytes, and the fertilization rate were significantly higher in the LzGA cycle ($p = 0.041$, $p = 0.019$, $p = 0.008$, $p = 0.01$, respectively). The rate of cycle cancellation was lower in the LzGA group (24.4%) than in the GA group (48.8%), ($p < 0.001$). Although LzGA administration demonstrated a trend toward improved implantation and clinical pregnancy rates, this was an insignificant trend ($p = 1.000$, $p = 0.177$, respectively).

Conclusions: Adjunctive letrozole administration seems to restore an IVF cycle by improving the cycle characteristics and reducing the total gonadotrophin dosage.

Key words: letrozole; ovarian stimulation; poor response; diminished ovarian reserve; antagonist cycle

Ginekologia Polska 2022; 93, 4: 267–272

INTRODUCTION

Diminished ovarian reserve (DOR) is defined as the reduced ability to achieve pregnancy and poor ovarian response to gonadotropin stimulation compared with women of a similar age [1]. In most patients, DOR remains unexplained but may be caused by advanced age, previous ovarian surgery, severe endometriosis, and environmental or genetic factors [2, 3]. DOR is often related to poor ovarian response to standard ovulation induction protocol and manifests as poor fertility results even when assisted reproductive techniques (ART) are used. Reduced implantation rate, decreased pregnancy rate, increased gonadotrophin use, and increased cycle cancellation are some of the main challenges in these patients.

Numerous investigations have been published about the management of poor ovarian responders, and they have proposed various stimulation protocols to improve pregnancy outcomes [4–7]. Increased gonadotrophin use, gonadotropin-releasing hormone (GnRH) agonist flare-up

protocols, natural cycle in vitro fertilization (IVF), and adjuvant therapies have been studied [8, 9]. Advised adjuvant therapies include growth hormone, aspirin, pyridostigmin, L-arginine, androgen supplements (testosterone and dehydroepiandrosterone), and androgen-modulating agents (letrozole and anastrozole). However, there is a lack of consensus regarding most interventions proposed to improve pregnancy rates in these patients.

Letrozole is a potent, highly selective, non-steroidal third-generation aromatase inhibitor. It prevents estrogen syntheses by inhibiting the aromatase enzyme activity. The resulting decrease in early follicular phase estrogen levels diminishes the negative feedback of estrogen on follicular stimulating hormone (FSH), consequently causing an augmentation in endogenous gonadotropin secretion and stimulation of ovarian follicular growth [10, 11]. The other proposed mechanism of action includes raised intraovarian androgens [10]. An increase in intraovarian androgens as a result of aromatase inhibition increases the expression

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Received: 2.09.2020 Accepted: 28.10.2020 Early publication date: 24.06.2021

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of FSH receptors on the follicle. Follicular sensitivity to FSH stimulation is thereby augmented [11].

Letrozole could successfully induce both ovulation and ovarian stimulation without any negative effects on the endometrium in women with polycystic ovary syndrome (PCOS) [11, 12]. A few initial studies also showed that, when letrozole is added to a gonadotropin ovulation induction in poor responder patients, the ovarian response to FSH increases and the gonadotropin doses required for stimulation are decreased [13–15]. However, recent studies on the use of letrozole in this patient group have reported inconsistent results [16–21]. This study therefore set out to assess whether adding letrozole in the early follicular phase of a GnRH antagonist (GA) stimulation cycle improves IVF outcomes in poor responders.

MATERIAL AND METHODS

Patient selection, stimulation protocol, oocyte retrieval and transfer

This retrospective study was conducted at the University of Health Sciences Tepecik Education and Research Hospital IVF Centre between January 2017 and December 2018. The medical records of 246 infertile patients with DOR according to the Bologna criteria of the 2011 European Society of Human Reproduction and Embryology consensus [22] were screened. To be included in this study, patients had to have had at least one previous GA cycle and a subsequent GA cycle with added early follicular phase letrozole (LzGA). A total of 41 patients with at least one previous GA cycle followed by an LzGA cycle were identified. Each patient was included only once. Women with multiple ART cycles who had previously had a GA cycle and then had several LzGA cycles were included only for the first GA cycle and the subsequent LzGA cycle. Patients with additional infertility factors, such as PCOS, tubal factors, and male factors, and who had received the treatment cycles more than six months apart were excluded from the study. The study protocol was approved by the Institutional Review Board of the University of Health Sciences, Tepecik Education and Research Hospital, Izmir, Turkey (approval number 2018/5-11).

Eligible women were evaluated on day two or three of their menstrual cycle by transvaginal ultrasound to measure endometrial lining, perform an antral follicle count, and exclude the presence of ovarian cysts. Blood samples were also taken for serum FSH, luteinizing hormone (LH), estradiol (E2), progesterone (P), and thyroid stimulating hormone (TSH). Subsequently, in GA cycle, recombinant FSH (Gonal-F; Merck-Serono, Istanbul) and highly purified hMG (Merional; IBSA, Istanbul, Turkey) were used at doses ranging between 225 and 375 IU/day. The dosages of FSH and hMG were adjusted according to the ovarian response. The ovarian response of patients was monitored by trans-

vaginal ultrasound and serum E2 levels. A flexible GnRH antagonist protocol (Cetrotide, 0.25 mg/day, Merc-Serono, Istanbul, Turkey) was initiated when the average diameter of the leading follicle was 13–14 mm and/or the serum E2 level was > 350 pg/mL, and the protocol was administered until the day of human chorionic gonadotropin (hCG). Recombinant hCG 250 µg (Ovitrelle; Merck-Serono, Istanbul, Turkey) was administered to trigger follicle maturation when at least two follicles measuring ≥ 17 mm in diameter.

In subsequent LzGA cycles of patients, hormonal and ultrasonographic measurements were taken on day two or three of the menstrual cycle. Letrozole (Femara; Novartis, Istanbul, Turkey) at a dose of 5 mg/day (2.5 mg \times 2) was initiated on day two or three and continued for five days. Ovarian stimulation, use of the GnRH antagonist protocol, and triggering of follicle maturation were performed, similarly to their preceding cycles. Oocyte retrieval was carried out by transvaginal ultrasonography under general anesthesia 35–36 hours after the ovulation trigger. Intracytoplasmic sperm injection (ICSI) was performed for all patients. The embryo quality was evaluated in the embryo cleavage stage (2–3 days) and morula-blastocyst stage (4–6 days) [23, 24]. Single or double embryos were transferred between day two and five under transabdominal ultrasound guidance. The luteal phase was supported with intravaginal progesterone gel (Crinone; Merck-Serono, Istanbul, Turkey) starting on the day of oocyte retrieval and continuing until the 12th week of pregnancy in cases with positive pregnancy tests. The β -hCG serum level was measured to confirm pregnancy on the 12th day after embryo transfer. Pregnancy was defined as blood β -hCG ≥ 20 IU/L.

Pregnancy

Clinical pregnancy was defined as presence of a fetal heartbeat detected by transvaginal ultrasound scan that was performed 4–5 weeks after embryo transfer. Implantation rate (IR) was calculated by dividing the number of gestational sacs transplanted embryos. Live birth was defined as an infant born alive after the 24th gestational week.

Outcome measurements

The mean cumulative gonadotrophin dosage, mean duration of gonadotrophin stimulation days, mean duration of GnRH antagonist stimulation days, mean serum estradiol concentration on the day of hCG administration, mean number of oocytes retrieved, mean number of mature oocytes (metaphase II oocytes), mean number of fertilized oocytes [mean number of 2 pronucleate (2PN) zygotes], fertilization rates, mean number of transferred embryos, mean number of transferred embryos for 2–3 days and 5–6 days, clinical pregnancy rates, and live birth rates were compared between patients who received the two stimulation protocols.

Statistics

Statistical calculations were done using SPSS for Windows version 20.0 (SPSS, Chicago, USA). The mean values are expressed as mean \pm standard deviation. Statistical comparison was carried out by Student's t-test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. A p value of < 0.05 was considered statistically significant.

RESULTS

A total of 41 patients, diagnosed as poor responders based on the Bologna criteria, were eligible for inclusion. A GA protocol and an LzGA protocol were applied to all patients. Table 1 shows the baseline characteristics of the study patients. Their age was 34.3 ± 4.25 . The duration of infertility was 7.47 ± 5.1 years. Basal hormone levels were similar among both cycles except for E2 levels on day three (Tab. 2).

The used gonadotroin units (2151.21 ± 649.61 vs 2807.62 ± 1125.55 ; $p = 0.001$), the number of stimulation days (7.63 ± 1.95 vs 8.87 ± 2.71 ; $p = 0.015$) and the duration of GnRH antagonist stimulation days (3.97 ± 1.42 vs 4.80 ± 1.92 ; $p = 0.033$) were significantly lower in the LzGA cycle than in

the GA cycle (Tab. 3). Although the mean number of follicles on the trigger day were similar in both cycles, the mean number of retrieved oocytes and the number of metaphase II oocytes were significantly higher in LzGA (Tab. 3). The mean peak E2 level on the trigger day was also found to be significantly lower in the LzGA cycle than in the GA cycle (Tab. 3). LzGA had a higher number of fertilized oocytes

Table 1. Demographic characteristics of patients

	n: 41
Age, years (mean \pm SD)	34.36 ± 4.25
BMI, kg/m ² (mean \pm SD)	25.12 ± 4.31
Gravida (mean \pm SD)	0.24 ± 0.43
Parity (mean \pm SD)	0
Live birth number (mean \pm SD)	0
Abortion (mean \pm SD)	0.17 ± 0.38
Menstrual cycle duration (mean \pm SD)	26.26 ± 4.82
Male age, years (mean \pm SD)	37.36 ± 5.26
Duration of infertility, years (mean \pm SD)	8.17 ± 5.10

Values are mean \pm SD; SD — standard deviation; BMI — body mass index

Table 2. Basal hormone concentrations in cycles letrozole + GnRH antagonist and GnRH antagonist

	LzGA cycle	GA cycle	p
Day-3 serum FSH [mIU/mL]	12.82 ± 4.38	11.24 ± 5.31	0.094
Day-3 serum LH [mIU/mL]	5.23 ± 3.49	4.64 ± 2.43	0.223
Day-3 serum E2 [pg/mL]	49.97 ± 23.78	39.69 ± 20.01	0.014
Day-3 serum progesterone [ng/mL]	0.82 ± 0.58	0.79 ± 0.49	0.763
TSH [mIU/L]	1.52 ± 0.75	1.49 ± 0.85	0.832
Prolactine [ng/mL]	14.89 ± 5.95	16.56 ± 8.10	0.060
AMH [ng/mL]	0.68 ± 0.33	0.68 ± 0.34	0.323
Antral follicle count (n)	4.5 ± 2.73	3.9 ± 2.42	0.276

Values are mean \pm SD; p value ≤ 0.05 was considered statistically significant; FSH — follicle stimulating hormone; LH — luteinizing hormone; E2 — estradiol; TSH — thyroid stimulating hormone; AMH — anti-mullerian hormone; LzGA — letrozole + GnRH antagonist; GA — GnRH antagonist

Table 3. Cycle characteristics in cycles letrozole + GnRH antagonist and GnRH antagonist

n: 41	LzGA cycle	GA cycle	p
Total gonadotrophin consumption [IU]	2151.21 ± 649.61	2807.62 ± 1125.55	0.001
Duration of stimulation [day]	7.63 ± 1.95	8.87 ± 2.71	0.015
Duration of GnRH antagonist stimulation [day]	3.97 ± 1.42	4.80 ± 1.92	0.033
Number of follicles on day of hCG	2.70 ± 2.13	2.43 ± 1.94	0.572
Oocytes retrieved (n)	2.82 ± 1.37	2.14 ± 0.041	0.041
MII oocytes retrieved (n)	2.56 ± 1.46	$1.85 \pm ???$	0.019
Peak E2 level [pg/mL]	557.43 ± 403.58	776.24 ± 376.46	0.002
Fertilized oocytes (n)	1.82 ± 1.37	1.19 ± 1.32	0.008
Fertilization rate [%]	68.55 ± 35.21	49.75 ± 44.86	0.01

Values are mean \pm SD; p value ≤ 0.05 was considered statistically significant; GnRH — gonadotropin releasing hormone; hCG — human chorionic gonadotropin; MII — metaphase II; E2 — estradiol; LzGA — letrozole + GnRH antagonist; GA — GnRH antagonist

and fertilization rate (1.82 ± 1.37 vs 1.19 ± 1.32 ; $p = 0.008$, 68.55 ± 35.21 vs 49.75 ± 44.86 ; $p = 0.01$, respectively) (Tab. 3). In the GA cycle, the cancellation rate was 48.8%, whereas in the LzGA cycle, it was 24.4% ($p < 0.001$). The causes of cancellation are summarized in Table 4. The day on which embryos were transferred and the pregnancy results are shown Table 5. Seven clinical pregnancies (17.07%) in the LzGA group and three clinical pregnancies (7.3%) in the GA group were recorded. Although LzGA administration was 2.6 times more common in clinical pregnancy when compared with GA administration, this was a non-significant trend toward higher clinical pregnancy rates in LzGA administration. Of the seven patients who had clinical pregnancy after LzGA administration, four patients (9.75%) had a live birth.

DISCUSSION

Our results show that, when compared with the previous GA cycle, the succeeding LzGA cycle resulted in a significant reduction in the gonadotrophin dose needed for ovarian stimulation (OS) and a reduction of the number of stimulation days and the number of GnRH antagonist stimulation days. Comprehensive analysis of the cycle characteristics showed that the number of oocytes, MII oocytes, and fertilized oocytes retrieved, as well as the fertilization rate, were significantly higher in the LzGA cycle. Moreover, lower cancellation rates were observed when adding letrozole to subsequent GnRH antagonist cycles. Letrozole's positive effects on cycle characteristics, implantation rates,

and clinical pregnancy rates between cycles were not statistically different.

Low response to OS, increased gonadotrophin use, reduced implantation rate, and decreased prospects of pregnancy are the clinical signs of DOR. The administration of ART cycles for these patient populations is one of the largest difficulties for the clinician. Although there is not a strong relationship between pregnancy results and the use of letrozole in the first days of the follicular phase of OS in poor responder patients, various positive effects on the cycles have been found [16–21, 25–27]. In a randomized study conducted by Ozmen et al. [21], ovarian stimulation with FSH plus letrozole along with GnRH antagonist in poor responder patients significantly reduces the necessary doses of gonadotrophin and the cost of gonadotrophin stimulation. More recently, Lee et al. [26], analyzed a total of 103 consecutive IVF cycles in poor responder patients performed with either FSH plus letrozole along with GnRH antagonist or with only FSH along with GnRH antagonist. They reported that the total doses of gonadotrophin and days of gonadotrophin administration were significantly lower in the letrozole group. In addition, the GnRH antagonist administration days was significantly decreased in the letrozole group in this study. A possible explanation is that letrozole increases ovarian sensitivity to gonadotrophins, and consequently, gonadotrophin consumption and the duration of stimulation days decrease. In the present study, we demonstrated that the addition of letrozole to the early follicular phase of an OS cycle significantly reduced gonadotrophin consumption and the duration of the cycle, which is consistent with the above-mentioned studies. The GnRH antagonist stimulation days was also shorter in the letrozole group.

When the cycle characteristics are examined, the results of the use of letrozole in the early follicular phase and in previous studies are slightly more conflicting [17, 18, 21, 25–27]. Ozmen et al., Goswami et al., and Ebrahimi et al., reported a comparable number of retrieved oocytes in the letrozole group, whereas Lee VC et al., Lee KH et al., and Garcio-Velasco et al., reported a significantly higher number of oocytes retrieved [17, 18, 21, 25–27]. Consistently with the studies performed by Lee VC et al., Lee KH et al., and Garcio-Velasco et al., the present research demonstrated that a significantly increased number of oocytes were retrieved in the LzGA cycle [25, 26, 17]. The number of metaphase II oocytes was significantly higher, consistent with the total number of oocytes retrieved. Similarly, both Ozmen et al., and Ebrahimi et al., found the number of metaphase II oocytes to be consistent with the total number of oocytes retrieved [21, 27]. However, in the study of Lee et al. [26], the number of oocytes retrieved and the number of MII oocytes were not consistent.

Table 4. The causes of cancellation

	LzGA	GA	p
Cycle cancellation rate n, (%)	10 (24.4)	20 (48.8)	< 0.001
Causes of cancellation (n=30)			
Total fertilization failure	4	10	
No oocyte in the OPU	2	5	
Arrest of embryo growth	3	1	
Poor morphology	1	4	

OPU — oocyte pick-up; LzGA — letrozole + GnRH antagonist; GA — GnRH antagonist

Table 5. Days of transferred embryos and pregnancy rates

	LzGA cycle	GA cycle	p
Day 2–3 embryo transfer, n (%)	28 (68.3)	19 (39.0)	0.015
Day 5 embryo transfer, n (%)	3 (7.3)	2 (4.9)	1.000
Clinical pregnancy n (%)	7 (17.07)	3 (7.3)	0.177
Implantation rate, n (%)	7 (17.07)	6 (12.5)	1.000
Live births, n (%)	4 (9.75)	0 (0)	0.124

LzGA — letrozole + GnRH antagonist; GA — GnRH antagonist

IVF treatment results can be expected to improve when significantly more oocytes are collected and more MII oocytes are obtained, probably because they augment embryo selection for embryo transfer [28, 29]. However, the most previous studies showed similar IVF outcomes in the letrozole group. [21, 25–27]. Pregnancy results in the letrozole group did not differ significantly in any of these studies. Ebrahimi et al. [27], added letrozole to a stimulation program for poor responder patients, identified based on the Bologna criteria, and there were no significant differences between groups regarding the number of oocytes retrieved, fertilization rate, implantation rate, total cycle cancellation rate, and clinical pregnancy rate. They suggested that the use of letrozole does not improve clinical outcomes in poor responder patients. A randomized, controlled trial conducted by Goswami et al. [18], reported similar numbers of oocytes retrieved and pregnancy rates between groups, except for the group with a significantly lower total dose of FSH. These results were consistent with what has been stated in various studies [21, 25–27]. Moreover, some studies had inconsistencies within themselves [17, 30]. In the Garcia-Velasco study [17], evaluating the impact of letrozole as an adjuvant treatment in IVF cycles on low responder patients, there was a significant improvement in the implantation rate and the number of oocytes retrieved in the group with added letrozole, but there was no significant difference between the groups regarding cycle cancellation, fertilization, or pregnancy rates. More recently, Moinid et al. [30], compared letrozole plus GnRH antagonist with a placebo plus GnRH antagonist in poor responders, and they showed that the total number of retrieved oocytes and of MII oocytes in the letrozole-treated group were significantly higher than in the control group. However, there were no marked differences regarding fertilization rate, implantation rate, or clinical pregnancy. In the current study, implantation rates and clinical pregnancy rates were comparable between groups despite the higher number of retrieved oocytes, MII oocytes, and fertilized oocytes, the increased fertilization rate, and the lower cycle cancellation rates in patients receiving letrozole. These results were in accordance with previous studies [17, 30].

In light of these findings, positive results of studies should be evaluated with caution because ovarian responses depend on cyclic fluctuations, and patients with a poor response in the first cycle might respond normally in the subsequent cycle [31]. Therefore, improvement in cycle results with letrozole might be linked to the fluctuation in ovarian response, not to the drug's effect. On the other hand, by blocking the conversion of androstenedione and testosterone to estrogen, letrozole might increase endogenous gonadotropin secretion and stimulate ovarian follicular growth, leading to a chance to produce more

oocytes [10, 11]. The outcomes of the present study support the literature that letrozole might improve the cycle characteristics and comparable pregnancy results in poor responder patients.

The main criticism of the current study are its retrospective nature and small sample size. Retrospective cohort studies are subject to selection bias, recall bias, and unknown confounding variables, which may negatively affect the accuracy of the results. Therefore, the results of the current study need to be interpreted carefully until well-designed, prospective randomized trials have been performed.

CONCLUSIONS

Our findings indicate that adding letrozole in the early follicular phase of a GnRH antagonist stimulation cycle has benefits in reducing the required dose of gonadotrophin and in improving the success of cycle characteristics in poor responders. Moreover, while there is a trend toward improved implantation and clinical pregnancy rates, these results are not statistically significant. Nevertheless, it is impossible to say that using letrozole as an adjuvant agent has no positive effects in poor responder patients during the stimulation cycle. However, further randomized controlled trials are required to confirm these findings.

Conflict of interest

The authors declare that they have no conflict of interest.

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