Effects of letrozole co-treatment on outcomes of gonadotropin releasing hormone antagonist cycles in POSEIDON group 3 and 4 expected poor responders

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Research Article

Keywords: infertility, letrozole, live birth rate, poor responder, POSEIDON

Posted Date: February 2nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1267736/v1>

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Abstract

Purpose: To evaluate the effects of adjuvant letrozole administration on outcomes of gonadotropin releasing hormone (GnRH) antagonist cycles with intracytoplasmic sperm injection in POSEIDON group 3 and 4 expected poor responder women.

Methods: This study was conducted by retrospective analysis of patients with expected poor ovarian response (POSEIDON group 3 and 4) that underwent GnRH antagonist cycles with intra-cytoplasmic sperm injection between 2010 and 2020. A total of 488 patients with letrozole co-administration and 2564 patients without any adjuvant treatment that underwent GnRH antagonist cycles within the selected period of time were included in the study.

Results: Implantation rates, clinical pregnancy rates and live birth delivery rates were significantly higher in letrozole administered patients in comparison to controls among POSEIDON group 3 women (0.39 ± 0.46 vs 0.27 ± 0.40 , $p=0.01$; 46.1% vs 33%, $p=0.014$; 42.7% vs 31.5%, $p=0.034$, respectively). Mean total doses of gonadotropins required per cycle were significantly lower in letrozole administered patients among both POSEIDON group 3 and 4 women (2864.65 ± 878.47 IU vs 3757.27 ± 1088.89 IU, $p<0.001$; 3286.06 ± 770.16 IU vs 3666.48 ± 959.66 IU, $p<0.001$, respectively).

Conclusion: Adjuvant letrozole co-administration in intracytoplasmic sperm injection cycles following GnRH antagonist protocol appears to improve implantation, clinical pregnancy and live birth delivery rates in women with POSEIDON group 3 expected poor ovarian response.

Introduction

Poor ovarian response (POR) is one of the major challenges in reproductive medicine that dramatically reduce the chance of success in assisted reproduction. For decades, several different criteria were used to define poor ovarian response until the definition suggested by ESHRE gained general acceptance and provide standardization for studies in literature. ESHRE defined poor ovarian response as ≤ 3 ovarian follicles on the day of oocyte maturation triggering or ≤ 3 oocytes obtained in a controlled ovarian stimulation cycle [1]. Particularly due to the heterogeneity in definition, prevalence of poor responder women ranges between 5.6% and 35.1% of in-vitro fertilization/embryo transfer cycles among studies in literature [2]. These patients have lower chance of conception and live birth rates are reported 6–23% in different studies [3, 4]. Determining unequivocally effective management modalities for poor responder women have been problematic due to heterogeneity of underlying conditions and etiologies among poor responder women. Considering the various drawbacks of former classification systems, POSEIDON study group introduced the concept of “low-prognosis” and stratified these patients considering the common etiologic factors in each group with a pragmatic approach in determining management strategies and in attempt to standardize studies [5].

Several management modalities are suggested to increase IVF success rates in studies using older classification systems and diagnostic criteria for poor responders, including higher doses of gonadotropin administration, mild stimulation with using clomiphene citrate, reduced dose protocols, modified natural cycles, adjuvant agents and more radical interventions like in-vitro oocyte activation with limited success [1, 6]. One of these modalities is adjuvant letrozole administration in controlled ovarian stimulation cycles.

Letrozole blocks conversion of androgens to estrogens by competitively inhibiting aromatase enzyme throughout the body that results with decreased estrogen and increased androgen levels [7]. Increase in androgen levels augments FSH activity and androgens are known to take crucial roles in pre-antral to early antral follicle transition [7]. Low androgen levels have been shown to be associated with impaired IVF success and transdermal testosterone pre-treatment has been demonstrated to increase live birth rates in poor responder women in previous studies [8, 9]. Unlike clomiphene citrate that depletes estrogen receptors, letrozole left negative estrogen feed-back mechanisms intact, therefore interferes less in the processes of normal follicular development, atresia of smaller follicles and dominant follicle selection hence induces mono-follicular growth that more closely resembles to a natural menstrual cycle and increases the chance of achieving a singleton pregnancy [10]. Besides these, letrozole has a short half-life of 45 minutes [7]. All these factors make letrozole an attractive option in infertility treatments. Utilization of letrozole to improve IVF outcomes in poor responder women was first proposed by Mitwally, and colleagues [11]. However, preliminary reports involving babies conceived via letrozole administration indicated an increased risk of cardiac and locomotor defects in

these infants [12]. Therefore effects of adjuvant letrozole administration on IVF success is a particularly understudied topic. Although not confirmed by larger studies, concerns about increased risk of congenital defects gave rise to a prudent approach to utilization of letrozole for infertility and its use stayed off-label for ovulation induction. However, recently, considering the latest safety data on letrozole, American College of Obstetricians and Gynecologists now recommends letrozole as first line therapy for infertile women with PCOS due to higher clinical pregnancy and live birth rates comparing to clomiphene citrate [13, 14]. Therefore, contemporarily, utilization of letrozole in reproductive medicine is increasing in frequency [7].

Here we conducted this study to evaluate the effects of adjuvant letrozole administration on IVF/ICSI outcomes in POSEIDON group 3 and POSEIDON group 4 expected POR women.

Materials And Methods

This study is conducted in a university affiliated infertility center (Memorial Atasehir Hospital affiliated with Uskudar University) in Istanbul by retrospective analysis of hospital records. Ethical approval for this study was obtained from Ethical Committee of Uskudar University at 28/04/2021 (Approval number: 61351342/APRIL 2021-84). Study protocol conforms to the "Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects" and the need for consent was omitted by ethical committee due to the retrospective design.

Study population

Patients with expected POR (POSEIDON group 3 and group 4) that underwent GnRH antagonist cycles with intra-cytoplasmic sperm injection (ICSI) between 2010 and 2020 were analyzed. POSEIDON group 3 consists of women younger than 35 years old with AMH<1,2 and AFC<5. POSEIDON group 4 consists of women ≥ 35 years of age and with AMH<1,2 and AFC<5. Patients with low (<18 kg/m²) or high (>30 kg/m²) body mass indexes (BMI), patients with endocrine co-morbidities (hyperprolactinemia, thyroid dysfunction, diabetes mellitus, Addison disease, congenital adrenal hyperplasia, Cushing syndrome), cycles with any adjuvant administration except from letrozole, patients with uterine anomalies or a history of surgical uterine anomaly correction, patients with male factor infertility due to azoospermia were excluded. Outcomes of the remaining patients with and without adjuvant letrozole administration were evaluated in the study. Expected POR patients (POSEIDON group 3 and group 4) that underwent GnRH antagonist cycles with adjuvant letrozole administration were included in letrozole group and patients without any adjuvant administration were included in control group. Cycle outcomes of these groups were compared. Subsequently, patients with adjuvant letrozole administration and controls were re-grouped in accordance with being POSEIDON group 3 or POSEIDON group 4 and data were re-analyzed.

Controlled ovarian stimulation and interventions

Controlled ovarian stimulation was initiated in 2nd day of menstrual cycle. Letrozole (Femara®; Novartis Pharmaceuticals Corporation) was administered 2.5 mg twice daily per-orally, starting from the 2nd day of menstruation for 5 days. Recombinant follicle stimulating hormone (rFSH, Gonal-F, Merck Serono S.p.A), human menopausal gonadotropin (hMG, Merional, IBSA Institut Biochimique S.A, Menopur® Ferring Pharmaceuticals) or combination of recombinant luteinizing hormone (rLH) and rFSH (Pergoveris, Merck Serono SA) were used for ovarian stimulation. Cycles were monitored for follicular growth with serial transvaginal ultrasounds and serum hormone levels throughout the course of stimulation. Gonadotropin doses were adjusted accordingly with each patient's follicular growth. GnRH antagonist (Cetrotide 0.25mg, Pierre Fabre Medicament Production) injections were initiated to suppress premature LH peak whenever the leading follicle have reached a diameter of 12-14 mm, and continued to the day of oocyte maturation triggering. Oocyte maturation was triggered when at least one follicle has reached a diameter of 18 mm. Either dual-trigger or traditional hcg-only trigger was used for final oocyte maturation triggering. GnRH agonist of 0.2 mg triptorelin acetate, (Gonapeptyl, Ferring Pharmaceuticals) and 250mcg recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono) were administered concomitantly in patients that dual-triggering was performed. 250mcg recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono) was used alone for oocyte maturation in women with hcg-only triggering. Oocytes were retrieved under transvaginal ultrasound guidance 35-36 hours after oocyte maturation trigger. Fertilization was conducted by intracytoplasmic sperm injection (ICSI). Embryo quality was graded according to The Society for Assisted Reproductive Technology (SART) grading system [15]. Regarding the SART grading system grade 1 embryos are referred

as good quality embryos, grade 2 embryos are referred as fair quality embryos and grade 3 embryos are referred as poor quality embryos in this study. Day 3 or day 5 embryos are transferred depending on the condition and physician preference. Embryo transfers were conducted via soft embryo transfer catheters under abdominal ultrasonography guidance. A maximum of 2 embryos were transferred in each attempt. Intravaginal progesterone is initiated in every patient with either 200 mg Lutinix twice a day or 200 mg Progesterone three times a day (Lutinix vaginal tablets, Ferring Pharmaceuticals; Progesterone Soft Capsules, Koçak Farma Pharmaceutical and Chemical Industry Co.) for luteal phase support and continued to the 8th-10th gestational weeks.

Outcome measures

Primary outcome of this study was determined as live birth delivery rates in POSEIDON group 3 patients, POSEIDON group 4 patients and overall expected POR patients. Secondary outcomes were total dose of required gonadotropins, number of retrieved oocytes, number of metaphase2 (M2) oocytes, oocyte maturation rate (number of M2 oocytes/number of retrieved oocytes), number of obtained 2 pronuclear (2PN) embryos, fertilization rates (number of 2PN embryos/number of M2 oocytes), embryo quality, implantation rates (gestational sacs observed/transferred embryos), clinical pregnancy rate, cycle cancellation rate and miscarriage rates. Outcome parameters were defined in accordance with The International Glossary on Infertility and Fertility Care, 2017 [16].

Statistical analysis

Statistical analysis were carried out by IBM SPSS statistics 23 (evaluation version). Descriptive statistics were expressed as mean $\pm$ standard deviations for normally distributed data. Categorical variables were expressed as numbers and percentages (%). Significance of differences between means were evaluated with Student's T test. Categorical variables were evaluated with Pearson's Chi-Squared test or Fisher's exact test. P values <0.05 are considered significant.

Results

Among the eligible subjects, adjuvant letrozole was administered in 488 women that constitute letrozole group, 2564 women were underwent GnRH antagonist cycles without any adjuvant administration within the selected period of time and included in control group. Eighty-nine women in letrozole group and 739 women in control group were found to be POSEIDON group 3 patients. Three-hundred-ninety-nine women in letrozole group and 1825 controls were found to be POSEIDON group 4 expected PORs.

Mean age, BMI and the method of oocyte maturation triggering in letrozole group and controls were similar among overall expected POR patients. Mean number of previous unsuccessful IVF attempts was significantly higher in letrozole group (2.33 vs. 2.03; $p=0.002$). Total dose of required gonadotropins (3209.21 ± 806.28 IU vs. 3692.65 ± 999.25 IU; $p<0.001$), days of stimulation (9.35 ± 1.51 vs. 9.87 ± 1.72 ; $p<0.001$), endometrial thickness (9.42 ± 1.83 mm vs. 10.19 ± 1.95 mm; $p<0.001$), peak estradiol (557.41 ± 474.16 pg/ml vs. 1014.09 ± 503.76 pg/ml; $p<0.001$) and progesterone levels (0.65 ± 0.39 ng/ml vs. 0.68 ± 0.31 ng/ml; $p=0.022$), number of retrieved oocytes (3.72 ± 1.82 vs. 3.97 ± 1.72 ; $p<0.001$) were significantly lower in letrozole group. Oocyte maturation rates (0.78 ± 0.23 vs. 0.75 ± 0.27 ; $p=0.001$), number of 2PN embryos (2.24 ± 1.37 vs. 1.87 ± 1.0 ; $p<0.001$), fertilization rates (0.82 ± 0.30 vs. 0.72 ± 0.29 ; $p<0.001$) were significantly higher in adjuvant letrozole administered women in comparison to controls. Comparison of baseline characteristics and outcome parameters of expected POR women with and without adjuvant letrozole administration were given in Table 1.

Table 1
Comparison of baseline characteristics and cycle outcomes of expected POR women with and without adjuvant letrozole administration.

		Letrozole Group	Controls	P value
Number of patients		488	2564	
Age (years)		37.77±3.85	37.42±4.73	0.137
BMI		24.74±3.16	24.91±2.87	0.413
Oocyte maturation triggering method	Dual trigger	177 (36.3%)	917 (35.8%)	0.837
	Hcg-only triggering	311 (63.7%)	1647 (64.2%)	
Day of embryo transfer	Day 3	336 (74.5%)	1699 (69.5%)	0.031
	Day 5	115 (25.5%)	747 (30.5%)	
Number of previous IVF attempts		2.33±1.64	2.03±1.36	0.002
Required gonadotropin doses (IU)		3209.21±806.28	3692.65±999.25	<0.001
Days of stimulation		9.35±1.51	9.87±1.72	<0.001
Peak estradiol level (pg/ml)		557.41±474.16	1014.09±503.76	<0.001
Peak progesterone level (ng/ml)		0.65±0.39	0.68±0.31	0.022
Endometrial thickness (mm)		9.42±1.83	10.19±1.95	<0.001
Cycle cancellations		35 (7.2%)	117 (4.6%)	0.017
Number of retrieved oocytes		3.72±1.82	3.97±1.72	<0.001
Maturation rate (M2 oocytes/Retrieved oocytes)		0.78±0.23	0.75±0.27	0.001
Number of M2 oocytes		2.80±1.43	2.86±1.38	0.078
Number of 2PN embryos		2.24±1.37	1.87±1.0	<0.001
Quality of obtained embryos	Good	280 (61.8%)	1492 (61%)	0.182
	Fair	118 (26%)	715 (29.2%)	
	Poor	55 (12.1%)	240 (9.8%)	
Number of frozen embryos		0.51±0.72	0.61±0.75	0.003
Fertilization rate per cycle		0.82±0.30	0.72±0.29	<0.001
Implantation rate per cycle		0.21±0.37	0.22±0.37	0.759
Clinical pregnancy rate		134 (27.5%)	684 (26.7%)	0.721
Live birth delivery rate (n)		17.5% (119)	26.7% (684)	0.474
Miscarriage		24 (17.9%)	176 (25.7%)	0.054
Multiple pregnancies		9 (1.8%)	79 (3.1%)	0.081

Analysis revealed no significant differences in terms of BMI, oocyte maturation trigger method and days of embryo transfer in letrozole groups and controls among POSEIDON group 3 and group 4 women. Number of previous IVF attempts were higher in letrozole groups comparing to controls in both POSEIDON group3 and 4 women. Required gonadotropin doses, peak estradiol levels, days of stimulation were all significantly lower in letrozole groups among POSEIDON group 3 and 4 patients. Implantation rates, clinical pregnancy rates and live birth delivery rates were found significantly higher in letrozole group in POSEIDON group 3 women however no significant differences between letrozole group and controls were found in these outcomes among

POSEIDON group 4 women. Comparison of cycle outcomes of POSEIDON group 3 and group 4 women with and without adjuvant letrozole administration were summarized in Table 2.

Table 2

Comparison of cycle outcomes of POSEIDON group 3 and group 4 women with and without adjuvant letrozole administration

		POSEIDON GROUP 3			POSEIDON GROUP 4		
		Letrozole Group	Controls	P value	Letrozole Group	Controls	P value
Number of patients		89	739		399	1825	
Age (years)		31.40±2.62	31.49±3.08	0.271	39.18±2.37	39.82±2.76	0.001
BMI		24.49±3.03	24.81±2.85	0.565	24.79±3.19	24.95±2.88	0.484
Oocyte maturation triggering method	Dual-trigger	30 (33.7%)	229 (31%)	0.601	147 (36.3%)	688 (37.7%)	0.749
	Hcg-only triggering	59 (66.3%)	510 (69%)		252 (63.2%)	1137 (62.3 %)	
Day of embryo transfer	Day 3	64 (75.3%)	496 (68.5%)	0.2	272 (74.3%)	1203 (69.9%)	0.089
	Day 5	21 (24.7%)	228 (30.9%)		94 (25.7%)	519 (30.1%)	
Number of previous IVF attempts		2.25±1.44	1.85±1.12	0.024	2.35±1.68	2.10±1.44	0.040
Required gonadotropin doses (IU)		2864.65±878.47	3757.27±1088.89	<0.001	3286.06±770.16	3666.48±959.66	<0.001
Days of stimulation		9.15±1.54	9.74±1.49	<0.001	9.40±1.51	9.93±1.80	<0.001
Peak estradiol level (pg/ml)		473.80±360.45	995.20±456.61	<0.001	576.15±494.42	1021.75±521.57	<0.001
Peak progesterone level (ng/ml)		0.73±0.41	0.69±0.31	0.534	0.63±0.39	0.67±0.31	0.009
Endometrial thickness (mm)		9.80±1.62	10.11±1.88	0.189	9.34±1.86	10.22±1.99	<0.001
Cycle cancellations		4 (4.5%)	15 (2%)	0.138	31 (7.8%)	102 (5.6%)	0.096
Number of retrieved oocytes		4.17±1.87	4.45±1.74	0.067	3.62±1.80	3.77±1.67	0.005
Maturation rate (M2 oocytes/Retrieved oocytes)		0.83±0.19	0.75±0.36	<0.001	0.77±0.23	0.75±0.23	0.077
Number of M2 oocytes		3.28±1.36	3.18±1.43	0.719	2.69±1.43	2.73±1.33	0.116
Number of 2PN embryos		2.65±1.41	1.98±0.86	<0.001	2.15±1.35	1.83±1.04	<0.001
Quality of obtained embryos	Good	57 (67.1%)	446 (61.6%)	0.402	223 (60.6%)	1046 (60.7%)	0.330
	Fair	19 (22.4%)	212 (29,3%)		99 (26.9%)	503 (29.2%)	
	Poor	9 (10.6%)	66 (9.1%)		46 (12.5%)	174 (10.1%)	
Number of frozen embryos		0.90±0.80	0.90±0.81	0.911	0.42±0.67	0.49±0.69	0.037
Fertilization rate per cycle		0.82±0.25	0.70±0.29	<0.001	0.81±0.30	0.72±0.29	<0.001
Implantation rate per cycle		0.39±0.46	0.27±0.40	0.010	0.18±0.35	0.20±0.36	0.593
Clinical pregnancy rate		41 (46.1%)	244 (33%)	0.014	93 (23.3%)	440 (24.1%)	0.734

Live birth delivery rate (n)	42.7% (38)	31.5% (233)	0.034	20,3% (81)	19.4% (354)	0.680
Miscarriages	7 (17.1%)	49 (20.1%)	0.654	17 (18.3%)	127 (28.9%)	0.037
Multiple pregnancies	4 (4.5%)	38 (5.1%)	0.217	5 (1.3%)	41 (2.2%)	0.151

Discussion

This study was conducted to assess the effects of adjuvant letrozole administration on IVF/ICSI outcomes of POSEIDON group 3 and 4 expected POR women. Due to the results obtained, letrozole administration seems to significantly improve implantation rates, clinical pregnancy rates and live birth delivery rates in POSEIDON group 3 patients whereas no significant differences were found in terms of these parameters among POSEIDON group 4 patients.

Studies evaluating the effects of letrozole administration on IVF outcomes in poor responder women have inconsistent results regarding implantation rates, clinical pregnancy rates and live birth rates in literature. Ebrahimi et al. conducted a randomized controlled trial to evaluate the effects of letrozole co-administration on IVF outcomes of poor responders underwent GnRH antagonist protocols [17]. They found no significant differences in implantation rates and clinical pregnancy rates therefore concluded that letrozole administration does not improve IVF outcomes in poor responders. Garcia-Velasco et al. demonstrated an increase in implantation rates with 2.5mg letrozole administration for first five days of the cycle [18]. Apart from these, most of the studies found increasing trends for clinical pregnancies in letrozole administered poor responder women in comparison to controls without reaching statistical significance [19–22]. Whereas Davar et al observed a decrease in clinical pregnancy rates with letrozole administration [23]. Up to 50% of IVF failures could be attributed to implantation failure [24]. Implantation rate is a reflection of conjoint effects of both endometrial receptivity and embryo quality. As letrozole blocks aromatase activity, its administration reduces estradiol levels reached following ovarian stimulation and limits deleterious effects of supraphysiological estradiol levels on endometrial receptivity [25]. Furthermore, endometrial integrin expression is a known marker of endometrial receptivity and previously, studies showed significant increase in endometrial integrin expressions with letrozole administration [26, 27]. However, low embryo quality is a major concern in older women and known as the dominant determinant of implantation rates therefore the clinical pregnancy rates in these patients [26]. Inconsistent findings in previous studies might be results of small sample sizes as well as the criteria used to define or classify poor responder women.

Previous studies demonstrated that letrozole administration reduces required gonadotropin doses by improving follicular sensitivity to FSH stimulation in poor responder women thus cutting the costs of ovarian stimulation [19, 28]. In our study we found significantly decreased gonadotropin requirements as well as shorter duration of stimulation in both POSEIDON group 3 and group 4 expected POR women that might render the process of ovarian stimulation relatively more patient friendly.

As we mentioned earlier embryo quality is a major determinant in achieving clinical pregnancies in older women. In our study, we observed that 5 mg/day of letrozole administration for five days does not create an improvement in embryo quality in both POSEIDON group 3 and POSEIDON group 4 women. However, Shapira et al. demonstrated that extended use of 5mg/day letrozole, starting from the first day of gonadotropin stimulation until the trigger day, increases embryo quality in poor responders [29]. They neither evaluated clinical pregnancy rates and live birth delivery rates in their study nor used the stratification system proposed by POSEIDON study group. However, improved embryo quality could induce an increase in clinical pregnancy rates particularly in POSEIDON group 4 patients. Nevertheless, further studies investigating the effects of extended letrozole administration in POSEIDON group 4 women could elucidate this issue.

In conclusion 5mg/day of adjuvant letrozole administration for five days appears to increase implantation rates, clinical pregnancy rates and live birth delivery rates in POSEIDON group 3 expected POR women.

Declarations

Authors' contributions: All authors contributed to the study conception and design. Data collection and analysis were performed by A Kahraman and F Tulek. The first draft of the manuscript was written by A Kahraman and F Tulek. All authors commented on

previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical approval for this study was obtained from Ethical Committee of Uskudar University at 28/04/2021 (Approval number: 61351342/APRIL 2021-84).

Funding: None

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