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Original Research

Comparison of an estradiol patch and GnRH-antagonist protocol with a letrozole/antagonist protocol for patients without oocyte development, fertilization and/or embryo development in previous IVF cycles

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Background: Diminished ovarian reserve (DOR) is a challenge for clinicians in IVF cycles and several treatment modalities have been suggested to improve outcomes. The aim of this study was to compare live birth rates following ovarian stimulation using a luteal phase estradiol (E2)/gonadotropin releasing hormone (GnRH) antagonist protocol (LPG group) with a letrozole/antagonist (LA group) protocol in patients with DOR. *Methods*: A total of 51 women with DOR, previously canceled IVF cycles and aged 40 years or less were investigated. In the LPG group (n = 26), a transdermal E2 patch was applied every other day starting 10 days after the luteinizing hormone (LH) surge. At day 11 after the LH surge, GnRH antagonists were administered for three consecutive days. In the LA group (n = 25), letrozole (5 mg/day) treatment was begun on the second day of menstruation and administered for 5 consecutive days. In both groups, gonadotropins were initiated on the second day of menstruation. Results for the two groups were compared using chi-square and Student's *t*-test, as appropriate. *Results*: Although the initial and total gonadotropin doses were significantly higher in the LPG group, the peak E2 levels, number of oocytes and fertilization rates and cancellation rates were similar. Trends toward improved live birth rates per transfer (23% vs. 11%) and per cycle (11.5% vs. 4%) were seen in the LPG group compared to the LA group, although the differences were not statistically significant. *Discussion*: DOR patients with previously canceled IVF cycles may be treated with either the LPG or LA protocols.

Keywords

Diminished ovarian reserve; Estradiol patch; Letrozole; GnRH-antagonist protocol

1. Introduction

Poor responders who show suboptimal response to controlled ovarian hyperstimulation (COH) comprise a challenging group for assisted reproductive techniques (ARTs). The reported prevalence of such patients ranges from 9% to 24% [1, 2]. Poor responders have been associated with advanced patient age, poor response to COH, high follicle-stimulating hormone (FSH) levels, low follicular response to exogenous gonadotropin therapy, low peak levels of estradiol (E2), high cancelation rates, low numbers of oocytes and embryos, and the need for high doses of gonadotropin. Despite major advances in ART, the management of poor responders remains difficult. Treatment goals for these patients include increasing the follicular sensitivity to gonadotropins and thus follicular development, with the aim of producing more mature oocytes and good quality embryos. Regardless of whether aggressive protocols or milder treatments are used, these patients still have high cycle cancelation rates and low pregnancy rates. The ideal protocol for improving the treatment results in these patients remains to be determined [1–4].

A common characteristic of poor responders is that early follicular development is asynchronous. The presence of various developmental stages of antral follicles due to different FSH receptor levels can cause different susceptibility to FSH and thus lead to heterogeneous follicular development. The resulting size heterogeneity causes asynchronous growth and poor follicular response at the beginning of COH treatment [5–7]. Estradiol is the main hormone involved in the negative regulation of FSH. The luteal estradiol/GnRH antagonist protocol aims to reduce follicle heterogeneity and synchronize follicular growth during COH by preventing follicular recruitment during the luteal phase. Estradiol administration during the late luteal phase has been shown to suppress an early increase in FSH and to increase the homogeneity of early antral follicles. Similarly, the use of a gonadotropin-releasing hormone antagonist (GnRHa) in the luteal phase induces luteolysis and coordinates follicular size. Subsequently, it induces follicular growth through inhibition of the luteal FSH increase [8–11].

Letrozole is a selective third-generation non-steroidal aromatase inhibitor (AI). It inhibits aromatase and blocks the conversion of androstenedione to E2, thus reducing the negative feedback of E2 to the hypothalamus, increasing FSH and enhancing follicular growth. The increased level of intrafol-

licular androgen also increases the expression of FSH receptors. Following the introduction of AIs for ovarian stimulation in poor responders, several studies have reported an increased response to FSH stimulation and a reduction in total gonadotropin dose and IVF cost [12–15].

The aim of this study was to compare the luteal E2 patch/GnRHa (LPG) protocol and the letrozole/antagonist (LA) protocol as potential treatment options for a subgroup of poor responders with diminished ovarian reserve (DOR). This subgroup did not show follicle development with standard antagonist protocol at maximum dose in previous treatment cycles. Moreover, less than three oocytes had been retrieved from this subgroup and/or embryo transfer was canceled because of fertilization failure or lack of embryo development.

2. Materials and methods

This retrospective study enrolled 51 women aged 40 years or less who were diagnosed with DOR according to the Bologna criteria (antral follicle count <5–7 follicles or anti-Müllerian hormone (AMH) <0.5–1.1 ng/mL or FSH level >12 mIU/L). The ovarian reserve tests were carried out in the IVF center of Selçuk University between January 2015 and January 2019. Participants in the study had a history of inability to perform embryo transfer because of fertilization failure and/or lack of embryo development and/or no follicle development or <3 oocytes retrieved, despite previous administration of an antagonist protocol with a starting dose of at least 300 IU. Excluded from the study were patients who had previously undergone ovarian surgery or received chemo-radiotherapy, had known endometriosis or male factors, or had received adjuvant herbal or vitamin supplements. Each patient was included in the study only once. The decision on which treatment protocol was to be given to each participant was made by the physician and approved by the patient. No vitamin supplements were administered, with the exception of 400 micrograms/day of folic acid. The scientific research and ethics board of Selçuk University approved this study. Patients in the LPG group (n = 26) began to use an E2 patch 10 days after the LH surge of the previous cycle (0.1 mg/day, replaced daily) (Climara forte, Bayer, Istanbul, Turkey) and were administered cetrorelix acetate 11 days after the LH surge (250 mg/day for three days, Cetrotide, Merck Serano, Istanbul, Turkey). On the second day of menstruation, a new patch was not applied and gonadotropin stimulation was initiated with 300 IU of hMG (Menopur, Ferring, Istanbul, Turkey) and 150–300 IU of recombinant FSH (Gonal-F, Merck Serono, Istanbul, Turkey).

The LA group was comprised of 25 patients. On the third day of spontaneous menstruation, gonadotropin stimulation was initiated with 300 IU/day of hMG (Menopur, Ferring, Istanbul, Turkey) and 150–300 IU/day of recombinant FSH (Gonal-F, Merck Serono, Istanbul, Turkey). At day 3, Letrozole (Femara, Novartis, Istanbul, Turkey) was initiated at a dose of 5 mg/day and administered for 5 consecutive days.

In both groups, cetrorelix acetate (0.25 mcg/day) (Cetrotide, Merck Serono, Istanbul, Turkey) was initiated when the follicle size reached 12 mm. In order to prevent premature ovulation, cetrorelix acetate was continued until administration of the ovulation trigger shot. For final oocyte maturation, choriogonadotropin alpha was administrated (HCG) (250 mcg/0.5 mL) (Ovitrelle, Merck Serano, Istanbul, Turkey). The cycle was canceled due to low E2 levels (<130 pg/mL) on the 6th day of the cycle or a low E2 concentration (E2 <250 pg/mL) on the day of HCG administration. Oocyte pick-up was performed under transvaginal ultrasound guidance and 34–36 hours after the HCG trigger shot. Intracytoplasmic sperm injection (ICSI) was performed with mature (metaphase II, MII) oocytes for fertilization. ICSI was canceled in cases where oocytes could not be retrieved or MII oocytes were not found. The luteal phase was supported with daily administration of vaginal progesterone gel (90 mg/day) (Crinone, Merck Serono, Istanbul, Turkey) starting from the first day after oocyte retrieval. In all cases embryo transfer was performed on day 3 of embryo development using a soft catheter under ultrasound guidance. Reasons for the cancelation of embryo transfer were total fertilization failure or the arrest of embryonic development.

Primary outcomes were the number of retrieved oocytes, the fertilization rate, the clinical pregnancy rate and the live birth rate. Secondary outcomes were the cancelation rate, total gonadotropin dose, and the duration of stimulation and peak serum E2 levels. Clinical pregnancy was defined as fetal heart activity using transvaginal ultrasound confirmation.

Data were analyzed using Statistical Package for the Social Sciences 20.0 (SPSS Statistics for Windows, Version 20.0. IBM Corp, Armonk, NY, USA). Student's *t*-test was used for continuous variables. Proportions were compared by the chi-square or Fisher exact tests. Data were presented as mean ± SD. A p-value < 0.05 was considered statistically significant.

3. Results

The study enrolled a total of 51 patients, with 26 in the LPG group and 25 in the LA group. Patient demographics were similar between the two groups (Table 1), including the mean age. Test results also showed that DOR was similar between the two groups. The average number of cycles previously canceled (as defined by the study criteria) was 1.3 ± 0.6 in the LPG group and 1.44 ± 0.5 in the LA group.

Table 2 shows the cycle characteristics and treatment results. The total gonadotropin dose used in the LPG group was higher than that used in the LA group ($p < 0.05$), even though the peak E2 levels were similar. The number of oocytes, the number of MII oocytes, the fertilization rate and the number of embryos transferred were similar between the two groups.

Fifty percent of the LPG cycles and 64% of the LA cycles were canceled. This difference was not statistically significant. Table 3 shows the reasons for cycle cancelations and their distribution.

Table 1. Demographic and clinical characteristics of the study population.

	LPG	LA	*p* value
Age, years	33.2 ± 5.2	33.4 ± 4.9	0.88
BMI, kg/m^2	24.4 ± 4.1	26.5 ± 5.4	0.10
Failed previous IVF cycles	1.3 ± 0.6	1.44 ± 0.5	0.40
Day 3 FSH, IU/L	17.8 ± 4.1	17.0 ± 6.3	0.61
AMH, ng/mL	0.35 ± 0.25	0.36 ± 0.27	0.88
Day 3 E2 (pg/mL)	55.3 ± 17.9	50.0 ± 22.3	0.29

Values are given as number, number (percentage), or mean ± SD. BMI, body mass index; FSH, follicle-stimulation hormone; AMH, anti-Mullerian hormone; E2, estradiol.

The LPG group showed a higher rate of clinical pregnancy (15.4% vs. 8%), pregnancy rate per cycle (11.5% vs. 4%), and pregnancy rate per embryo transfer (23% vs. 11%) compared to the LA group. However, none of these differences was statistically significant.

4. Discussion

DOR remains difficult to manage in the field of IVF and results in poor response to gonadotropin stimulation, low number of oocytes retrieved and low pregnancy rates. This condition is considered normal in women of advanced age. However, when detected in younger women DOR can impact the treatment results obtained from ART. High-dose and aggressive stimulation protocols are generally preferred for the treatment of this group in order to increase the number of mature oocytes and embryos [11, 16]. Here, we compared the efficacy of a luteal estradiol patch/GnRHa protocol used to increase follicular synchronization against that of a letrozole/antagonist protocol that increases the response to gonadotropin therapy by increasing the endogenous FSH level. This comparison was made in relatively young patients who experienced cycle cancelation due to poor response to gonadotropin treatment at maximum dose in earlier therapy cycles.

The important feature of this study is that while examining a subgroup of patients in who the treatment was canceled due to poor ovarian response in their previous cycles and who are not eligible for embryo transfer, our patient group was composed of patients with ovarian reserve tests that resulted as DOR. Even in patients with normal ovarian reserve test results, a poor response to ovarian stimulation can sometimes also occur [17, 18].

Poor response to ovarian stimulation is associated with cycle cancelation rates as high as 75% and pregnancy rates as low as 3–14%. There is no clear consensus on which treatment protocol could improve these results for subsequent cycles. However, younger patients who are poor responders are known to have better oocyte quality and therefore better pregnancy outcomes compared to patients with a more advanced age [11, 19]. For this reason, we studied a homogeneous subgroup of younger, poor responders with DOR by including women aged less than 40 years.

The first report on the LPG protocol investigated a poor responder group with a mean patient age of 39 years [10]. Outcomes with the LPG protocol were compared to previous treatment cycles, with the authors reporting increased oocyte count, fertilization rate and number of transferred embryos, as well as a decreased cycle cancelation rate.

A subsequent study of poor responders also with a mean patient age of 39 years compared the LPG protocol with the standard antagonist protocol. The authors reported better oocyte counts and rates of fertilization, pregnancy and live births with the LPG protocol [20]. Weitzman *et al.* [21] compared the LPG protocol and the microdose agonist protocol in poor responder patients with a mean age of 37 years. They reported no difference in oocyte counts, pregnancy rates and cycle cancelation rates, and concluded that both protocols may be used in this patient group. In another study of poor responders with a mean age of 38 years, the LA protocol decreased the gonadotropin dose, cost and cycle cancelation rates, but the pregnancy rate was the same as that of the classical antagonist protocol [13]. Finally, another study compared the LA protocol with microdose flare-up protocol in poor responders with a mean age of 36 years [22]. Although the total gonadotropin dose and oocyte count were lower with the LA protocol, clinical pregnancy rates were similar in both groups.

In a Cochrane meta-analysis, Farquhar *et al.* [23] compared the cycles with and without estrogen pretreatment in GnRH antagonist cycles. Although they reported that live birth rates did not change, the patients in this meta-analysis were responders and women who had been diagnosed with premature ovarian failure were not included in the study. A second meta-analysis studied the effects of clomiphene citrate and AIs on IVF treatment cycles in poor responders [24]. These oral medications changed the live birth or pregnancy rates compared to patients who received gonadotropin alone. They also led to increased cycle cancelation rates and a decrease in the total dose of gonadotropin used in LA protocols.

In the present study, the cycle cancelation rate was higher with the LA protocol compared to the LPG protocol, but this was not statistically significant (64% vs. 50%, respectively, $p = 0.313$).

To our knowledge, this study is only the second that compared LPG and LA protocols in patients with poor prognosis who responded poorly to ovarian stimulation in earlier treatment cycles. In the first study, the LPG and LA protocols were compared in women younger than 42 years who had poor response (defined as less than 5 oocytes retrieved following ovulation induction with 300 IU or more gonadotropin, or cycle cancelation due to low follicular response) in their previous cycles [15]. Clinical pregnancy rates per cycle and per embryo transfer were reported as 26.9% and 42.4% for LPG, and 25.5% and 50% for LA, respectively. There were no significant differences between the groups. In our study, no significant difference in the pregnancy rate was found between the groups, but the clinical pregnancy rates

Table 2. Cycle characteristics and outcomes.

	LPG (n = 26)	LA (n = 25)	*p* value
No. of days of FSH	10.0 ± 1.5	9.1 ± 2.1	0.52
Initial IU of gonadotropins	542.31 ± 74.42	450 ± 0	<0.05
Total IU of gonadotropins (IU)	5411.53 ± 853.73	4164.16 ± 1027.81	<0.05
Peak E2 (pg/mL)	482.30 ± 186.97	387.92 ± 253.48	0.136
No. of oocytes retrieved	2 (1–4)	1 (1–7)	0.806
No. of mature oocytes retrieved	1 (1–4)	1 (1–7)	0.592
Fertilization rate (n, %)	13 (50 %)	9 (36%)	0.468
No. of embryos transferred	1 (1–2)	1 (1–2)	0.264
Clinical pregnancy rate per cycle (n, %)	4/26 (15.4%)	2/25 (8%)	0.413
Clinical pregnancy rate per transfer (n, %)	4/13 (31%)	2/9 (22%)	0.409
Live birth rate per started cycle (n, %)	3/26 (11.5%)	1/25 (4%)	0.371
Live birth rate per transfer (n, %)	3/13 (23%)	1/9 (11%)	0.359

Values are given as number, number (percentage), or mean ± SD. FSH, follicle-stimulation hormone; E2, estradiol.

Table 3. Details of the cycle cancelation rates.

Cycles	LPG	LA	*p*
Total cycles canceled	13/26 (50%)	16/23 (64%)	0.313
Canceled OPU[a]	3/13 (23.1%)	2/16 (12.5%)	
Canceled ICSI[b]	4/13 (30.8%)	12/16 (75%)	
Canceled embryo transfer	6/13 (46.2%)	2/16 (12.5%)	

Values are given as a number (percentage). [a]OPU, oocyte-pick up; [b]ICSI, intracytoplasmic sperm injection.

per cycle and per embryo transfer were lower in both the LPG group (26% and 42%, respectively) and in the LA group (8% and 22%, respectively) than in the study by Elassar *et al.* [15]. The major reason why the results of the Elassar study are different to those of the present study is likely to be that our patients had a poorer prognosis. This can be explained by the inclusion criteria for our study, which allowed patients with poor prior responses, previously abandoned oocyte pick-up due to lack of follicle development, or no embryo transfer due to the absence of eggs, fertilization or transfer. In addition, the lower ovarian reserve of patients in our study may have contributed to the divergent results. In our study, FSH was 17.8 ± 4.1 mIU/mL in the LPG group and 17.0 ± 6.3 mIU/mL in the LA group, whereas in the study by Elassar *et al.* it was 4.07 ± 3.0 and 9.2 ± 2.8 mIU/mL in the LPG and LA groups, respectively. In addition, Elassar *et al.* administered 5 mg/day of letrozole for 5 days starting from the second day of menstruation. They did not administer gonadotropins simultaneously but started these three days after treatment with letrozole.

One limitation of the present study is its retrospective nature. A second limitation is the small cohort size due to the strict exclusion criteria such as age >40 years, prior surgery, endometriosis and use of adjuvant therapies. Trends for higher live birth rates per transfer (23% vs. 11%) and per cycle (11.5% vs. 4%) were observed in the LPG group compared to the LA group, however these were not statistically significant. Because no significant differences in terms of pregnancy and live birth rates were observed between the LPG and LA protocols, we conclude that both are viable treatment options for women with a history of poor prognosis for IVF and when egg donation is not an option.

5. Conclusions

Outcomes for young women with DOR and a history of IVF failure because of poor response to ovarian stimulation could potentially be improved by the use of LPG or LA protocols in subsequent cycles.

Author contributions

AP and ÖSK designed the research study. AP, ÖSK, AGK and EÇ performed the research. AP analyzed the data. AP and ÖSK wrote the manuscript. All of the authors have read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Selcuk University (approval number: 2020/184).

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Conflict of interest

The authors declare no conflict of interest.

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