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Comparison of the standard GnRH antagonist protocol and the luteal phase estradiol/ GnRH antagonist priming protocol in poor ovarian responders

Mehmet Fırat MUTLU[1,*], İlknur MUTLU[2], Mehmet ERDEM[3], İsmail GÜLER[3], Ahmet ERDEM[3]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Yüksek İhtisas University Ankara, Turkey
[2]IVF Unit, Novaart IVF and Women Health Center, Ankara, Turkey
[3]Department of Obstetrics and Gynecology, Faculty of Medicine, Gazi University, Ankara, Turkey

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Background/aim: The aim of the study was to compare the luteal estradiol patch/GnRH antagonists priming protocol (LPP) with the standard GnRH antagonist protocol in poor ovarian responders (PORs) in terms of the outcomes of in vitro fertilization (IVF) treatment

Materials and methods: IVF outcomes of 265 cycles in 265 patients (106 in the LPP group, 159 in the standard GnRH antagonist group) were evaluated retrospectively.

Results: Mean length of stimulation (11.4 ± 2.7 vs. 10.0 ± 2.7 days; $P < 0.05$) and the total gonadotropin dose (3403 ± 1060 vs. 2984 ± 1112) used were significantly greater in the LPP group than in the standard GnRH antagonist protocol group. The mean number of oocytes retrieved (3.5 ± 2.6 vs. 3.7 ± 2.8), the number of mature oocytes (2.8 ± 2.2 vs. 2.6 ± 2.2), fertilization rates (65% vs. 62%), the number of embryos transferred (1.6 ± 0.6 vs. 1.7 ± 0.6), and implantation rates (16% vs. 13%) were similar. The cancellation rate did not significantly differ between the groups (9.4% vs. 13.2%). There were no significant differences in the clinical pregnancy (11.3% vs. 13.2%) or live birth rates per patient (3.8% vs. 9.4%) and clinical pregnancy (18.8% vs. 22.6%) or live birth rates per embryo transfer (6.3% vs. 12.9%) between the groups.

Conclusion: LPP does not improve IVF outcomes when compared with the standard GnRH antagonist protocol in PORs.

Key words: In vitro fertilization, poor ovarian response, GnRH antagonists, estradiol

1. Introduction

Poor ovarian responders (PORs) are the most compelling infertile population subgroup in assisted reproduction, with a prevalence ranging from 9% to 24% (1). In PORs, total number of growing follicles, retrieved oocytes, and transferred embryos; peak estradiol levels; and implantation and pregnancy rates are all lower whereas cycle cancellation rates are higher than they are in women who respond normally to ovarian hyperstimulation (COH) (2,3).

Poor ovarian response to COH is a result of decreased numbers of mature oocytes due to follicular asynchronization as well as low ovarian reserve (4,5). In PORs, a rise in FSH in the late luteal phase induces development of sensitive and larger antral follicles and causes asynchronization among follicle diameters. As a result, the number of follicles ready for recruitment and the number of retrieved oocytes decrease (6,7). In this patient group, various protocols have been used to increase success rates, but none of these has prevailed (8,9). Dragisic et al. were the first who used the luteal estradiol patch/GnRH antagonists priming protocol (LPP) in an attempt to overcome early FSH rise in the luteal phase without suppressing ovarian functions (10). In this protocol, simultaneous administration of a transdermal estradiol patch and GnRH antagonist helps to achieve dual suppression of FSH and subsequently gonadotropin stimulation with addition of flexible antagonist is started in the cycle (10). In some studies, LPP has been compared with well-known protocols (GnRH antagonist and microdose flare-up) (6,7,10–15). A recent meta-analysis shows that LPP has significantly higher clinical pregnancy rates when compared with other protocols. However, the results were limited by uncertain definitions of poor responders in the included studies (16).

Our search of the current literature revealed no trials comparing the standard GnRH antagonist protocol with the luteal estradiol patch/GnRH antagonists priming protocol. The aim of our study was to examine whether adding luteal estradiol and GnRH antagonist pretreatment

* Correspondence: firatmutlu78@hotmail.com

to GnRH antagonist protocols can improve in vitro fertilization (IVF) outcomes in PORs.

2. Materials and methods

A retrospective review of records between May 2014 and September 2015 was performed at our clinic. Two hundred and sixty-five PORs according to the European Society of Human Reproduction and Embryology (ESHRE) Bologna criteria were included (17). Women were classified as PORs if they met at least two of the following three criteria: (i) advanced maternal age (≥40) or any other risk factor for poor ovarian response; (ii) a previous poor ovarian response (cycles cancelled or ≤3 oocytes retrieved with a conventional protocol); (iii) an abnormal ovarian reserve test (ORT) (antral follicle count <5–7 follicles or anti-Müllerian hormone (AMH) <0.5–1.1 ng/mL). Women who had two episodes of poor ovarian response after maximal stimulation in the absence of advanced maternal age or an abnormal ORT were also defined as poor responders (fourth item of the Bologna criteria). Patients aged ≤25 and ≥43 were excluded. The institutional review board approved the study.

Patients in the LPP group started using one 0.1 mg/day transdermal estradiol patch (Climara forte, Bayer, İstanbul, Turkey) 7 days after ovulation, which was proved by transvaginal ultrasonography (TVU), and changed the patch every other day three times. The last estradiol patch was removed on the second day of menses. Even if menstrual bleeding did not start, the last estradiol patch stayed on for no longer than 1 week. Following the day on which the first patch was applied, 0.25 mg/day subcutaneous GnRH antagonist cetrorelix (Cetrotide; Merck–Serono, İstanbul, Turkey) was started and applied for 3 days. The patients on the GnRH antagonist protocol did not receive any hormonal pretreatment during the luteal period.

In both groups, ovarian stimulation with 150 IU recombinant FSH (Gonal-F; Merck Serono, İstanbul, Turkey) and 150 IU hMG (Merional; IBSA, Turkey) was started on the second day of menses. Monitoring of follicular development and gonadotropin dose adjustments were performed with serial ultrasound and levels of serum E_2. Cetrorelix (Cetrotide; Merck Serono) was started 0.25 mg/day subcutaneously when the leading follicle was >13 mm or E_2 >300 pg/mL and was continued through the day of human chorionic gonadotropin (hCG) injection. Cycle cancellations were performed due to lack of ovarian response (when there were no follicular recruitment and/or peak E_2<100 pg/mL despite adequate gonadotropin). When one or more follicles were 17 mm or more in mean diameter, hCG (Ovitrelle 250 μg; Merck Serono) was given for final oocyte maturation. Endometrial thickness was measured by TVU on the day of hCG administration and recorded.

TVU-guided oocyte retrieval was performed 35 h after hCG administration. ICSI was carried out in all cases. One to three embryos were transferred under ultrasonographic guidance 48–72 h after oocyte pick-up, depending on the quality and number of embryos. For luteal support, 90 mg daily intravaginal progesterone gel (8% Crinone gel; Merck Serono) and 4 mg daily oral estradiol hemihydrate (Estrofem 2 mg, Novo Nordisk, İstanbul, Turkey) were administered starting on the day after oocyte pick-up and, if the pregnancy test performed 12 days after embryo transfer (ET) was positive, continued until 9 weeks of gestation.

Pregnancy was tested by measuring serum βhCG level 12 days after ET and intrauterine pregnancy was confirmed by TVU examination 2 weeks after a positive pregnancy test. Clinical pregnancy was defined as a pregnancy confirmed by ultrasound visualization of the gestational sac between the 5th and 6th weeks of gestation. Implantation rate was defined by the number of gestational sacs on ultrasound divided by the number of embryos transferred. Live birth rate was defined as delivery of a viable baby after 24 weeks of gestation.

Primary outcomes were number of oocytes retrieved and live birth rate. The secondary outcome was cycle cancellation rate.

All statistical analyses were performed using SPSS version 20 (Chicago, IL, USA). Student's t-test was used for continuous variables, and the chi-square test was used for categorical variables. P values <0.05 were considered statistically significant.

3. Results

A total of 265 patients were included in the study; 106 women used the LPP and 159 women were given the standard GnRH antagonist protocol. The baseline characteristics of patients are presented in Table 1. The groups were similar with respect to age, body mass index (BMI), basal FSH, duration of infertility, basal antral follicle count, and number of prior IVF attempts.

The cycle characteristics and outcomes are presented in Table 2. Mean length of stimulation (11.4 ± 2.7 vs. 10.0 ± 2.7 days; P = 0.01) and the total gonadotropin dose (3403.7 ± 1060.6 vs. 2984.4 ± 1112.1 IU; P < 0.05) used were significantly higher in the LPP group than in the standard GnRH antagonist protocol group. The mean number of oocytes retrieved (3.5 ± 2.6 vs. 3.7 ± 2.8), number of mature oocytes (2.8 ± 2.2 vs. 2.6 ± 2.2), fertilization rates (65% vs. 62%), number of embryos transferred (1.6 ± 0.6 vs. 1.7 ± 0.6), and implantation rates (16% vs. 13%) were similar. The cancellation rates were not significantly different between the groups (9.4% vs. 13.2%). There were no significant differences in the clinical pregnancy (11.3% vs. 13.2%) or live birth rates (LBR) per patient (3.8% vs.

Table 1. Comparison of patient characteristics of the LPP and standard GnRH antagonist protocol.

	LPP group (n = 106)	Standard GnRH antagonist group (n = 159)	P value
Age (years)	38.7 ± 4.8	39 ± 3.7	NS
BMI (kg/m^2)	24.5 ± 3.4	23.8 ± 3.6	NS
Basal antral follicle count	4.1 ± 1.9	4.5 ± 2.1	NS
Basal FSH (mIU/mL)	12.2 ± 7.6	12.5 ± 6.1	NS
Duration of infertility	7.1 ± 5.7	7.8 ± 6.5	NS
Prior IVF attempts	1.7 ± 1.5	2.0 ± 2.2	NS

NS: nonsignificant

Table 2. Comparison of cycle characteristics and outcomes.

	LPP group (n = 106)	Standard GnRH antagonist group (n = 159)	P value
Total length of stimulation (day)	11.4 ± 2.7	10.0 ± 2.7	0.01
Total dose of gonadotropin (IU)	3403.7 ± 1060.6	2984.4 ± 1112.1	0.04
Peak E_2 (pg/mL)	892.5 ± 624.2	1098.0 ± 757.1	NS
Endometrial thickness on hCG day (mm)	10.9 ± 2.0	10.3 ± 1.9	NS
No. of oocytes retrieved (n)	3.5 ± 2.6	3.7 ± 2.8	NS
No. of mature oocytes (n)	2.8 ± 2.2	2.6 ± 2.2	NS
No. of normally fertilized oocytes	2.0 ± 2.1	1.7 ± 1.8	NS
Fertilization rate	65%	62%	NS
No. of embryos transferred	1.6 ± 0.6	1.7 ± 0.6	NS
Cancellation rate	9.4% (10/106)	13.2% (21/159)	NS
Implantation rate	16%	13%	NS
Clinical pregnancy rate per ET	18.8% (12/64)	22.6% (21/93)	NS
Clinical pregnancy rate per patient	11.3% (12/106)	13.2% (21/159)	NS
Live birth rate per ET	6.3% (4/64)	12.9% (12/93)	NS
Live birth rate per patient	3.8% (4/106)	9.4% (15/159)	NS

NS: Nonsignificant
ET: Embryo transfer

9.4%) and clinical pregnancy (18.8% vs. 22.6%) or live birth rates per embryo transfer (6.3% vs. 12.9%) between the groups.

4. Discussion

Management of PORs is a compelling issue in assisted reproduction practice because of diverse and unclear definitions and controversial, heterogeneous data regarding the optimal protocol (16). To the best of our knowledge, this is the first study based on the ESHRE Bologna criteria and in which outcomes of a GnRH antagonist protocol combined with luteal estradiol patch and GnRH antagonist pretreatment were compared with those of a standard GnRH antagonist protocol. The results of our study demonstrated no significant improvement in IVF treatment outcomes in the LPP group.

In PORs, due to the effect of FSH rise during the late luteal phase, advanced growth of fewer and more sensitive follicles can cause asynchronism and ultimately result in a smaller cohort available for recruitment and decreased oocyte yield (5,18). In order to suppress FSH rise in the preceding luteal phase, prevent asynchronous follicular stimulation, and obtain a larger and more coordinated cohort of follicles responding to the stimulation, oral

contraceptive pills, microdose agonist flare protocol (MDP), and LPP are commonly used in clinical practice (15). On the other hand, standard GnRH antagonist protocols have been used increasingly more often in PORs because of their lower costs, shorter durations of stimulation, absence of pituitary downregulation, decrease in the total amount of gonadotropins, and at least equivalent pregnancy and implantation rates when compared with GnRH agonist protocols (19). Therefore, we aimed to examine whether luteal estradiol and GnRH antagonist pretreatment could provide additional advantages to outcomes in PORs treated with the standard GnRH antagonist protocol. We preferred to design a study in which we could reveal the effect of priming treatment more accurately by comparing LPP with the standard GnRH antagonist protocol instead of MDP, which was the control protocol in most studies (7,11,12,14,15)

Our findings are consistent with earlier studies demonstrating similar numbers of oocytes retrieved in the LPP group when compared with controls (7,11,12,14,15). A recent and single randomized study assessing IVF outcomes in 54 poor responders undergoing a MDP or a LPP showed no significant differences in number of oocytes retrieved, cancellation rates, or pregnancy rates between the two protocols (14). Ata et al. compared 57 anticipated poor responders who underwent LPP or MDP retrospectively and showed that oocyte yield and pregnancy outcomes were similar in the two groups (12). Previously, a retrospective study in which 45 poor responders using LPP were compared with 76 patients using MDP showed similar number of oocytes retrieved, cancellation rates, or pregnancy rates (15). In a recent retrospective study that compared the IVF outcomes of luteal estrogen priming and letrozole co-treatment in an antagonist protocol in PORs according to the Bologna criteria revealed no significant difference in terms of pregnancy outcome (20). Recently, a meta-analysis determined that there was no significant improvement in the number of oocytes retrieved in PORs treated with luteal estradiol priming protocols (16). Furthermore, it was shown that initiation of FSH in the luteal phase yielded similar retrieved oocyte number and pregnancy rates as compared to initiation of FSH in the follicular phase in two studies (21,22). These results suggest that in fact follicular recruitment has already been initiated before the last menstrual luteal phase and so the pretreatment can synchronize the follicles but cannot improve oocyte yield (23). However, in two other studies luteal phase priming treatment improved ovarian response to ovarian hyperstimulation (10,13). Dragisic et al. investigated the effect of the luteal estradiol patch/GnRH antagonist protocol by comparing the outcomes with previous cycles of the same patients without LPP. They reported a significantly lower cancellation rate, a higher mean number of oocytes retrieved and fertilized, and a higher mean number of embryos transferred in the LPP group (10). The heterogeneity of the previous treatment protocols used in comparison might yield inconclusive results. Chang et al. examined PORs undergoing stimulation with the luteal estradiol protocol and standard GnRH antagonist protocol retrospectively and found that the luteal estradiol protocol had significantly higher peak estradiol levels, numbers of oocytes retrieved, and pregnancy rates and lower cancellation rates (13). These results may be the consequence of heterogeneity in the luteal estradiol group in which most of the patients had used estradiol through the day of hCG administration. It is known that estrogen induces FSH receptor proliferation in granulosa cells and stimulates follicular growth and granulosa cell proliferation. Extended use of estradiol can cause higher peak estradiol levels and numbers of oocytes retrieved.

The successful outcomes in PORs were closely linked to increased numbers of retrieved oocytes and transferred embryos (24,25). In relation to this, we could not determine any statistically significant differences between the two groups in terms of cycle cancellation rate or LBR. In the current literature, the only study in which the primary outcome was LBR, similar to ours, demonstrated that estradiol priming protocol did not improve IVF outcomes (7). Our results also showed a significant increase in the total dose of gonadotropins and duration of stimulation in the LPP group as reported in many other trials (7,11–13). Peak E_2 level was lower in the LPP than in the standard GnRH antagonist protocol most probably due to dual suppression with antagonist and estradiol in the late luteal phase. Suppression of FSH in the preceding luteal phase and synchronic antral follicles small in diameter at the beginning of stimulation may be the reason for the increase in the total dose of gonadotropins and days of stimulation (7,13). Although the higher gonadotropin doses were used in the LPP group in our study, this situation did not improve cycle outcomes as it has been demonstrated in the literature (26–28).

The limitations of this study are its retrospective design and small sample size as with most published trials in which luteal estradiol priming protocol was compared with other protocols in poor responders. Despite these limitations, comparison of two protocols that differ only in the pretreatment component gave us an advantage to demonstrate the impact of luteal estradiol and antagonist pretreatment on IVF outcomes more precisely.

In conclusion, the luteal phase estradiol/GnRH antagonist priming protocol does not improve IVF outcomes compared with the standard GnRH antagonist protocol in PORs. Concerning the issue of cost-effectiveness, adding luteal phase estradiol and GnRH

antagonist pretreatment to GnRH antagonist protocols seems to increase the cost of the COH cycle, which results in an additional financial burden for poor responders, who generally participate in multiple trials. Ultimately, randomized controlled trials with larger sample sizes are required.

References

1. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update 2003; 9: 61-76.
2. Hendriks DJ, te Velde ER, Looman CW, Bancsi LF, Broekmans FJ. Expected poor ovarian response in predicting cumulative pregnancy rates: a powerful tool. Reprod Biomed Online 2008; 17: 727-736.
3. Ulug U, Ben-Shlomo I, Turan E, Erden HF, Akman MA, Bahceci M. Conception rates following assisted reproduction in poor responder patients: a retrospective study in 300 consecutive cycles. Reprod Biomed Online 2003; 6: 439-443.
4. Fanchin R, Salomon L, Castelo-Branco A, Olivennes F, Frydman N, Frydman R. Luteal estradiol pre-treatment coordinates follicular growth during controlled ovarian hyperstimulation with GnRH antagonists. Hum Reprod 2003; 18: 2698-2703.
5. Fanchin R, Cunha-Filho JS, Schonauer LM, Kadoch IJ, Cohen-Bacri P, Frydman R. Coordination of early antral follicles by luteal estradiol administration provides a basis for alternative controlled ovarian hyperstimulation regimens. Fertil Steril 2003; 79: 316-321.
6. Elassar A, Engmann L, Nulsen J, Benadiva C. Letrozole and gonadotropins versus luteal estradiol and gonadotropin-releasing hormone antagonist protocol in women with a prior low response to ovarian stimulation. Fertil Steril 2011; 95: 2330-2334.
7. Hill MJ, McWilliams GD, Miller KA, Scott RT, Jr, Frattarelli JL. A luteal estradiol protocol for anticipated poor-responder patients may improve delivery rates. Fertil Steril 2009; 91: 739-743.
8. Kyrou D, Kolibianakis EM, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Fertil Steril 2009; 91: 749-766.
9. Loutradis D, Drakakis P, Milingos S, Stefanidis K, Michalas S. Alternative approaches in the management of poor response in controlled ovarian hyperstimulation (COH). Ann N Y Acad Sci 2003; 997: 112-119.
10. Dragisic KG, Davis OK, Fasouliotis SJ, Rosenwaks Z. Use of a luteal estradiol patch and a gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation for in vitro fertilization in poor responders. Fertil Steril 2005; 84: 1023-1026.
11. Shastri SM, Barbieri E, Kligman I, Schoyer KD, Davis OK, Rosenwaks Z. Stimulation of the young poor responder: comparison of the luteal estradiol/gonadotropin-releasing hormone antagonist priming protocol versus oral contraceptive microdose leuprolide. Fertil Steril 2011; 95: 592-595.
12. Ata B, Zeng X, Son WY, Holzer H, Tan SL. Follicular synchronization using transdermal estradiol patch and GnRH antagonists in the luteal phase; does it increase oocyte yield in poor responders to gonadotropin stimulation for in vitro fertilization (IVF)? A comparative study with microdose flare-up protocol. Gynecol Endocrinol 2011; 27: 876-879.
13. Chang EM, Han JE, Won HJ, Kim YS, Yoon TK, Lee WS. Effect of estrogen priming through luteal phase and stimulation phase in poor responders in in-vitro fertilization. J Assist Reprod Genet 2012; 29: 225-230.
14. DiLuigi AJ, Engmann L, Schmidt DW, Benadiva CA, Nulsen JC. A randomized trial of microdose leuprolide acetate protocol versus luteal phase ganirelix protocol in predicted poor responders. Fertil Steril 2011; 95: 2531-2533.
15. Weitzman VN, Engmann L, DiLuigi A, Maier D, Nulsen J, Benadiva C. Comparison of luteal estradiol patch and gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation versus microdose gonadotropin-releasing hormone agonist protocol for patients with a history of poor in vitro fertilization outcomes. Fertil Steril 2009; 92: 226-230.
16. Reynolds KA, Omurtag KR, Jimenez PT, Rhee JS, Tuuli MG, Jungheim ES. Cycle cancellation and pregnancy after luteal estradiol priming in women defined as poor responders: a systematic review and meta-analysis. Hum Reprod 2013; 28: 2981-2989.
17. Ferraretti AP, Gianaroli L. The Bologna criteria for the definition of poor ovarian responders: is there a need for revision? Hum Reprod 2014; 29: 1842-1845.
18. Frattarelli JL, Hill MJ, McWilliams GD, Miller KA, Bergh PA, Scott RT, Jr. A luteal estradiol protocol for expected poor-responders improves embryo number and quality. Fertil Steril 2008; 89: 1118-1122.
19. Mahutte NG, Arici A. Role of gonadotropin-releasing hormone antagonists in poor responders. Fertil Steril 2007; 87: 241-249.
20. Pabuccu EG, Caglar GS, Pabuccu R. Estrogen or anti-estrogen for Bologna poor responders? Gynecol Endocrinol; 2015; 31: 955-958.
21. Kansal Kalra S, Ratcliffe S, Gracia CR, Martino L, Coutifaris C, Barnhart KT. Randomized controlled pilot trial of luteal phase recombinant FSH stimulation in poor responders. Reprod Biomed Online 2008; 17: 745-750.
22. Rombauts L, Suikkari AM, MacLachlan V, Trounson AO, Healy DL. Recruitment of follicles by recombinant human follicle-stimulating hormone commencing in the luteal phase of the ovarian cycle. Fertil Steril 1998; 69: 665-669.

23. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev 1996; 17: 121-155.

24. De Vries MJ, De Sutter P, Dhont M. Prognostic factors in patients continuing in vitro fertilization or intracytoplasmic sperm injection treatment and dropouts. Fertil Steril 1999; 72: 674-678.

25. Sharma V, Allgar V, Rajkhowa M. Factors influencing the cumulative conception rate and discontinuation of in vitro fertilization treatment for infertility. Fertil Steril 2002; 78: 40-46.

26. Lefebvre J, Antaki R, Kadoch IJ, Dean NL, Sylvestre C, Bissonnette F, Benoit J, Ménard S, Lapensée L. 450 IU versus 600 IU gonadotropin for controlled ovarian stimulation in poor responders: a randomized controlled trial. Fertil Steril 2015; 104: 1419-1425.

27. van Hooff MH, Alberda AT, Huisman GJ, Zeilmaker GH, Leerentveld RA. Doubling the human menopausal gonadotrophin dose in the course of an in-vitro fertilization treatment cycle in low responders: a randomized study. Hum Reprod 1993; 8: 369-373.

28. Cedrin-Durnerin I, Bstandig B, Herve F, Wolf J, Uzan M, Hugues J. A comparative study of high fixed-dose and decremental-dose regimens of gonadotropins in a minidose gonadotropin-releasing hormone agonist flare protocol for poor responders. Fertil Steril 2000; 73: 1055-1056.