Original Article

Microdose Flare-up Gonadotropin-releasing Hormone (GnRH) Agonist Versus GnRH Antagonist Protocols in Poor Ovarian Responders Undergoing Intracytoplasmic Sperm Injection

Aysen Boza [1*], Erbil Cakar [2], Barış Boza [2], Murat Api [2], Semra Kayatas [2], Kenan Sofuoglu [2]

1- Goztepe Research and Training Hospital, Kadikoy, Istanbul, Turkey
2- Zeynep Kamil Maternity and Children Research and Training Hospital, Uskudar, Istanbul, Turkey

Abstract

Background: Microdose flare-up GnRH agonist and GnRH antagonist have become more popular in the management of poor ovarian responders (POR) in recent years; however, the optimal protocol for POR patients undergoing *in vitro* fertilization has still been a challenge.

Methods: In this observational study design, two hundred forty four poor ovarian responders were retrospectively evaluated for their response to GnRH agonist protocol (group-1, n=135) or GnRH antagonist protocol (group-2, n=109). Clinical pregnancy rate was the primary end point and was compared between the groups. Student t-test, Mann Whitney U test and χ^2-test were used to compare the groups. The $p<0.05$ was considered to show a statistically significant result.

Results: The mean total gonadotropin doses were 3814±891 *IU* in group 1 and 3539±877 *IU* in group 2 (p=0.02). The number of metaphase-II oocytes (3.6±2.4 *vs*. 2.8±1.9, p=0.005) and implantation rates (27.8% *vs*. 18.8%, p=0.04) in group 1 and group 2, respectively were significantly different. The fertilization rate in group 1 and group 2 was 73% *vs*. 68%, respectively (p=0.5) and clinical pregnancy rate was 19.8% *vs*. 14.4%, respectively (p=0.13).

Conclusion: The GnRH agonist microdose flare-up protocol has favorable outcomes with respect to the number of oocytes retrieved and implantation rate; nevertheless, the clinical pregnancy rate was found to be similar in comparison to GnRH antagonist protocol in poor ovarian responders. GnRH antagonist protocol appears to be promising with significantly lower gonadotropin requirement and lower treatment cost in poor ovarian responders.

** Corresponding Author:*
Aysen Boza, Goztepe Training and Research Hospital, Department of Obstetrics and Gynecology, Doktor Erkin Street, Postal code: 34730, Kadikoy, Istanbul, Turkey
E-mail:
aysenboza@hotmail.com

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Introduction

The optimal protocol for poor ovarian responders (POR) undergoing *in vitro* fertilization (IVF) has still been a challenge. Poor response to ovarian stimulation occurs in 9%-24% of women undergoing IVF which results in poor clinical pregnancy rates (CPR) and many of these cycles are cancelled without proceeding to egg collection (1). For preventing cycle cancellation, several strategies have been suggested such as decreasing the dosage and timing of gonadotropin releasing hormone agonist (GnRH-a) (2) or the use of GnRH-a flare up regimens (3). Theoretically, these strategies should eliminate excessive ovarian suppression while capitalizing on the initial stimulatory effect of GnRH-a on pituitary gonadotropin release. The overall CPR when using flare protocols in POR has ranged between 12% and 26.3% (4, 5). However, some reports on

outcomes using a decreased dose of GnRH-a (the so-called microdose flare protocol) have revealed conflicting results in cycle cancellation rate (CCR) and CPR despite improved cycle outcomes (3, 6).

In recent years, GnRH antagonists (GnRH-ant) have been used in the treatment of POR to prevent the LH surge. The action of GnRH-ant does not result in early folliculogenesis inhibition, which is a critical point for patients with a limited cohort of follicles (4, 7) because GnRH-ant can be administered during the late follicular phase. As a consequence, they promptly suppress pituitary gonadotropin secretion, which allows their use without the need for a desensitization period.

After GnRH-ant was introduced into clinical practice, these two protocols became popular in terms of treatment of POR but several studies compared flare-up and antagonist protocols have still reported conflicting results (8-10). The aim of this study was to compare the effectiveness of GnRH-a microdose flare-up and multiple doses GnRH-ant protocols in patients with POR undergoing intra-cytoplasmic sperm injection (ICSI) and embryo transfer (ET) cycles.

Methods

A total of 285 patients diagnosed with POR and treated in our IVF center (Zeynep Kamil Training and Research Hospital, Assisted Reproduction Unit) with either GnRH-a microdose flare-up or GnRH-ant protocols between January 2011 and January 2014 were enrolled in this observational retrospective study. All patients had fulfilled three criteria for poor response; patients with less than 4 oocytes in all previous IVF cycles, who needed at least 375 *IU* of daily gonadotropin in the study cycle or having totally ≤4 antral follicle count (AFC) on bilateral ovaries, patients with previous ovarian surgery, stage III-IV endometriosis according to the revised American Fertility Society classification (1985) or with inflammatory, autoimmune, or chromosomal disorders. Also, patients with endocrine or metabolic diseases who lacked medical records were all excluded. Of 285 patients, 244 patients were included to the final analysis. For 135 patients (176 cycles), GnRH-a microdose flare-up protocol (group 1) was administered and for 109 patients (138 cycles) GnRH-ant protocol (group 2) was used.

None of the patients received any hormonal pretreatment. Patients in the flare-up protocol were started on 50 *μg* SC of leuprolide acetate (Lucrin; Abbott, Cedex, France) twice daily on cycle day (CD) 2 followed by high dose gonadotropin stimulation on CD 3. In the antagonist protocol, the gonadotropin was started on CD 3 and 0.25 *mg* cetrorelix (Cetrotide; Asta Medica, Frankfurt, Germany) was administered daily when two or more follicles reached 13-14 *mm* in diameter. In both regimes, at least 375 *IU* of gonadotropins [recombinant FSH (Gonal-F; Serono, Istanbul, Turkey) and/or human menopausal gonadotropin (Menogon; Ferring, Istanbul, Turkey)] was administered to all patients by adjusting the doses according to the ovarian response. GnRH-a, GnRH-ant and gonadotropin administration continued until the day of hCG administration. HCG (6,500 *IU* IM, Ovitrelle, Merck Serono, Germany) was administered when at least two leading follicles reached a mean diameter of 17 *mm* and serum estradiol (E2) concentration was over 500 *pg/ml*. Transvaginal oocyte retrieval was scheduled 36 hours after hCG injection. ICSI was performed for all metaphase II oocytes according to our clinical approach, and ET for good-quality embryos was performed under ultrasound guidance on days 3, 4 and 5. After the transfer, luteal support was provided by administering 90 *mg* of vaginal progesterone gel (Crinone 8% gel, Serono) once daily.

CPR was defined as the presence of gestational sac with accompanying fetal heart beat by ultrasound 4 weeks after ET for each ICSI cycle. Implantation rate (IR) was defined as the ratio of gestational sac to the number of ETs and fertilization rate (FR) was defined as the proportion of oocytes resulting in two pro-nuclei formation.

Statistical analyses were performed using the SPSS software version 15.0 for Windows (SPSS, Chicago). Data were expressed as the mean±SD or percentages. The Student t-test, Mann Whitney U test, and χ^2-test were used to compare the groups. The $p<0.05$ was considered to show a statistically significant result.

Results

The mean age of the patients, mean duration of infertility, body mass index, the number of previous IVF cycles, basal AFC, serum basal FSH and E2 level were similar between groups (Table 1).

The comparison of cycle parameters and clinical outcomes including total gonadotropin doses used, duration of stimulation, mean number of follicles, mean number of oocytes retrieved, mean number of embryos transferred and FR, CCR and CPR are given in table 2. The mean total gonadotropin doses were 3814±891 *IU* in group 1 and

Table 1. Demographic and baseline characteristics of the groups

	GnRH-a (group-1) (n=135)	GnRH-ant (group-2) (n=109)	P-value
	Mean±SD	Mean±SD	
Age (years)	35±3.1	34.3±3.9	0.23
Duration of infertility (years)	6.8±3	6.6±2.9	0.4
BMI (*kg/m²*)	24±1	24±1.1	0.59
N. of previous IVF cycles (n)	3±1	3±0.9	0.6
Antral follicle count (n)	3.2±1.6	3.3±1.7	0.6
Basal FSH (*IU/l*)	10.7±4.1	10.7±4.6	0.9
Basal E2 (*pg/ml*)	66.1±88.4	59.6±39	0.8

GnRH-a: Gonadotropin Releasing Hormone Analog, GnRH-ant: Gonadotropin Releasing Hormone Antagonist, BMI: Body Mass Index, IVF: *In vitro* Fertilization, FSH: Follicle Stimulating Hormone, E2: Estradiol

Table 2. Comparison of the cycle characteristics and outcomes between groups

	GnRH-a (group-1) (n=176)	GnRH-ant (group-2) (n=138)	P-value
Total gonadotropine dose (*IU*)	3814±891	3539±877	0.02
Duration of stimulation (days)	8.7±1.6	8.4±1.5	0.25
N. of follicles	3±2	2.7±1.7	0.08
Cycle cancellation rate (n, %)	21 (11.9)	22 (19.9)	0.19
Peak E2 level (*pg/ml*)	1263±938	1276±983	0.9
Endometrial thickness (*mm*)	9.2±1.6	9.3±2	0.66
N. of oocytes (metaphase II) retrieved	3.6±2.4	2.8±1.9	0.005
Fertilization rate (%)	73	68	0.5
N. of embryos transferred	1.5±0.6	1.3±0.5	0.07
Embryo quality, (%)			
Grade 1	60.3	51.4	0.11
Grade 2	39.7	48.6	
Implantation rate (%)	27.8	18.8	0.04
Clinic pregnancy rate (%)	19.8	14.4	0.13

Values are given as mean±SD, unless otherwise indicated; GnRHa: Gonadotropin Releasing Hormone Analog, GnRHant: Gonadotropin Releasing Hormone Antagonist, hCG: Human Chronionic Gonadotropine, E2: Estradiol

3539±877 *IU* in group 2 (p=0.02). No statistical significance between the protocol types with respect to the duration of stimulation, mean number of follicles, peak E2 level was found. CCR was not different between the groups. The number of metaphase-II oocytes (3.6±2.4 *vs*. 2.8±1.9, p=0.005) and IR (27.8% *vs*. 18.8%, p=0.04) in group 1 and group 2, respectively were significantly different.

The FR in group 1 and group 2 was 73% *vs*. 68%, respectively (p=0.5) and CPR was 19.8% *vs*. 14.4%, respectively (p=0.13).

Discussion

Despite the improvements in the success of IVF in all age groups, the treatment of POR remains a significant problem in assisted reproductive techniques. The best stimulation protocol for POR should have an acceptable rate of cycle cancellation, yield the maximum number of mature, good-quality oocytes, at a reasonable cost with optimal side effects and duration of therapy and have reasonable pregnancy and delivery rates. According to the results of a recent survey (11) in which 272 IVF units from 45 countries responded, the preferred protocol for POR was the GnRH-ant protocol (52%), the short GnRH-a flare-up (20%) and the microdose protocols (15%). In this retrospective study, an attempt was made to compare the most common protocols, microdose flare-up and antagonist, used in the treatment for POR.

A microdose flare-up regimen has been developed to minimize the ovarian suppression of the

GnRH-a long protocols while taking the advantage of the initial up-regulatory effect of leuprolide on follicular recruitment which was used successfully in POR. Toth et al. (12) reported that using flare protocols in patients with basal FSH level >15 *IU/l* significantly increased CPR over long agonist protocols. Leonodris et al. (6) retrospectively analyzed 170 patients in either GnRH-a long or flare protocol, and found no statistically significant differences in regard to CPR but found a higher cancellation rate in microdose flare group. Conversely, Surrey et al. (3) reviewed different regimens used in POR and concluded that a microdose flare-up protocol was more beneficial to the cycle outcomes than other regimens.

The addition of GnRH-ant to the ovarian stimulation in POR brought new hope to the clinicians. The studies about the GnRH-ant in the treatment of POR resulted in reducing the amount of gonadotropins used, the length of the stimulation, the number of cancelled cycles, and the overall cost normally associated with the long protocol and in increasing the number of oocytes retrieved with satisfactory pregnancy rates (10, 13-17). GnRH-ant characteristics will always be reasonable for POR since a high gonadotropin requirement is commonly associated with high cancellation rates and low numbers of oocytes retrieved.

Prior randomized controlled trials (RCTs) compared microdose flare-up to the GnRH-ant protocols in POR and they offered varied results. The reason of these may be due to the lack of a uniform definition of poor responders. This makes it difficult to compare treatment outcomes and develop and assess protocols for prevention and management. Demirol et al. (8) showed that using flare-up protocols when compared with using antagonist yielded significantly higher mature oocytes, higher IR in flare protocols with similar CPR. Kahraman et al. (9) found higher peak E2 level in flare protocols, apart from the fact that no statistically significant differences were noted in cycle outcomes or CPR. A meta-analysis in 2006 (18) reported higher number of retrieved oocytes in the GnRH-a protocols when the meta-analysis was applied to the four trials that had used GnRH-ant *vs*. flare-up protocols. Also, it was reported that there was no difference between GnRH-ant and GnRH-a (long and flare-up protocols) with respect to CCR and PR. Similarly, in our study, significantly higher numbers of oocytes retrieved and higher implantation rates in microdose flare-up were found compared to GnRH-ant protocol with similar FR and CPR. These results may show that the number of oocytes is not always predictive of pregnancy rates.

In a recent RCT, Ibrahim et al. (10) examined the use of microdose triptorelin (30-35 μg S.C. once a day) starting on the first day of the cycle compared with using cetrorelix when the leading follicle was >14 *mm*. Although the FR, IR and ongoing pregnancy rate were higher and cancellation rate was lower in antagonist protocol, they did not achieve statistical significance. It was noted that when using GnRH-ant protocol, significantly less gonadotropin was required. In a Cochrane review (19), it was reported that the GnRH-ant produced higher number of oocytes and used lower dose of gonadotropins compared with GnRH-a long protocol and GnRH-a flare up protocol had an increased frequency of IVF cancellation compared with the GnRH-a long protocol. It was concluded that the evidence was not sufficient to support any IVF protocol in routine practice. Pu et al. (20) reported that GnRH-ang protocols resulted in significantly lower duration of stimulation compared to GnRH-a protocols in POR; however, CCR and CPR were similar between the groups. In our study, the total gonadotropin doses were significantly lower in the GnRH-ant protocol without any difference in the length of stimulation.

Our study included 244 patients (314 cycles) who received either the GnRH-a flare or the GnRH-ant protocol. One advantage of our study was having large sample size. To our knowledge, it had the largest sample size among the previous researches published in the literature. One of the limitations of our study was that the choice of the protocol, whether to use GnRH-a flare or the antagonist protocol, was based on the individual preference of the clinicians carrying out the treatment. Although the patients cannot be randomized in a retrospective analysis such as this and the administered gonadotropins were different, the similarity in the baseline characteristics of the groups can make it possible to compare the outcomes. The results of our study appear to confirm the conclusion of some already published reviews indicating higher number of oocyte retrieved, higher implantation rate in GnRH-a cycles compared with GnRH-ant cycles (8, 18) with similar CPR (9, 18, 21). Nevertheless, additional RCT with better planning and larger sample size are still needed to further confirm these results.

Conclusion

The GnRH-a microdose flare-up protocol has favorable outcomes with respect to the number of oocytes retrieved and IR; nevertheless, the CPR was found to be similar in comparison to GnRH-ant protocol in POR. GnRH-ant protocol appears to be promising with significantly lower gonadotropin requirement and lower treatment cost in POR.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Keay SD, Liversedge NH, Mathur RS, Jenkins JM. Assisted conception following poor ovarian response to gonadotrophin stimulation. Br J Obstet Gynaecol. 1997;104(5):521-7.
2. Scott RT, Navot D. Enhancement of ovarian responsiveness with microdoses of gonadotropin-releasing hormone agonist during ovulation induction for in vitro fertilization. Fertil Steril. 1994;61(5):880-5.
3. Surrey ES, Bower J, Hill DM, Ramsey J, Surrey MW. Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. Fertil Steril. 1998;69(3):419-24.
4. Akman MA, Erden HF, Tosun SB, Bayazit N, Aksoy E, Bahceci M. Comparison of agonistic flare-up-protocol and antagonistic multiple dose protocol in ovarian stimulation of poor responders: results of a prospective randomized trial. Hum Reprod. 2001; 16(5):868-70.
5. Fasouliotis SJ, Laufer N, Sabbagh-Ehrlich S, Lewin A, Hurwitz A, Simon A. Gonadotropin-releasing hormone (GnRH)-antagonist versus GnRH-agonist in ovarian stimulation of poor responders undergoing IVF. J Assist Reprod Genet. 2003;20(11):455-60.
6. Leondires MP, Escalpes M, Segars JH, Scott RT Jr, Miller BT. Microdose follicular phase gonadotropin-releasing hormone agonist (GnRH-a) compared with luteal phase GnRH-a for ovarian stimulation at in vitro fertilization. Fertil Steril. 1999;72(6):1018-23.
7. Akman MA, Erden HF, Tosun SB, Bayazit N, Aksoy E, Bahceci M. Addition of GnRH antagonist in cycles of poor responders undergoing IVF. Hum Reprod. 2000;15(10):2145-7.
8. Demirol A, Gurgan T. Comparison of microdose flare-up and antagonist multiple-dose protocols for poor-responder patients: a randomized study. Fertil Steril. 2009;92(2):481-5.
9. Kahraman K, Berker B, Atabekoglu CS, Sonmezer M, Cetinkaya E, Aytac R, et al. Microdose gonadotropin-releasing hormone agonist flare-up protocol versus multiple dose gonadotropin-releasing hormone antagonist protocol in poor responders undergoing intracytoplasmic sperm injection-embryo transfer cycle. Fertil Steril. 2009;91(6):2437-44.
10. Zakia Mahdy Ibrahim, Heba Younes Mohamed Youssef, Magda Moustafa Elbialy, Mohamed Mouselhy Farrag. Micro-dose flare-up gonadotrophin-releasing hormone (GnRH) agonist vs. flexible gonadotrophin-releasing hormone (GnRH) antagonist protocol in patient with poor ovarian reserve. Middle East Fertil Soc J. 2011;16(4):272-7.
11. Patrizio P, Vaiarelli A, Levi Setti PE, Tobler KJ, Shoham G, Leong M, et al. How to define, diagnose and treat poor responders? Responses from a worldwide survey of IVF clinics. Reprod Biomed Online. 2015;30(6):581-92.
12. Toth TL, Awwad JT, Veeck LL, Jones HW Jr, Muasher SJ. Suppression and flare regimens of gonadotropin-releasing hormone agonist. Use in women with different basal gonadotropin values in an in vitro fertilization program. J Reprod Med. 1996;41 (5):321-6.
13. Al-Inany H, Aboulghar M. GnRH antagonist in assisted reproduction: a Cochrane review. Hum Reprod. 2002;17(4):874-85.
14. Chang PL, Zeitoun KM, Chan LK, Thornton MH 2nd, Sauer MV. GnRH antagonist in older IVF patients. Retrieval rates and clinical outcome. J Reprod Med. 2002;47(4):253-8.
15. Craft I, Gorgy A, Hill J, Menon D, Podsiadly B. Will GnRH antagonists provide new hope for patients considered 'difficult responders' to GnRH agonist protocols? Hum Reprod. 1999;14(12):2959-62.
16. Marci R, Caserta D, Dolo V, Tatone C, Pavan A, Moscarini M. GnRH antagonist in IVF poor-responder patients: results of a randomized trial. Reprod Biomed Online. 2005;11(2):189-93.
17. Nikolettos N, Al-Hasani S, Felberbaum R, Demirel LC, Kupker W, Montzka P, et al. Gonadotropin-releasing hormone antagonist protocol: a novel method of ovarian stimulation in poor responders. Eur J Obstet Gynecol Reprod Biol. 2001;97(2): 202-7.
18. Franco JG Jr, Baruffi RL, Mauri AL, Petersen CG, Felipe V, Cornicelli J, et al. GnRH agonist versus GnRH antagonist in poor ovarian responders: a meta-analysis. Reprod Biomed Online. 2006;13(5): 618-27.
19. Pandian Z, McTavish AR, Aucott L, Hamilton MP, Bhattacharya S. Interventions for 'poor responders'

to controlled ovarian hyper stimulation (COH) in in-vitro fertilisation (IVF). Cochrane Database Syst Rev. 2010;(1):CD004379.

20. Pu D, Wu J, Liu J. Comparisons of GnRH antagonist versus GnRH agonist protocol in poor ovarian responders undergoing IVF. Hum Reprod. 2011;26 (10):2742-9.

21. Malmusi S, La Marca A, Giulini S, Xella S, Tagliasacchi D, Marsella T, et al. Comparison of a gonadotropin-releasing hormone (GnRH) antagonist and GnRH agonist flare-up regimen in poor responders undergoing ovarian stimulation. Fertil Steril. 2005;84(2):402-6.