Oral contraceptive pretreatment does not improve outcome in microdose gonadotrophin-releasing hormone agonist protocol among poor responder intracytoplasmic sperm injection patients

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Abstract

Purpose To compare oral contraceptive (OC) pretreatment plus microdose GnRH-a in flare-up protocol and non-OC microdose GnRH-a in flare-up protocol among poor responder ICSI patients.

Methods A retrospective analysis of poor responder ICSI patients. Patients were divided into two groups according to used microdose protocol. Precycle treatment with OC followed by follicular phase administration of 40 µg sc leuprolide acetate (LA) every 12 h beginning on after 2 day pill-free period and rFSH administration was begun on the third day of LA administration (OC-Group, $n=26$). Alternatively on day 2 after menses, patients were administered similar stimulation regime (non-OC Group, $n=27$).

Results There were no significant differences between groups in the number of oocytes, peak estradiol levels, endometrial thickness, fertilization rates and embryo quality. Implantations and pregnancy rates per embryo transfer were similar.

Conclusion OC pretreatment plus microdose GnRH-a in flare-up protocol does not offer advantages over non-OC microdose GnRH-a in flare-up protocol among poor responder ICSI patients.

Capsule Oral contraceptive pretreatment plus microdose GnRH-a in flare-up protocol does not offer advantages over non-OC microdose GnRH-a in flare-up protocol among poor responder ICSI patients.

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Introduction

The popular “microdose GnRH agonist flare” stimulation protocol consists of utilizing oral contraceptive (OC) priming followed by diluted doses of leuprolide acetate (LA) 40 µg given twice daily. Two days later, stimulation is initiated by adding high doses of gonadotropins. The microdoses of LA and the high doses of gonadotropins are then continued until the day of human chorionic gonadotropin (HCG) administration. Microdose GnRH-agonist (GnRH-a) flare protocol is especially preferred for poor responders who are undergoing in vitro fertilization and embryo transfer (IVF-ET) [1]. Basically, microdose GnRH-a protocol takes the advantages of both the agonist and down-regulation properties of GnRH agonist analogues. In the first few days GnRH-a stimulate endogenous gonadotrophins secretion from pituitary gland during the early follicular phase which increases the substrate of aromatization and results in higher estradiol levels, particularly in poor responders. Then, continuing administration of the analogue inhibits subsequent premature luteinizing hormone (LH) surges.

Currently the percentage of poor responders, out of all infertile patients undergoing IVF-ET programs, is varies from 9% to 24% [2, 3]. Poor responders include women in whom a previous cycle yielded three or fewer oocytes or was cancelled because of observations of three or fewer follicles 16 mm or greater, a single dominant follicle or a peak serum estradiol less than 500 pg/ml. Despite tremendous improvements in stimulation protocols in IVF pro-

gram, the stimulation of poor responders still remains a challenging task for the clinician. Scott and Navot described a novel approach to the poor responder patients, using a follicular phase administration of microdoses of GnRH-a after OC pretreatment. OC pretreatment in this population has many putative benefits, such as preventing rescue of the corpus luteum from the previous cycle or a rise in progesterone with initiation of the follicular phase microdose GnRH-a [4]. Although there is a general feeling that OC pretreatment may improve the ovarian response of poor responders, only a minimal amount of published data exist confirming this hypothesis.

The aim of this study is to compare two microdose GnRH-a flare protocols, one is pretreatment with OC and the other is without OC pretreatment microdose GnRH-a flare protocol. To our knowledge this is the first report to compare pretreatment OC-microdose GnRH-a flare protocol with non-OC microdose GnRH-a flare protocol.

Materials and methods

This study was a retrospective analysis of 53 ICSI cycles of poor responder patients. Cycles were performed at the Ankara University and Fatih University IVF Center from 1/1/2006 to 1/1/2007 in which the patient was identified as a poor responder. All diagnoses of infertility were included. Determination of a “poor response” was based on a combination of some of the following factors; recruitment of fewer than four mature follicles, maximal estrogen value of <500 pg/ml, had prior cancellation with an agonist flare cycle, a cycle day 3 FSH level >10 pg/ml, age >39. Two treatment regimens were used during the course of this study. Assignment to the treatment groups was arbitrary, and not randomized. Patients initially received 21 days of OCs (Myralon 150 mcg desogestrel, 20 mcg ethinyl estradiol, Organon) and after 2 day pill-free period (OC Group, $n=26$), LA was administered every 12 h at a dose of 40 µg SC and rFSH (Gonal F, Serono; Puregon, Organon) administration was begun on the third day of LA administration and continued until at least three follicle reached a mean diameter of 17 mm. Alternatively, on day 2 after menses patients administered LA every 12 h at a dose of 40 µg SC and rFSH administration was begun on the third day of LA administration and continued until hCG day (non-OC Group, $n=27$). Follicular development was monitored by serial transvaginal ultrasonography (TV-USG) and determination of serum estradiol (E_2) levels. When at least three follicles were greater than 17 mm, patients received 10,000 IU HCG IM (Pregnyl 5,000 IU, Organon group, Turkey). Oocyte retrieval was scheduled for 35.5 h after HCG injection. Embryo transfer was performed on day 2 or 3 of culture after all embryos using

a Wallace catheter. All patients received luteal phase progesterone supplementation in the form of vaginally gel (90 mg progesterone). Pregnancy was confirmed 12 days after the embryo transfer with a serum HCG assay. Clinical pregnancy was defined by a normal gestational sac measurement via TV-USG that was consistent with the gestational age performed between 5 and 7 weeks.

Baseline characteristics and cycle outcomes were compared between groups. All patients were evaluated with E_2 levels and transvaginal ultrasonography at their initial evaluation before stimulation.

E_2 was measured by (electrochemiluminescence) immunoassay method on the Roche Elecsys 2010 analyzer. Results were given as pg/ml. For E_2 , the interassay and intraassay coefficients of variation were 3.8% and 3.1%. FSH was measured by a chemiluminescent immunometric assay with Immulite One, Bio-DPC, Siemens, USA. Analytical sensitivity: 0.1 mIU/ml. The inter assay and intra assay coefficients of variation were 5.6% and 3.1%.

Data analysis was performed by using SPSS for Windows, version 11.5. Whether the distributions of continuous variables were normally or not were determined by using Shapiro Wilks test. Data were shown as mean±SD for continuous variables and percentages for categorical ones. Means were compared by using Student's t or Mann Whitney U test and chi-square or Fisher's exact test was used for categorical comparisons, where appropriate. A p value less than 0.05 was considered statistically significant.

Results

This retrospective analysis included 53 cycles of poor responders and all were offered the microdose GnRH-a stimulation protocol. The mean age of the patients in the study was 37.4 ± 3.5 years. All patients in the study had a complete cycle, including ovarian stimulation, oocytes retrieval and the transfer of at least a single embryo. There were no significant differences between two groups in the main baseline characteristics. Baseline characteristics of the patients were presented in Table 1.

Table 1 Baseline characteristics of the groups

	OC Group	Non-OC Group	P value
Age (year)	36.3 ± 5.1	36.5 ± 4.6	NS
BMI (kg/m ²)	25.9 ± 2.8	26.8 ± 5.1	NS
Infertility period (year)	11.2 ± 6.9	9.8 ± 6.2	NS
Day 3 FSH (mIU/ml)	9.1 ± 3.0	7.8 ± 3.1	NS
Day 3 Estradiol (pg/ml)	42.4 ± 22.0	46.5 ± 18.0	NS
Cycle number	1.4 ± 0.7	1.4 ± 0.6	NS

Values are means±SD

There were also no significant differences between the groups in the mean peak E_2 level and endometrial thickness on the day of HCG administration; in the number of follicles that developed, mean numbers of oocytes retrieved and mature oocytes. There were, however, significant differences between the groups in the total dose of gonadotrophins used (3925 ± 1201 , 2882 ± 1523 , respectively) and days of stimulation (9.5 ± 1.9 , 7.8 ± 2.3 , respectively).

The stimulation parameters are compared for those patients receiving the standard OC plus microdose GnRH-a flare protocol (OC Group) versus the non-OC microdose GnRH-a flare protocol (non-OC Group) in Table 2.

Clinical pregnancy rates per ET were 15% (4/26) for the OC Group and 14% (4/27) for the non-OC Group, which were not statistically different. Implantation rates were 8% (4/47) and 7% (3/38) for the OC Group and non-OC Group, respectively ($P > 0.05$). Pregnancy and implantation rates based on OC use are presented in Table 3.

Discussion

The purpose of this study was to compare popular OC pretreatment plus microdose GnRH-a in flare-up protocol with non-OC microdose GnRH-a flare-up protocol for ovarian stimulation in poor responder ICSI patients. Women undergoing in vitro fertilization with low ovarian reserve and poor response to controlled ovarian hyperstimulation (COH) still present a management dilemma. Microdose GnRH-a flare protocols have been proven to be efficient in women with a history of a poor response to luteal phase protocols [1, 4].

During IVF-ET cycles, most women undergo hypothalamic pituitary suppression prior to the initiation of ovarian stimulation [5]. Hypothalamic pituitary suppression before ovarian stimulation in IVF-ET cycles prevents premature luteinization, increases the number of follicles stimulated, synchronization of follicular development, allows patients

Table 3 Comparison of implantation and pregnancy rates in groups

	OC Group	Non-OC Group	P value
Grade A embryo (%)	32 (15/47)	29 (11/38)	NS
Implantation rate (%)	8 (4/47)	7 (3/38)	NS
Pregnancy rate per attempt (%)	15 (4/26)	14 (4/27)	NS

scheduling and improves the pregnancy rates [5–7]. Generally, gonadotropin suppression is achieved by the use of a gonadotropin releasing hormone agonist (GnRH-a). However, it becomes challenging to utilize the benefits of hypothalamic-pituitary suppression in poor responders. Various hypothalamic pituitary suppression protocols such as GnRH-a stop, low-dose suppression and GnRH-a flare have been used to treat these patients [8, 9]. The administration of GnRH-a has several disadvantages and side effects such as ovarian hyperstimulation, hot flushes, headaches, ovarian cyst formation, as well as the need for luteal phase support [10–13]. In addition, luteal phase administration of GnRH-a in poor responders may result in excessive pituitary suppression and hence inhibit ovarian response to exogenous gonadotrophins. Microdose GnRH-a flare regimens were designed to take advantage of the release of endogenous gonadotrophins induced by administration of GnRH-a in the early follicular phase while inhibiting subsequent LH surges [12, 14]. Hypothalamic-pituitary suppression in IVF patients may also be achieved with the use of OCs or progestins alone [13, 15]. Among the treatments proposed to improve the ovarian response in poor responders, OC pretreatment is considered as a possible option [14]. The use of OCs allows easy cycle scheduling, prevents endogenous LH surges as effectively as a GnRH-a, may lead to “lighter” suppression and could synchronize follicular development leading to a better response. Currently, OCs is efficiently used in normo and high responder IVF-ET patients for these advantages. For example, in a study of Gonen et al. [16] clearly demonstrate that OCs are useful in in vitro fertilization stimulation protocols to facilitate scheduling of cycles and to prevent spontaneous LH surges in normo-responder patients. They revealed suppression performed by OCs, required less gonadotropin to achieve optimal follicle maturation as a result of ovarian stimulation. In another study, Burry et al. [17] evaluate the effect of short-term oral contraceptive suppression on the recovery of pituitary gonadotropin function and subsequent controlled ovarian hyperstimulation. They suggested that short-term oral contraceptive suppression did not oversuppress the ovarian stimulation in normo-responders. Similarly, Biljan et al. [18] reported the advantage of OC pretreatment during the long standard protocol of GnRH-a administration. Damario et al. [19] also revealed that dual suppression with oral contraceptives and

Table 2 The stimulation parameters in groups

Parameter	OC Group	Non-OC Group	P value
Peak E_2 (pg/ml)	1837 ± 997	1547 ± 1132	NS
Endometrial thickness (mm)	8.7 ± 2.9	9.8 ± 1.9	NS
Follicles ≥ 14 mm (n)	5 ± 2.6	6 ± 2.6	NS
Day of stimulation (n)	9.5 ± 1.9	7.8 ± 2.3	0.01
Dose of gonadotrophins (U)	3929 ± 1201	2882 ± 1523	0.01
No of oocytes retrieved (n)	6.1 ± 3.5	6.1 ± 3.6	NS
Mature oocytes retrieved (n)	4.7 ± 3.2	5.2 ± 3.4	NS
Mean fertilization rate (%)	75 ± 25	62 ± 27	NS
Pre-embryos transferred (n)	2.1 ± 0.7	2.4 ± 1.1	NS

Values are means $\pm$ SD

gonadotrophin releasing-hormone agonists improves in-vitro fertilization outcome in high responders.

All of the above mentioned studies evaluated OCs used in normo and/or high responder patients during long luteal protocol. However, only limited number of studies evaluated OC use in poor responder IVF-ET patients. For example, in a retrospective observational study of Al-Mizyen et al. [20] compared the pretreatment use of the gestogen medroxyprogesterone acetate with an oral contraceptive pill and they conclude that pre-IVF treatment with oral contraceptive pill or gestogen combined with the flare protocol in women at high risk of or with a history of poor ovarian response did not appear to result in an improvement in outcome of IVF-embryo transfer. Similarly, our results did not show any improvement in cycle outcomes or pregnancy rates, when OC plus microdose protocol was used instead of a non-OC microdose protocol in poor responders. The differences of our study from Al-Mizyen et al. [20], we have compared OC plus microdose GnRH-a protocol with control group (non-OC) during ICSI cycle. Likewise, in a retrospective study of Kovacs et al. [21] revealed no improvement in IVF cycle outcomes in poor responders who received OCs to achieve hypothalamic-pituitary suppression instead of GnRH-a. Contrary to us they did not use OC with microdose flare protocol in poor responder patients. Cycles with no suppression or with GnRH-a flare use were not included to avoid another factor potentially influencing the outcome. However, Lindheim et al. [22] reported that short-term gonadotropin suppression with oral contraceptives benefits in poor responders prior to controlled ovarian hyperstimulation. Differences may be resulting from the study protocol. Although they compared four treatment regimens, none of them were consisting of microdose GnRH-a flare protocol in poor responders. In addition, Benavida et al. [23] studied ovarian response to human menopausal gonadotropin following suppression with oral contraceptives. They compared normo and poor responders with control OC Group and conclude that IVF patients who received oral contraceptives as part of treatment had undesirable responses such as an inadequate response which may require a longer or different regimen of stimulation to human menopausal gonadotropin when the contraceptives were administered for only a short period. In another study Klein and Mishell [24] observed that the follicular phase is prolonged after discontinuing OC and suggested that a brief interval is needed to reestablish normal ovarian–pituitary relationships in a spontaneous cycle.

In our study we did not detect any advantages of OC plus microdose GnRH-a flare over the non-OC microdose GnRH-a flare protocol in poor responders. On the contrary while implantation and pregnancy rates were similar, day of stimulation and used gonadotrophin doses were significant-

ly increased in OC-group. This may be due to the excessive suppressive effect of the OCs in poor responders opposite to normo-responders. The standard flare regimen commonly associated with significantly increases in serum progesterone and androgen levels, presumably resulting from apparent corpus luteum rescue, which may explain the reported deleterious effects on oocyte quality, fertilization and pregnancy rates. Pretreatment with OC agents before initiation of GnRH-a therapy may have eliminated the potential for corpus luteum rescue by administration of GnRH-a in the follicular phase. On the contrary, we did not find any differences in the implantation and pregnancy rates between groups. This may be a function of the lower doses of GnRH-a.

In our knowledge this is the first report comparing OCs' effectiveness in microdose GnRH-a flare regimen with non-OC microdose GnRH-a flare in poor responder ICSI patients. Given the results, OC plus microdose GnRH-a did not have any advantages over the non-OC control group in poor responders. Actually, OC-plus microdose GnRH-a contrary to normo-responders may cause excessive suppression and causes both longer stimulation day and higher gonadotrophin doses in this population. The drawback of our study is the absence of power analysis because of its retrospective design. The clarification of the dilemma on this subject can be achieved by performing more prospective randomized trials.

In summary, the beneficial effect of OCs in poor responders still remains controversial issue. At the moment, it does not seem possible to draw conclusive indications regarding the use of OCs in microdose GnRH-a flare protocol because of a paucity of clear data in the literature on OC pretreatment in poor responders.

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