Clinical Effects of a Microdose GnRH Agonist Flare Regimen Administered to Poor Responders Undergoing ART Cycles

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Abstract- The microdose GnRH agonist (GnRH-a) flare protocol may have a particular value for previously poor responders in whom it has been observed to stimulate dramatic increases in serum FSH. The Purpose of this study was to determine the effects of microdose GnRH-a in poor responders. This is a clinical trial with before and after design. This study was done in Research and Clinical Center for Infertility (Shahid Sadoughi University, Yazd, Iran) and Madar Hospital, Yazd, Iran. In this study, 61 poor responders volunteered for in vitro fertilization (IVF) or intracytoplacmic sperm injection (ICSI). The volunteers were divided into two age groups (group A, 20 - 34; group B, 35 - 40) and received low dose oral contraceptive pills for 21 days, then 40µg of subcutaneous buserelin 2 times/day from day 3 of the cycle and human menopausal gonadotropin (hMG) 3 ampoules/day from day 5. Main Outcome measures were number of follicles, oocytes and embryos, and pregnancy rate (PR). These measures were then compared with those of the previous cycle. There were significant differences in all parameters ($P < 0.05$). Pregnancy occurred in 3 women (5%). There was no significant difference in number of follicles, oocytes and embryo between two age groups ($P > 0.05$). Use of microdose GnRH-a plus HMG for controlled ovarian hyperstimulation in IVF or ICSI cycles can lead to formation of more follicles, oocyte and embryo in poor responders.

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Introduction

The use of GnRH agonist **(**GnRH-a) along with gonadotropins as adjunctive agents in controlled ovarian hyperstimulation (COH) for IVF was first described by Porter *et al.* (1984) (1). The GnRH-a has since been shown to prevent premature luteinization, decrease cancellation rates, increase the number of follicles stimulated, facilitate patient scheduling and improve pregnancy rate (2,3,19). Treatment with GnRH-a initially causes brief pituitary stimulation followed by pituitary desensitization and ovarian quiescence (4).The GnRH-a has been used as an adjunct to gonadotropin therapy in the luteal and follicular phases of the menstrual cycle. Filicorim *et al.* described the administration of the GnRH-a therapy in the early follicular phase concomitant with the administration of exogenous gonadotropins (5). Such flare protocols were designed to use the initial rise in endogenous gonadotropins after the initiation of GnRH-a treatment (6, 7). Most reported flare protocols consist of GnRH-a in the early follicular phase at a dose of 0.5-1.0 µg/day. Some reports have suggested that a flare regimen is associated with a reduction in fertilization and embryo quality, decreased preovulatory follicle numbers, higher spontaneous abortion rates, and lower pregnancy rates (8, 9). Conversely, other investigators observed that follicular phase flare protocols produce clinical results similar to luteal phase protocols (7, 10). There is no clear consensus whether initiation of GnRH-a in the follicular phase is superior to a luteal phase with respect to pregnancy rate. Despite widespread clinical use of GnRH-a, there are insufficient data in human to define the lowest effective dose of GnRH-a. Scott and Novat (1994) studied the effect of very low doses of GnRH-a in cynomologus monkeys and humans and established that 10 µg of historelin in four divided doses

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(microdoses) could induce ovarian hyperstimulation in humans. This study aimed to determine if women, who previously had demonstrated poor ovarian responsiveness during ovulation induction for IVF, would obtain an improved follicular response by the administration of microdoses of GnRH-a. Microdose GnRH-a administration beginning in the early follicular phase may result in an augmented ovarian response when compared with traditional GnRH-a-exogenous gonadotropin stimulations. Additionally, it may decrease gonadotropin requirements while effectively prevent premature LH surges (11, 12). In support of these findings, patients classified as poor responders were reported to have lower cancellation rates, improved cycle quality, and pregnancy rates after being given the follicular phase microdose GnRH-a (13-16). The microdose flare protocol for poor responders demonstrated a trend toward higher delivery rates. In our study, we used microdose GnRH-a for IVF cycles in poor responders.

Patients and Methods

In this clinical trial (before and after design), 61 infertile poor responder patients, who had referred for conventional IVF or ICSI from April 2002 to March 2003, were studied. We define a low response cycle as one in which less than 3 oocytes are retrieved and the oestradiol level on the day of HCG administration is less than 300 pg/ml, and she has had an inadequate response in at least 2 previous IVF cycles. Their age ranged between 20 - 45 years and they were divided into age groups of 20 - 34 (A) and 35 - 45 years (B) accordingly.

All patients received oral contraceptive pills for one cycle and then 40µg of subcutaneous buserelin (Superfact® injectable; Hoechest AG) 2 times a day from the 3rd day and HMG (Menogon®, FERRING®, 225IU) per day from the 5th day of cycle, which was known as. Follicular growth was monitored by transvaginal sonography from the 9th day of cycle. HCG (Pregnyl®; NV Organon®, Oss, The Netherlands) 10000 unit was injected when at least 4 follicles ≥ 18mm were noted on sonography. Oocyte retrieval was done 34 - 36 hours later and embryo transfers performed 48 - 72 hours after retrieval. Chemical pregnancy was assessed by measurement of serum βHCG 2 weeks later. The clinical pregnancy was verified by the presence of gestational sac and fetal heart activity on the 6th week of pregnancy.

Data as mean numbers of follicles, oocytes, embryos, as well as clinical and chemical pregnancies were analyzed by SPSS software using Chi square, Fisher exact test and one sample *t*-test.

Results

Sixty-one infertile poor responder patients were studied whose age ranging from 20 to 45 years. 42 patients were in group A and 19 in group B.

The causes of infertility in 39 cases were female factor, 14 cases were both male and female and 8 cases were unexplained. Relative frequency of different ovarian stimulation regimen in previous cycle was HMG, HMG + Buserelin, HMG + Clomiphene, unknown was 34 (55.7%), 15 (24.6%), 10 (16.4%) and 2 (3.3%), respectively. The mean number of follicles, oocytes and embryos after using microdose protocol were 7.93 ± 4.9, 4.49 ± 4.1 and 2.26 ± 1.9, respectively. The mean number of follicles, oocytes, and embryos in previous in comparison with present were significant ($P < 0.05$). The chemical pregnancy rate in group A and B was 1/42 (2.4%) cases and 2/19 (10.5%) cases respectively and there was no statistically significant differences in chemical pregnancy rate between two age groups ($P > 0.05$).

The mean number of follicles in group A and B were 8.48 ± 5.03 and 6.47 ± 4.6 respectively ($P < 0.05$). The mean number of oocytes in group A was 4.95 ± 4.71 and in group B was 3.47 ± 2.46 ($P < 0.05$).The mean number of embryos in group A and B was 2.4 ± 2.21 and 1.95 ± 1.08 respectively ($P < 0.05$). No statistically significant difference was found between groups in respect to number of follicles, oocytes and embryos (Table 1).

In pregnant patients, the mean number of follicles, oocytes, and embryos were 7.67 ± 7.23, 3.33 ± 2.52 and 2.67±1.53 respectively. In the non-pregnant, the corresponding values were 7.88±4.88, 4.49 ± 4.26,2.21 ± 1.96 respectively ($P > 0.05$). The difference between the mean number of follicles, oocytes and embryos in pregnant patients and those of non- pregnant were not significant (Table 2).

Table 1. Mean number of follicle, oocyte and embryo in two age group of participants

Variable	A (20-34yrs)	B (35-45yrs)	Mean ± SD	*P Value*
Mean no.of follicles	8.48 ± 5.03	6.74 ± 4.6	8.48 ± 5.03	*P = 0.2*
Mean no.of oocytes	4.95 ± 4.71	3.47 ± 2.46	6.74 ± 4.6	*P = 0.2*
Mean no. of embryo	2.4 ± 2.21	1.95 ± 1.08	4.95 ± 4.71	*P = 0.3*

Table 2. Chemical pregnancy in relation to number of follicle, oocyte and embryo

Chemical pregnancy	Positive N=3	Negative N=57	*P Value*
	Mean±SD	Mean±SD	
Mean no.of follicles	7.67 ± 7.23	7.88 ± 4.88	*P = 0.9*
Mean no.of oocytes	3.33 ± 2.52	4.49 ± 4.26	*P = 0.6*
Mean no.of embryos	2.67 ± 1.53	2.21 ± 1.96	*P = 0.6*

Discussion

The first successful IVF was seen in an unstimulated cycle but now the majority of IVF programs are with stimulated cycles in order to obtain more follicles and oocytes. More follicles and oocytes were produced using gonadotropins with or without clomiphene citrate (CC), but these are associated with a high prevalence of premature LH surge (5-25%). In 1984, for the first time, Buserelin was used along with gonadotropin in IVF cycles. Many research studies reported that GnRH-a inhibited spontaneous LH surge and increased the response of ovaries, implantation and PR per cycle (2, 3, 17). Based on world collaboration report in 1995, the most popular regimen for ovarian stimulation in IVF was GnRH-plus gonadotropins. This regimen has two important effects: the inhibition of the hypophysis, and stimulation of ovaries. Both effects have special advantages and disadvantages, but the result is desirable (11). The management of poor responders in IVF has always been a big problem. The ideal approach has yet to be formulated (20). Most initial studies using combined treatment were carried out on poor responder patients. However, later on, this regimen has been used for all of the patients undergoing IVF cycles. It is clear that GnRH-a is useful for poor responders, abnormal responders, patients who had failed to respond to either gonadotropin alone or with clomiphene citrate (12). There are two regimens: GnRH-a either in follicular or luteal phase. Some researches claim that the use of GnRH-a at follicular phase causes a decrease in fertilization, poor embryo quality, decreased number of follicles, increased chance of miscarriage and decreased pregnancy rate (8,9). However, some of them suggested that the use of GnRH-a at follicular or luteal phase has similar clinical results (7, 10). There are controversies on the use of microdose GnRH-a in poor responders. In 1999, Leondires treated two groups of patients, one group with GnRH-a (usual dose) and another one with microdose. The rate of pregnancy was not different between the two groups, but cancellation rate were higher in microdose group (4). Surrey *et al.* (1998) gave oral contraceptive pills to poor responder patients for 21 days, added leuprolide acetate (40µg Sc Bid) from day 3 and HMG from day 5. He used the long protocol for the second group. There was higher pregnancy rate and lower cancellation rate in the latter group (17). In Akman *et al*. (2001), a total of 48 poor responder patients described from previous cycles were included and grouped into two: group I consisted of 24 patients in 24 cycles in which leuprolide acetate (40 µg s.c. per day) was initiated on day 2 of the cycle followed by exogenous gonadotrophins on cycle day 3; group II consisted of 24 patients in 24 cycles in which ovarian stimulation included GnRH-a (cetrorelix, 0.25 mg daily during late follicular phase) administration. While only ~~the~~ oestradiol concentrations on the day of HCG were lower in group II compared with group I, the clinical pregnancy and implantation rates among groups were not significantly different. The impact of these two regimens in ovarian stimulation of poor responders seem to be the same and in order to confirm these results, further randomized studies with larger sample sizes are required (20). In our study, there was a significant difference in the number of follicles, oocyte and embryo compared with their previous cycles. The present study showed that there were significant differences between the number of follicles, oocyte and embryos after and before using microdose regimen, so a microdose regimen stimulates the growth of more follicles, higher oocyte retrieval and more embryos compared with other treatment. However, the response to microdose regimen had no significant difference in two age groups. There have been various reports for designation of the ideal stimulation protocol for these patients. It has been documented that cycle cancellation is common for this particular group of patients, mostly due to premature LH surges. To overcome the extra suppression while preventing the premature LH surges, various researchers have advocated decreasing the dosage and the timing of GnRH- a, such as in microdose GnRH agonist flare-up regimens (20). Detti et al. evaluated the efficacy of three different GnRH-a stimulation regimens to improve the ovarian

response in poor responders undergoing IVF. They compared three different stimulation regimens during IVF cycles: (1) stop protocol: GnRH-a 500 microg/d administered from the midluteal phase to the start of menses, then gonadotropins from day 2 of the cycle, (2) microdose flare: GnRH-a 20 microg administered twice daily with gonadotropins from day 2 to the day of hCG administration, or (3) regular dose flare: gonadotropins beginning with GnRH-a on day 2 at 1 mg/d doses for 3 days, followed by 250 microg/d until the day of hCG administration. In this study, sixty-one IVF cycles were included in the study.

None of the comparisons reached statistical significance; however, the microdose group demonstrated a trend toward a higher pregnancy rate (14). Surrey et al. assessed the efficacy of various COH regimens in the prior poor-responder patients preparing for assisted reproductive techniques. A lack of uniformity in definition of the poor responder and of prospective randomized trials makes data interpretation somewhat difficult. Of the varied strategies proposed, those that seem to be more uniformly beneficial are microdose GnRH-a flare and late luteal phase initiation of a short course of low-dose GnRH-a discontinued before COH (17).

'Micro-dose' GnRH protocols are mainly suggested for the so-called 'poor responder' patients. However, as proper dose finding studies are lacking, there is no proper use of the word, and the so-called 'normal dose' may well turn out to be a 'macro- dose'. None of the studies are randomized, and it now seems that GnRH antagonists have become the newest means in the treatment of 'poor responders (12).

Scott and Navot argued that microdose regimen increased endogenous FSH secretion at early follicular phase and there was no increase in androgens, which can explain more success rate of this regimen. By this regimen, it is possible that microdose regimen causes an increase in gonadotropins and inhibits spontaneous LH surge. However, GnRH-a down regulates gonadotropins and inhibits ovarian response to exogenous gonadotropins. This study suggests that microdose regimen in any patient who does not respond to other formal stimulation treatments, cause more folliculogenesis, oocytes, embryos and pregnancy (11).

Women underwent either a standard long luteal leuprolide acetate protocol or a modified microdose LA flare protocol. It was concluded that a small ovarian volume necessitates a change in stimulation protocol. Women with a small ovarian volume at baseline ultrasound can have comparable implantation and pregnancy rates to those with larger ovarian volumes by the use of a higher dose gonadotrophin, microdose GnRH agonist stimulation (21). In conclusion, in this study we assessed GnRH-a with the microdose GnRH-a flare-up regimen in poor responders. The clinical outcome is almost the same and in order to confirm these results, further randomized studies with larger sample sizes are required.

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