GnRH Antagonist/Letrozole Versus Microdose GnRH Agonist Flare Protocol in Poor Responders Undergoing *In Vitro* Fertilization

Robab Davar[1], Homa Oskouian[2]*, Shahnaz Ahmadi[2], Razieh Dehghani Firouzabadi[1]

[1]*Department of Obstetrics and Gynecology,* [2]*Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Science, Yazd, Iran.*

SUMMARY

Objective: To evaluate and compare the efficacy of microdose gonadotropin-releasing hormone (GnRH) agonist flare (MF) and GnRH antagonist/letrozole protocols in poor responders undergoing *in vitro* fertilization.

Materials and Methods: A total of 94 poorly responding patients were randomized in an ovarian stimulation protocol with a MF, or a letrozole and high dose follicle-stimulating hormone/ human menopausal gonadotropin and flexible GnRH antagonist protocol.

Results: There was no significant difference in mean age, body mass index, basal serum follicle-stimulating hormone and estradiol levels, duration of infertility, distribution of etiology of infertility, and the number of previously failed *in vitro* fertilization cycles. The days of stimulation, mean gonadotropin dose, the number of mature follicles, and oocytes retrieved and metaphase II oocytes retrieved, serum estradiol level on the day of human chorionic gonadotropin administration, and the percentage of top and good quality embryos were significantly higher in the MF group. The endometrial thickness, fertilization rate, and the number of embryos transferred were similar in both groups. The implantation and clinical pregnancy rates were higher in the MF group and the total cancellation rate was higher in the GnRH antagonist/letrozole group, but these findings were not statistically significant.

Conclusion: The addition of letrozole to the GnRH antagonist for poor responders does not improve the outcome of assisted reproductive technology cycles. The MF protocol remains the most appropriate protocol in poor responders. [*Taiwan J Obstet Gynecol* 2010;49(3):297–301]

Key Words: controlled ovarian hyperstimulation, GnRH agonist, GnRH antagonist, *in vitro* fertilization, letrozole, poor responders

Introduction

Failure to respond to controlled ovarian hyperstimulation (COH) is still a major concern in assisted reproduction and there is no consensus on the choice of ovarian stimulation regimen for poor responders. Many strategies have been assayed to improve outcomes. Strategies have ranged from high follicle stimulating hormone (FSH) dose [1], a combination of clomiphene citrate and human menopausal gonadotropin (hMG) [2], microdose gonadotropin-releasing hormone (GnRH) agonist flare protocol (MF) [3], stop GnRH-agonist protocol [4], addition of growth hormone [5], use of GnRH antagonists [6], and even a natural cycle [7], but the improvement in pregnancy rate has been quite small. The MF protocol has been used widely in poor responders [8].

Recently GnRH antagonists have been administered to poor responders with contradictory results [9,10]. In the absence of prior pituitary gonadotropin down

Correspondence to: Dr Homa Oskouian, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Science, Bouali Ave, Safaieh, Yazd, 8916877391 Iran.
E-mail: homaoskouian@gmail.com
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regulation, the dose of gonadotropins may be reduced in GnRH antagonist protocols. Letrozole is an aromatase inhibitor and by blocking estradiol (E2) synthesis decreases negative feedback and increases endogenous gonadotropin secretion [11]. Furthermore, letrozole causes an increase in intraovarian androgens and FSH receptor expression on granulosa cells, thus improving ovarian response to FSH in poor responders [12]. Co-administration of letrozole and GnRH antagonists for COH in poor responders may enhance the pregnancy rate in assisted reproductive technology cycles, therefore we designed a randomized controlled trial study to evaluate the GnRH antagonist/letrozole (A/L) and MF protocols in poor responders undergoing *in vitro* fertilization (IVF) cycles.

Materials and Methods

Patients

This study was a prospective controlled trial with 94 poor responders who were admitted to our IVF center between November 1, 2007, and November 1, 2008. The study was reviewed and approved by the ethics committee of the Research and Clinical Center for Infertility (Yazd University of Medical Science). Written informed consent was obtained from all patients. The inclusion criteria were to have at least one previous failed IVF cycle in which three or fewer follicles with a mean diameter of 16 mm were achieved and/or serum E2 levels measured on the day of human chorionic gonadotropin (hCG) administration was 500 pg/mL or less. Patients exhibiting a day 3 serum FSH level greater than or equal to 12 mIU/mL were excluded; there was no age limit for inclusion in the study. Patients were randomized to an ovarian stimulation regime with either an MF or A/L protocol. A method of computer generated randomization was used. The primary outcome was to compare the clinical pregnancy rate in those protocols.

Treatment protocols

All women received 21 days of an oral contraceptive. A MF protocol was used for ovarian stimulation in 49 patients. Three days after the last pill, subcutaneous administration of a GnRH-agonist, buserelin (Suprefact; Aventis Pharma, Frankfurt, Germany), at a dose of 50 μg twice daily was initiated. Two days after that, recombinant FSH (Gonal-F; Serono, Aubonne, Switzerland) or hMG (Merional; IBSA, Lugano, Switzerland) at 300–450 IU/day was administered. Forty-five patients were assigned to an A/L protocol. After oral contraceptive withdrawal bleeding on day 3 of the cycle, recombinant FSH or hMG at 300–450 IU/day was initiated and letrozole (Femara; Novartis, East Hanover, NJ, USA) at 5 mg/day was administered for 5 days. When the dominant follicle reached 14 mm in mean diameter, subcutaneous ganirelix acetate (Antagon; Organon, West Orange, NJ, USA) treatment at 0.25 mg daily was started.

Patients were monitored by serial vaginal ultrasonography and measurement of serum E2 levels. When at least two follicles with a mean diameter of 18 mm were observed, 10,000 IU hCG (Pregnyl; Organon, Oss, the Netherlands) was administered. Cycle cancellation was considered when fewer than two follicles with normal growth pattern were noted.

Oocyte retrieval was performed 34–36 hours after hCG administration. Conventional IVF or intracytoplasmic sperm injection was performed as appropriate. Embryos with 4–6 equal-sized and evenly shaped blastomeres on day 2 with 20% fragmentation or less and no multinucleation were considered top quality embryos. Embryos with 2–6 even or uneven blastomeres with 20% fragmentation or less and no multinucleation were considered good quality embryos. Embryos were transferred on day 2 or 3 under ultrasound guidance with a C.C.D. embryo transfer catheter (Laboratoire C.C.D., Paris, France). Luteal support with intramuscular administration of progesterone in oil (Progesterone; Aburaihan Co., Tehran, Iran) at 100 mg daily was started on the day of oocyte retrieval. Serum β-hCG level was measured 14 days after embryo transfer and a transvaginal ultrasonography was performed 3 weeks after a positive β-hCG result for documentation of gestational sac and fetal heart activity. Clinical pregnancy was considered as the presence of a gestational sac with fetal heart activity.

Statistical analysis

Data were expressed as mean ± standard deviation. The Student's *t* test and χ^2 test were used for analysis as appropriate. A *p* value less than 0.05 was considered statistically significant. SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

Results

Patient characteristics are shown in Table 1. There were no significant differences among the groups with respect to women's age (33.8 years *vs.* 33.5 years), body mass index (23.8 kg/m^2 *vs.* 24.3 kg/m^2), basal FSH (8.3 mIU/mL *vs.* 7.9 mIU/mL) and E2 (66.2 pg/mL *vs.* 63.5 pg/mL) levels, duration of infertility (9.9 years *vs.* 8.8 years), and number of previous failed IVF cycles (1.64 *vs.* 1.63). Infertility etiology distribution did not differ between groups.

The results of COH are shown in Table 2. Mean FSH/hMG dose expressed as the number of 75 IU ampoules administered (42.1 *vs.* 46.1), the length of stimulation (8.5 days *vs.* 9.2 days), mean E2 level on the day of hCG administration (477 pg/mL *vs.* 1,065 pg/mL), the number of mature follicles (4.2 *vs.* 5.6), the number of oocytes retrieved (2.8 *vs.* 4.4), the number of metaphase II oocytes retrieved (2.4 *vs.* 4.1), and the percentage of top and good quality embryos (31.4% *vs.* 55.8%) were significantly higher in the MF group. There was no significant difference in the endometrial thickness (8.3 mm *vs.* 8.4 mm), percentage of intracytoplasmic sperm injection performance (48.8% *vs.* 51.2%), fertilization rate (67.3% *vs.* 70.7 %), and the number of embryos transferred (1.7 *vs.* 2.0) between two groups.

Cycle outcomes are shown in Table 3. The implantation rate (3.8% *vs.* 7.7%) and clinical pregnancy rate (4.4% *vs.* 12.2%) was higher in the MF group. The total cancellation rate (22.2% *vs*. 12.2%) was higher in the A/L group but these findings were not statistically significant.

Table 1. Basal characteristics of patients*

	A/L (n=45)	MF (n=49)	p
Age (yr)	33.8±5.9	33.5±6.0	NS
BMI (kg/m^2)	23.8±2.8	24.3±2.4	NS
Day 3 FSH (mIU/mL)	8.3±2.2	7.9±2.4	NS
Day 3 E2 (pg/mL)	66.2±34.0	63.5±30.7	NS
No. of previous IVF	1.64±0.7	1.63±0.7	NS
Duration of infertility (yr)	9.9±5.1	8.8±5.3	NS
Causes of infertility (%)			
Male factor	46.7	46.9	NS
Tubal factor	15.6	16.3	NS
Anovulation	11.1	10.2	NS
Endometriosis	8.9	10.2	NS
Unexplained	17.8	16.3	NS

**Data are presented as mean±standard deviation or %. A/L=antagonist/letrozole protocol; MF=microdose GnRH flare protocol; BMI=body mass index; E2=estradiol; FSH=follicle stimulating hormone; IVF=in vitro fertilization; NS=not significant.*

Discussion

The results of this prospective randomized trial showed that the A/L protocol did not improve the implantation and pregnancy rates in poor responders. Although the days of stimulation and the dose of gonadotropin administered were lower in the A/L group, the total cancellation rate was higher in this group than the MF group. Furthermore, the stimulation outcomes did not improve.

There is no consensus on the use of GnRH antagonists in poor responders. In a randomized controlled trial performed in poor responders, ongoing pregnancy rate was significantly higher in the antagonist group, compared with the flare GnRH agonist group [13].

A meta-analysis found no difference between GnRH antagonist and agonist (long and flare up) protocols with respect to the cycle cancellation, number of mature

Table 3. Cycle outcome characteristics*

	A/L (n=45)	MF (n=49)	p
Implantation rate (%)	3.8	7.7	NS
Clinical pregnancy rate (%)	4.4	12.2	NS
Clinical pregnancy rate/ET (%)	5.3	14.3	NS
Total cancellation rate (%)	22.2	12.2	NS

**Data are presented as %. A/L=antagonist/letrozole protocol; MF=microdose GnRH flare protocol; ET=embryo transfer; NS=not significant.*

Table 2. Outcomes of ovarian stimulation*

	A/L (n=45)	MF (n=49)	p
Duration of stimulation (d)	8.5±1.1	9.2±1.2	0.007
Gonadotropin dose (No. of 75 IU ampoules)	42.1±7.5	46.1±7.1	0.009
E2 level on day of hCG administration (pg/mL)	477±54	1,065±706	0.000
Endometrial thickness (mm)	8.3±1.3	8.4±1.2	NS
Mature follicle (n)	4.2±3.4	5.6±3.0	0.035
Oocytes retrieved (n)	2.8±2.7	4.4±2.7	0.008
M II oocyte retrieved (n)	2.4±2.4	4.1±2.4	0.001
Fertilization rate (%)	67.3	70.7	NS
ICSI performance (%)	48.6	51.2	NS
Embryo transferred (n)	1.7±1.0	2.0±0.7	NS
Top and good quality embryo (%)	31.4	55.8	0.040

**Data are presented as mean±standard deviation, n or %. A/L=antagonist/letrozole protocol; MF=microdose flare GnRH agonist protocol; E2=estradiol; M II oocytes=metaphase II oocytes; ICSI=intracytoplasmic sperm injection; NS=not significant.*

oocytes, and clinical pregnancy rate in poor responders [14].

Demirol et al showed that a MF protocol appeared to have a better outcome in poor responders who had a significantly higher implantation rate when compared with an antagonist protocol [15]. Mittwally and Casper reported that aromatase inhibition with letrozole reduced the FSH dose required for COH and improved the ovarian response to FSH in poor responders [11]. Garcia-Velasco et al showed that the addition of letrozole to an antagonist gonadotropin protocol in poor responders improved the pregnancy rate, but this finding was not statistically significant. In their study the implantation rate was enhanced significantly [16]. Schoolcraft et al demonstrated that ongoing pregnancy rates were significantly higher in the MF group than the A/L group with trends toward increased implantation and lower cancellation rates also noted, but these were not statistically significant [17].

Recently, Yarali et al in a retrospective study showed that the total gonadotropin consumption, duration of stimulation, E2 level on the day of hCG administration, and the number of oocytes retrieved were significantly lower with the A/L protocol compared with the MF protocol. However, the fertilization rate and the rate of at least one top-quality embryo transferred was higher with the A/L protocol compared with the MF protocol. The clinical pregnancy rates were comparable between the two groups [18].

The results of our study demonstrated poorer outcome in the A/L group. Lossl et al demonstrated short-term androgen priming by the use of an aromatase inhibitor in COH caused an increased testosterone level and decreases in both the E2:testosterone ratio and inhibin B level in follicular fluid (FF) [19]. A higher ratio of E2:androgen in pre-ovulatory FFs reflects both good follicular health and the viability of oocytes [20], and the pre-ovulatory FF level of inhibin B was positively associated with embryo score. This suggests that the granulosa cells of the androgen-primed follicle functioned sub-optimally and the aromatase inhibitor was perhaps not fully cleared from the follicular environment [19]. These data emphasize the importance of qualitative changes in these patients' oocytes. Therefore, the lower fertilization, implantation, and pregnancy rates in the A/L group may be related to impaired oocyte quality and lower embryo score. It is unclear whether the change of protocol and reduction in the dose and duration of letrozole administration may improve outcomes. Further studies with a larger sample size would be necessary to verify these questions.

The safety of aromatase inhibitors for pregnancy outcome in assisted reproduction has been raised. Tulandi et al compared 514 children born after ovarian stimulation including letrozole with 397 children born after the use of clomiphene citrate and found no increase in the number of malformations when letrozole was administered [21].

In conclusion, our study showed that the addition of letrozole to the GnRH antagonist protocol did not improve outcomes in poor responders, and we recommend the MF protocol as a preferred regimen for COH in patients undergoing assisted reproductive technology cycles. Much work remains to be done in optimizing the A/L protocol and individualizing it to different cycle characteristics. Furthermore, large, prospective randomized studies are needed to introduce a regimen for COH in poor responders that not only increases the quantitative ovarian response but also enhances the quality of the developing cohort of oocytes.

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