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GnRHa stop protocol versus long protocol in poor responder IVF patients

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Abstract

Background: Recently different studies suggested that discontinuation of gonadotrophin releasing hormone analogue (GnRHa) at beginning of ovarian stimulation (improvement of ovarian response to gonadotrophins) may have some benefit to poor responder patients in invitro fertilization (IVF) cycles.

Objective: The efficacy of GnRHa stop protocol in poor responder patients in IVF cycles was assessed.

Materials and Methods: This study was a prospective, randomized controlled trial that 40 poor responder patients (less than three mature follicles in a previous cycle) with normal basal follicle stimulating hormone (FSH) were randomly allocated into two protocols: 1) Non-stop protocol: long GnRHa suppression, and start gonadotrophins from day 3 of mense. 2) Stop-protocol: GnRHa is stopped with the onset of menses, and gonadotrophin doses remained similar to group 1.

Results: A significantly higher number of follicles, oocytes, embryos and fertilization rate also shorter stimulation days and lower human menopausal gonadotropins (HMG) ampoules were recorded in the stop protocol compared to the control group. Both protocols resulted in a similar cancellation rate, pregnancy rate, estradiol level and LH level.

Conclusion: Early follicular cessation of GnRHa permitted the retrieval of a significantly higher number of follicles, oocytes and embryos, and can reduce the number of HMG and stimulation days.

***Key words**: GnRHa long protocol, IVF, Poor respondr, GnRHa stop protocol.*

Introduction

A reasonable percentage of women undergoing infertility treatment respond poorly to the usual gonadotrophin stimulation protocol (1). Garcia et al (1983) first described poor responders as patients with peak estradiol (E2) level of <300 pg/ml and decreased follicular response, who expressed low number of retrieved and fertilized oocytes and transferred embryos. These patients have lower pregnancy rates compared to normal responders (2).

Among patients undergoing IVF treatment, prevalence of low response is 9% to 24% (3). The optimal approach for poor responders to ovarian stimulation is still controversial. There are different ways for this, such as using high doses of gonadotrophins, co-treatment with estrogens, growth hormone or contraceptive pills, and even natural cycle (4, 5).

In a prospective analysis in 182 low responders undergoing 224 IVF-ET cycles, down-regulation was obtained with the administration of leuprolide acetate, beginning in the mid-luteal phase and ending with the onset of menses. Daily

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administration of 6 ampoules of FSH alone or in combination with hMG was initiated on cycle day 3. The clinical PR per transfer, the ongoing PR per transfer, and the implantation rate were 32%, 24%, and 9%, respectively. Short-term ovarian suppression begun in the luteal phase and discontinued with the onset of menses followed by high-dose stimulation with gonadotropins yields favorable pregnancy results in low responders. In another study, Drinfield (1999) studied 63 patients with previous poor response to COH and/or high basal FSH level (>=9 mIU/mL) undergoing 78 IVF-ET cycles.

In both groups, administration of GnRH-a was started in the midluteal phase. Whereas in the study group (40 cycles), it ended before administration of gonadotropins, in controls (38 cycles) GnRH-a treatment was continued throughout the follicular phase. A significantly higher cancellation rate was noted in the study group than in the controls. The new and control regimens resulted in similar stimulation characteristics and clinical pregnancy rates (7). Recent evidence confirms that early GnRHa cessation is still effective in the prevention of a premature rise in LH (9).

In Garcia study (2000) 70 low responder patients (less than three mature follicles in a previous cycle) with normal basal follicle stimulating hormone concentrations and a previous cancelled IVF cycle were randomly allocated into two protocols: 1) non-stop protocol: long GnRHa suppression with high doses of gonadotrophins, and 2) stop protocol, in which GnRHa administration was stopped with the onset of menses, while gonadotrophin doses remained similar to the non-stop protocol. A significantly higher number of mature oocytes were obtained in the study group (stop protocol) compared to the control group (non-stop protocol). The stop protocol reduced the number of ampoules of gonadotrophins required. Both protocols resulted in a similar cancellation rate, pregnancy rate, and implantation rate (10).

In Detti retrospective study, women diagnosed as poor responders underwent three different stimulation regimens during IVF cycles: 1) stop protocol: GnRH-a 500 µg/d administered from the midluteal phase to the start of menses, then gonadotropins from day 2 of cycle, 2) microdose flare: GnRH-a 20 µg 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 for 3 days, followed by 250 µg/d until the day of hCG administration. None of the comparisons reached statistical significance; however, the microdose group demonstrated a trend toward a higher completed pregnancy rate (8). In Schachter study 63 patients enrolled IVF program were treated in two consecutive cycles. Starting with a standardized protocol utilizing midluteal administration of Nafarelin (N) 600 µg/d continued throughout the stimulation phase with human menopausal gonadotropin (hMG) until follicles of 20 mm were identified by transvaginal ultrasound (standard group).

Patients with a poor response in the standard cycle were treated in the subsequent cycle with N and hMG initially in a similar manner, and then N was stopped after 5 days of hMG stimulation (N-stop group). The change in each parameter in the N-stop cycle was expressed as the percent change as compared with the standard protocol cycle for each patient. Peak estradiol (E2) and the number of aspirated oocytes were increased in the N-stop cycle, but insignificantly. The percentag of cleaving embryos was significantly increased in the N-stop cycle, as embryo morphology was improved. The efficacy of gonadotropin treatment was enhanced in the N-stop cycle, as expressed by a 32.5% increase in oocytes retrieved per hMG ampoule administered (21).

In this prospective randomizes clinical trial the benefit of withholding GnRHa in early follicular phase in women who previously had insufficient ovarian response to complete an IVF attempt was evaluated.

Materials and methods

This study was performed on 40 poor responders' patients (previous poor response) undergoing IVF cycle treated at Vali-e-Asr Reproductive Health Center, Tehran University, between November 2004, and February 2006. All couples were required to sign a written informed consent after the provision of complete information. Including criteria were: patients with at least one previous cancelled IVF cycle, with fewer than three follicles>18mm in diameter and basal FSH <12mIu/ml. There was no age limit. In our standard long protocol, after pituitary desensitization with Buserelin 0.5 mg daily subcutaneously (Suprefact, 1mg/cc, Aventis, Germay), on days 3 of cycle four ampoules of human menopausal gonadotrophin (HMG 75 Iu, Merional, IBSA) was administered to patients and number of HMG were regulated according to transvaginal ovarian ultrasound on 7th day of stimulation. Twenty patients were treated with a stop protocol with Buserelin cease on the onset of mense and twenty patients stimulated with long protocol that Buserelin was continued until HCG administration (half dose after mense). The criteria for HCG 10,000 Iu (Pregnyl, ORGANON, Iran) administration, was at least three follicles measuring ≥ 18mm in diameter. Oocyte retrieval was scheduled for 34-36 hours after HCG injection and Cyclogest 400mg (Actoverco, Iran) BD was administrated as luteal support. Then standard IVF procedure was done (11). Three types of embryos were established, ranging from type A to C. Type A embryos were the best and were defined as round and well-shaped blastomeres without fragments. Type C was defined as irregular blastomeres with many fragments, and type B was intermediate. Only patients with freshly transferred embryos were included in the study. Serum estradiol and LH level were analyzed on day of HCG injection.

Statistical analysis

Data were expressed as mean ±SEM. Statistical analysis was performed using commercially available software packages (SPSS). Number of follicles, oocytes, embryos, fertilization rate and cancellation rate were compared between two groups with Mannwhitney U test. Chemical and clinical pregnancy rate and quality of embryos were compared with chi-square.

Results

After randomization, 20 patients were included in 2 groups. Group I non- stop protocol and group II stop protocol. Table 2 shows that in patients undergoing ovarian stimulation with the stop protocol a significantly higher number of follicles, oocyetes embryos and fertilization rate were found, no differences were found in cancellation rate (5% versus 0%) and grade of embryos. Obviously, a higher number of HMG ampoules and Stimulation days were found in the non–stop protocol. Pregnancy rate was higher in stop protocol but the differences were not significant.

Table I. Comparison of demographic characteristic in two groups.

variations	Group 1 (mean ± SD)	Group 2 (mean ± SD)	p-value
Age (years)	33.9±6.797	37.2±6.42	0.123(NS)
Duration of infertility (years)	8.3±5.04	11.17±7.68	0.17(NS)
BMI	26.67±3.28	25.92±2.22	0.404(NS)

BMI=Weight ⁄ $(\text{Height})^2$
GroupI: non stop protocol
GroupII: stop protocol

Table II. Comparison of quantities' outcomes in two groups.

variations	Group 1 (mean ± SD)	Group 2 (mean ± SD)	p-value
Number of HMG	45.2±9.8	36.6±9.3	0.019
Number of stimulation days	10.8±2.4	9.3±2.4	0.04
Number of follicles	3.6±1.3	5.5±2.9	0.022
Number of oocytes	2.3±1.04	4±2.3	0.034
Number of M2 oocytes	1.25±0.91	2.7±2.02	0.042
Number of embryos	0.8±0.83	1.90±1.4	0.033
Number of transferred embroys	0.8±0.83	1.7±1.08	0.025
Fertilization rate	60±38.3	83.9±27.6	0.043
Serum estradiol (pg/ml)	675.15±139.3	754.2±264.17	NS
Serum LH (mIu/ml)	0.7947±0.135	0.72±0.12	NS
Endometrial thickness (mm)	9.1±1.7	9.7±1.6	NS

GroupI: non stop protocol
GroupII: stop protocol

Table III. Comparison of grade of embryos in two groups. (p-value=1)

Grade of embryos	Group 1		Group 2	
	number	**percent**	**number**	**percent**
Good (A)	7	63.6	11	64.7
Middle (B)	4	36.4	5	29.9
Bad (C)	0	0	1	5.9

Table IV. Comparison of pregnancy between two groups. (p=NS)

outcome	Group 1		Group 2	
	number	**percent**	**number**	**percent**
Chemical pregnancy	1	5%	4	20%
Clinical pregnancy	1	5%	4	20%

Discussion

The results of this prospective, randomized trial showed that early cessation of GnRHa combined with gonadotropins, lead to a significantly higher number of follicles, mature oocytes, embryos and fertilization rate, than a conventional non-stop, long down-regulation protocol but the IVF outcomes were the same in both groups. No significant differences were found comparing the two groups regarding patients age, duration of infertility, BMI, cause of infertility, serum estradiol, LH and endometrial thickness on the day of HCG injection.

GnRHa is routinely used until oocyte retrieval, but even with short-acting molecules pituitary down regulation continues following cessation of GnRHa during ovarian stimulation for IVF (12). In Becker's study, early follicular phase cessation of GnRHa was still effective in the prevention of a premature rise in LH or progesterone(9).This is in accordance with the fact that most of the cancellations in the study group in the current study as well as others (6) were not due to a premature ovulation. Several mechanisms may contribute to the improved ovarian response observed with the stop protocol. The pituitary gonadotrophin down regulation induced by GnRHa, decreases cancellation rates by suppressing endogenous LH surge, although this may require significantly higher requirement of gonadotrophins (13).

A direct effect of GnRHa on the ovaries has been proposed; therefore reducing the dose or even stopping the administration would remove this suppression, and improve ovarian responsiveness (14). This hypothesis is based on the presence of GnRH receptors on the ovaries (15). On the other hand GnRHa have been proven to decrease blood flow assessed by pulsed Doppler analysis (16) .

Some investigators believe that follicular growth is dependent on an appropriate vascular network responsible for the distribution of circulating gonadotrophins(17).

In Schachter study, similar to our study, embryo cleavage rates and morphology were significantly improved, this may be due to improved oocyte quality, which may have been responsible for achieving pregnancies. The efficacy of gonadotropin treatment was enhanced when GnRH-a was discontinued (21). This results show that GnRH-a may have a direct negative effect on folliculogenesis and oocytes, which is apparent especially in poor responder patients. In fact, ovarian blood flow velocity after pituitary suppression has been shown to be predictive of ovarian responsiveness and the outcome of IVF treatment (18).

Thus it could be postulated that GnRHa early cessation while maintaining pituitary suppression, restores the diminished perifollicular blood flow, which correlates with the number of oocytes retrieved and IVF outcome (19). The primary efficacy result, such as a significantly higher number of mature oocytes, lower gonadotrophin ampoule consumption in the stop protocol and GnRHa showed that the cost of the cycle in terms of medication is significantly reduced. The reduced gonadotrophin usage together with the higher number of mature oocytes retrieved and higher number of embryos, make this protocol appealing, something that would be definitive if it allowed higher pregnancy rates. Although a trend was observed (20% versus 0%) in this study, another prospective study with pregnancy rate as primary end-goal should be designed to prove this hypothesis.

The stop protocol, in spite of having significantly more oocytes, had similar estradiol concentration to those observed in the non-stop protocol, such as Garcia study (10).

In fact, serum concentrations of estradiol were higher in the stop protocol, although the value did not reach statistically significant differences, probably due to the limited sample size of this study. When clinicians observe a low response to IVF stimulation (less than three follicles), cycle cancellation is usually counseled to the patients, in the hope that a better response might be obtained in a subsequent cycle. This trend is based on the assumption that poor responders have a poor IVF outcome as they usually have a low oocyte quality. According to their based FSH concentrations, young low responders with high basal FSH concentrations have a poor outcome based on the low quality of the oocytes retrieved. However, young low responders with normal day 3 FSH concentrations; although their ovarian reserve may be compromised (19), might benefit from alternative protocols such as the stop protocol, as their chances of achieving a pregnancy seem to be similar to those of normal responders (20). In Detti study, flare protocol versus stop protocol demonstrated a trend toward higher delivery rates (8), this may be because of the number of HMG or higher dose of GnRHa.

The stop protocol increases the number of available embryos and decrease the quantity of gonadotrophin required. Further studies with more number of patients are required to determine weather early cessation of GnRHa leads to increase in implantation rate and pregnancy rate.

References

1. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF. *Hum Reprod Update* 2003; 9: 61-76.
2. Garcia JE, Jones GS, Acosta AA, Wright GJr. Human menopausal gonadotropin/human chorionic gonadotropin follicular maturation for oocyte aspiration. *Fertil Steril* 1983; 39(2): 174-179.
3. Keay SD, Liversedge NH, Mathur RS, Jenkins JM. Assisted conception following poor ovarian response to gonadotrophin stimulation. *Br J Obstet Gynaecol* 1997; 104: 521-527.
4. Scott RT Jr. Evaluation and treatment of low responders. *Semin Reprod Endocrinol* 1996; 14: 317–337
5. Karande V, Gleicher N. A rational approach to the management of low responders in in-vitro fertilization. *Hum Reprod* 1999; 14: 1744–1748.
6. Faber BM, Mayer J, Cox B, Jones D, Toner JP, Oehninger S, et al. Cessation of gonadotrophin-releasing hormone agonist therapy combined with high-dose gonadotrophin stimulation yields favorable pregnancy results in low responders. *Fertil Steril* 1998; 69: 826–830.
7. Dirnfield M, Fruchter O, Yshai D. Cessation of gonadotrophin-releasing hormone analogue (GnRH-a) upon down-regulation versus conventional long GnRH-a protocol in poor responders undergoing in vitro fertilization. *Fertil Steril* 1999; 72: 406–411.
8. Detti L, Williams DB, Robins JC, Maxwell RA, Thomas MA. Comparison of three downregulation approaches for poor responders undergoing in vitro fertilization, *Fertil Steril* 2005; 84(5): 1401-1405.
9. Beckers NGM, Laven JSE, Eijkemans MJC. Follicular and luteal phase characteristics following early cessation of gonadotrophin-releasing hormone agonist during ovarian stimulation for in-vitro fertilization. *Hum Reprod* 2000; 15: 43–49.
10. Garcia-Velasco JA, Isaza V, Requena A, Martínez-Salazar FJ, Landazábal A, Remohí J, et al. High doses of gonadotrophins combined with stop versus non-stop protocol of GnRH analogue administration in low responder IVF patients: a prospective, randomized, controlled trial. *Hum Reprod* 2000; 15: 2292-2296.
11. Gil-Salom M, Mínguez Y, Rubio C, De los Santos MJ, Remohí J, Pellicer A. Efficacy of intracytoplasmic sperm injection using testicular spermatozoa. *Hum Reprod* 1995; 10: 3166–3170.
12. Sungurtekin U, Jansen RPS. Profound Latinizing hormone suppression after stopping the gonadotrophin releasing hormone-agonist leuprolide acetate. *Fertil Steril* 1995; 63: 663–665.
13. Horvath PM, Styler M, Hammond JM, Shelden RM, Kemmann E. Exogenous gonadotropin requirements are increased in leuprolide suppressed women undergoing ovarian stimulation. *Fertil Steril* 1988; 49: 159–162.
14. Kowalik A, Barmat L, Damario M, Liu HC, Davis O, Rosenwaks Z. Ovarian estradiol production in vivo. Inhibitory effect of leuprolide acetate. *J Reprod Med* 1998; 43: 413–417.
15. Latouche J, Crumeyrolle-Arias M, Jordan D, Kopp N, Augendre-Ferrante B, Cedard L, et al. GnRH receptors in human granulosa cells: anatomical localization and characterization by autoradiographic study. *Endocrinology* 1989; 125: 1739–1741.
16. Aleem, FA, Predanic M. The hemodynamic effect of GnRH agonist therapy on uterine leiomyoma vascularity: a prospective study using transvaginal color Doppler sonography. *Gynecol Endocrinol* 1995; 9: 253-528.
17. Zeleznik AJ, Schuler HM, Reichert LEJ. Gonadotrophin-binding sites in the Rhesus monkey ovary: role of the vasculature in the selective distribution of human chorionic gonadotrophin to the preovulatory follicle. *Endocrinology* 1981; 109: 356–362.
18. Engmann L, Sladkevicius P, Agrawal R, Bekir JS, Campbell S, Tan SL. Value of ovarian stromal blood flow velocity measurement after pituitary suppression in the prediction of ovarian responsiveness and outcome of in vitro fertilization treatment. *Fertil Steril* 1999; 71: 22–29.
19. Bhal PS, Pugh ND, Chui DK, Gregory L, Walker SM, Shaw RW. The use of transvaginal power Doppler ultrasonography to evaluate the relationship between perifollicular vascularity and outcome in in-vitro fertilization treatment cycles. *Hum Reprod* 1999; 14: 939–945.
20. Lashen H, Ledger W, Lopez-Bernal A, Barlow D. Poor responders to ovulation induction: is proceeding to in-vitro fertilization worthwhile? *Hum Reprod* 1999; 14: 964–969.
21. Schachter M, Friedler S, Raziel A, Strassburger D, Bern O, Ron-el R. Improvement of IVF outcome in poor responders by discontinuation of GnRH analogue during the gonadotropin stimulation phase--a function of improved embryo quality. *J Assist Reprod Genet* 2001; 18: 197-204.