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SHORT COMMUNICATION

BRONX, NEW YORK

Day 6 Estradiol Level Predicts Cycle Cancellation Among Poor Responder Patients Undergoing In Vitro Fertilization—Embryo Transfer Cycles Using a Gonadotropin-Releasing Hormone Agonist Flare Regimen

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***Purpose*:** To compare two GnRHa flare protocols among poor responders undergoing IVF-ET and to evaluate if a Day 6 estradiol level can predict outcome.

***Methods*:** Retrospective analyses of GnRHa flare IVF cycles among poor responders. Group A ("miniflare," $N = 36$) 40 μg GnRHa s.c. b.i.d. from Day 3; Group B ("standard flare," $N = 24$) 1 mg GnRHa on Days 2–3; 0.5 mg GnRHa from Day 4. ROC analysis was performed to find a Day 6 estradiol value that is predictive of cycle outcome.

***Results*:** With the standard flare, patients required less gonadotropins and tended to have fewer cancellations and higher pregnancy rates. A Day 6 estradiol level $\leq$75 pg/mL was predictive of cycle cancellation, but not of pregnancy outcome.

***Conclusions*:** Standard GnRHa flare offers some advantages over the miniflare. Day 6 estradiol $\leq$75 pg/mL is predictive of cycle cancellation. When the estradiol level is low on Day 6 (no flare), early cancellation should be considered.

KEY WORDS: Cycle cancellation; GnRHa flare; IVF-ET; poor responder; pregnancy.

INTRODUCTION

Women with diminished ovarian reserve undergoing assisted reproduction typically respond poorly to standard ovarian hyperstimulation regimens. Poor responders frequently require higher doses of gonadotropins, are more likely to get cancelled, have fewer oocytes and embryos during IVF, and have lower pregnancy rates. To optimize ovarian stimulation, several alternative stimulation protocols have been suggested. The use of lower dose gonadotropin-releasing hormone agonist (GnRHa), a "GnRHa stop" protocol, and the use of GnRHa flare have all been suggested to be associated with better outcome (1,2). GnRHa is used to suppress the pituitary, thereby preventing an endogenous luteinizing hormone (LH) surge. It also allows better cycle scheduling, and the use of suppression could lead to better synchronization of the cohort of follicles.

When GnRHa is started in the beginning of a cycle, it has an initial stimulatory effect and then provides adequate suppression of the endogenous LH surge. The presence of this "flare effect" should be evident by increased serum estradiol levels after a few days. GnRHa can be given in several ways, and studies report the use of different doses (3–7). The aims of our study are to compare outcomes with two different GnRHa flare protocols and to assess if Day 6 estradiol levels (flare effect) can be used as a predictor for cycle outcome with the flare protocols.

MATERIALS AND METHODS

We identified 71 IVF-ET cycles where a GnRHa flare protocol was used at our IVF Center during the year 2000. Sixty patients were poor responders (identified based on elevated Day 3 FSH (follicle-stimulating hormone) level (>9 IU/mL) and/or previous cancelled cycle). All patients received oral contraceptive pills (Demulen: 35 μg ethinyl estradiol + 1 mg ethynodiol diacetate; Searle) for at least 21 days prior to ovarian stimulation. Patients were treated with either the "GnRHa miniflare" or "GnRHa standard flare" regimen. Patients in Group A ("miniflare") started 40 μg GnRHa (Lupron, Tap Pharmaceutical) s.c. b.i.d. on the 2nd day of their menstrual cycle and continued with the same dose until the day of human chorionic gonadotropin (HCG) injection. Gonadotropins were started on Day 4. Patients in Group B ("standard flare") took 1 mg Lupron on Days 2 and 3 of their menstrual cycle and 0.5 mg from Day 4 onwards until the day of HCG injection. Gonadotropins were started on Day 2 with the GnRHa. All patients returned on Day 6 for a serum estradiol level and a transvaginal ultrasound to assess ovarian response. From Day 6 on they returned daily or every other day for monitoring. The dose of gonadotropins was adjusted based on ovarian response.

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Cycles with inadequate estradiol rise (<200 pg/mL on Day 8) or <3 mature follicles were cancelled or converted to intrauterine insemination. When at least two follicles were >17 mm in diameter, 10,000 U im HCG was given. Transvaginal oocyte retrieval was performed 34 h later. Oocytes were inseminated on the day of retrieval or injected if severe male factor infertility was identified as well. Embryos were cultured for 3 days and were transferred transvaginally on Day 3. The luteal phase was supported with im progesterone 50 mg in oil daily. Patients returned for a serum βHCG level 12 days after the transfer to establish pregnancy. Data was collected for baseline characteristics, cycle parameters, and pregnancy outcome. Embryo quality was assessed based on cell number and fragmentation (8).

Statistical analysis was performed using the Student's t test, chi-square test, and receiver operating characteristic (ROC) curve analysis. $p < 0.05$ was considered statistically significant.

RESULTS

Seventy-one cycles were identified where one of the flare protocols was used. Sixty patients were identified as poor responders (Group A: $n = 36$, Group B: $n = 24$). Baseline characteristics (age, baseline FSH) and cycle parameters (length of stimulation, Day 6 estradiol, peak estradiol, number of oocytes retrieved, number of embryos available, quality of embryos) were not different between the two groups. Patients with the standard flare required fewer ampoules of gonadotropins. Cycles were cancelled in 16/36 (44.4%) cases with the miniflare and 7/24 (29.2%) cases with the standard flare ($p =$ ns). Pregnancies were achieved in 2/36 (5.6%) cycles with the miniflare and 5/24 (20.8%) cycles with the standard flare ($p =$ ns) (see Table I).

To evaluate if the Day 6 estradiol level was predictive of cycle cancellation or pregnancy outcome, we performed an ROC analysis including all the flare cycles. This analysis showed that a Day 6 estradiol level ≤75 pg/mL was predictive of cycle cancellation when all 71 cycles were included (estradiol >75 pg/mL 7/45 [15.5%] vs. estradiol ≤75 pg/mL 15/26 [57.7%], $p < 0.001$). A separate ROC analysis using pregnancy results did not find a cutoff value that could predict pregnancy outcome.

The same cutoff value among poor responders also predicted cycle cancellation (estradiol >75 pg/mL 7/35 [20%] vs. estradiol ≤75 pg/mL 15/25 [60%], $p = 0.002$), but did not predict pregnancy outcome. Twenty-five patients with a Day 6 estradiol level ≤75 pg/mL completed the IVF cycle and had an embryo transfer. There were two clinical pregnancies among them (2/25; 8%). Patients with a Day 6 estradiol level >75 pg/mL achieved five pregnancies (5/35; 14.3%); this was not statistically different though (see Table II).

Table I. Baseline Characteristics, Cycle Parameters, and Cycle Outcome Among Poor Responders

	Standard flare ($N = 24$)	Miniflare ($N = 36$)
Age (years)[a]	38.92 ± 0.91	39.83 ± 0.66
Baseline FSH (IU/mL)[a]	8.2 ± 0.34	9.2 ± 0.55
Days of stimulation[a]	12.2 ± 0.49	11.53 ± 0.45
Day 6 estradiol (pg/mL)[a]	154.22 ± 30.25	183.03 ± 38.33
Peak estradiol (pg/mL)[a]	1621.8 ± 182.29	1271.5 ± 150.6
Number of ampoules of gonadotropins*	64.7 ± 8.12	89.5 ± 6.49
Number of oocytes retrieved[a]	7.2 ± 0.99	5.67 ± 0.64
Number of embryos available[a]	2.82 ± 0.26	2.39 ± 0.27
Embryo quality (MCS)[a]	17.33 ± 2.54	16.61 ± 2.06
Cycle cancellation[a]	7/24 (29.2%)	16/36 (44.4%)
Positive pregnancy[a]	5/24 (20.8%)	2/36 (5.6%)

Note. Values are reported as mean ± SEM.
[a] Nonsignificant.
* $p = 0.02$.

Table II. Cycle and Pregnancy Outcome Among Poor Responders with Day 6 Estradiol ≤75 pg/mL or >75 pg/mL

	Estradiol ≤75 pg/mL	Estradiol >75 pg/mL
Cycle cancellation*	15/25 (60%)	7/35 (20%)
Cycle not cancelled*	10/25 (40%)	28/35 (80%)
Positive pregnancy[a]	2/25 (8%)	5/35 (14.3%)
Negative pregnancy[a]	23/25 (92%)	30/35 (85.7%)

[a] Nonsignificant.
* $p = 0.002$.

DISCUSSION

There is no consensus on how to define a "poor responder." Studies evaluating ovarian response use various entry criteria. Poor responders have been identified based on age (>40 years of age), previous poor performance (cancelled cycle secondary inadequate estradiol rise or few mature follicles), or elevated baseline FSH levels (1,9–12). Ovarian

reserve tests include measuring baseline hormone (FSH, FSH/LH, estradiol, inhibin B) levels, dynamic testing (clomiphene challenge test), or measuring ovarian volume or preantral follicle number. None of these tests are associated with a great positive predictive value though (13,14). We have identified our patients on the basis of previous poor response and/or elevated baseline FSH levels.

Hypothalamic-pituitary suppression, to prevent premature endogenous LH surges, is an integral part of ovarian hyperstimulation. It can be achieved with the use of GnRHa, GnRH antagonist, or OCPs. Several stimulation protocols were developed with the aim of trying to reduce this suppression and thereby improve outcome among poor responders. There are controversial results with the use of OCP suppression for poor responders (15,16). Reduced dose GnRHa can be initiated in the luteal phase of the prior cycle. Alternatively GnRHa initiated in the luteal phase can be discontinued once ovarian stimulation with gonadotropins is started (GnRHa stop). (1) When GnRHa is administered in the early follicular phase, initially it will lead to increased gonadotropin release from the pituitary before suppressing its release. This initial stimulatory flare effect is utilized by the flare protocols.

The effects of different flare protocols have been evaluated. These studies use different inclusion criteria, different type, and dose GnRHa, and there are differences in the protocols and the IVF procedures as well. This makes it very difficult to compare the studies to each other. Surrey *et al.* evaluated the flare regimen among IVF patients who previously showed poor response with luteal GnRHa downregulation stimulation. With GnRHa flare there was less cancellation, estrogen levels were higher, and more patients reached an embryo transfer when compared to their previous luteal phase downregulation cycles. Interestingly they found no difference in the early follicular phase estradiol levels questioning the presence of a flare effect (3). Schoolcraft *et al.* evaluated the flare regimen among 32 women who previously showed poor response with the conventional long protocol. With the flare regimen there was a significantly lower cancellation rate, the Day 5 estradiol level was significantly higher, and more follicles were visualized on ultrasound. The ongoing pregnancy rate was 50% per oocyte retrieval (17).

Studies also evaluated the flare protocol among normal responder patients. Frydman *et al.* compared the luteal phase downregulation protocol to the flare protocol in normal responder women. Ovarian response (estradiol level, number of oocytes retrieved) and pregnancy rates were similar with the two stimulation protocols (4). Cramer *et al.* evaluated the use of the flare protocol and compared it to the luteal downregulation protocol based on 1244 cycles. They found lower pregnancy rates among patients using the flare regimen (5).

Several studies evaluated the presence of the flare effect and its prognostic value. Padilla *et al.* used 1 mg Lupron from Day 2 and gonadotropins from Day 5. Serum estradiol measurements were performed on Days 2, 3, and 4. Pregnancy rates were higher when a transient or persistent serum estradiol rise was seen when compared to no increase in serum estradiol levels. Cancellation rate was higher when there was no estradiol rise, suggesting no flare effect (6). Winslow *et al.* also evaluated the significance of early estradiol patterns on stimulation outcome. Participants were given 1 mg Lupron s.c. on Days 2, 3, and 4. On Day 5 the dose of Lupron was decreased to 0.5 mg. Gonadotropin stimulation was initiated on Day 4. They also observed poor outcome when the serum estradiol level did not rise. This group had the highest cancellation rate and fewest cycles progressing to embryo transfer. They reported the best outcome in cycles where the estradiol level was persistently rising, consistent with a flare effect (7). Loumaye *et al.* studied hormonal changes during GnRHa luteal phase downregulation and GnRHa flare protocols. Patients on the flare regimen received buserelin 300 μg t.i.d. intranasally on Day 1 and started gonadotropins on Day 3. LH levels initially rose in the flare group and then plateaued, while they remained low in the long-protocol group. FSH levels in the flare group also rose on Day 2 and then dropped but stabilized at a higher level than in the long-protocol group. Estradiol levels started to rise sooner in the flare group and remained higher from Days 3 through 11; however, preovulatory estradiol levels were similar. The number of oocytes retrieved was similar, but the fertilization rate was higher in the long-protocol group leading to higher number of embryos. Pregnancy rates were similar, but this is probably due to the low number of participants (18).

Since patients undergoing IVF-ET are usually not seen until Day 6 of stimulation, we tried to find a discriminatory Day 6 estradiol value that will predict cycle outcome. In this study we compared two different flare protocols and tried to establish a Day 6

estradiol level predictive of outcome. The flare protocol is usually offered to poor responders, but we have no specific guidelines to determine which flare protocol should be used. That choice is primarily physician dependent. There were however similar number of patients in both groups, and they had similar baseline characteristics. Cycle management is similar among the physicians at our center, and therefore we would not expect significant differences because of management differences. We found that a Day 6 estradiol level lower than 75 pg/mL was predictive for cycle cancellation but did not predict pregnancy outcome. Patients had similar responses to the two different protocols; however, patients on the standard flare regimen required less gonadotropins and had a tendency towards lower cancellation rates and higher pregnancy rates.

Since our study is a retrospective analysis, it has its limitations. In a retrospective analysis there are potential biases. We have identified all cycles where the flare regimen was used and the poor responders were identified based on accepted criteria. Our patients were not randomized to treatment; however, they had similar baseline characteristics. The treating physician was not blinded to treatment, but physicians at our center managed cycles similarly. Based on our results, there are several benefits of the standard flare protocol. Patients require less gonadotropins, they have fewer cancelled cycles, and they require less injections as well. Our findings also confirm findings of other studies that showed that when there is no early flare response outcome is poor. Cancellation rate was high, and pregnancy rate was low.

In conclusion, the standard flare protocol offers some advantages for poor responder patients over the miniflare regimen. In cycles where the flare effect is not present, early cycle cancellation should be considered.

REFERENCES

1. Faber BM, Mayer J, Cox B, Jones D, Toner JP, Oehninger S, Muasher S: Cessation of gonadotropin releasing hormone agonist therapy combined with high-dose gonadotropin stimulation yields favorable pregnancy results in low responders. Fertil Steril 1998;69:826–830
2. Surrey ES, Schoolcraft WB: Evaluating strategies for improving ovarian response of the poor responder undergoing assisted reproductive techniques. Fertil Steril 2000;73:667–676
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;69:419–424
4. Frydman R, Forman R, Belaisch-Allart J, Hazout A, Parneix I, Testart J: Comparison between flare up and down regulation effects of luteinizing hormone-releasing hormone agonists in an in vitro fertilization program. Fertil Steril 1988;50:471–475
5. Cramer DW, Powers DR, Oskowitz SP, Liberman RF, Hornstein MD, McShane PM, Barbieri RL: Gonadotropin-releasing hormone agonist use in assisted reproduction cycles: The influence of long and short regimens on pregnancy rates. Fertil Steril 1999;72:83–90
6. Padilla SL, Bayati J, Garcia JE: Prognostic value of the early serum estradiol response to leuprolide acetate in in vitro fertilization. Fertil Steril 1990;53:288–294
7. Winslow KL, Toner JP, Brzyski RG, Oehninger SC, Acosta AA, Muasher SJ: The gonadotropin-releasing hormone agonist stimulation test—a sensitive predictor of performance in the flare-up in vitro fertilization cycle. Fertil Steril 1991;56:711–717
8. Hu Y, Maxson WS, Hoffman DI, Ory SJ, Eager S, Dupre J, Lu C: Maximizing pregnancy rates and limiting higher-order multiple conceptions by determining the optimal number of embryos to transfer based on quality. Fertil Steril 1998;69:650–654
9. Karande V, Morris R, Rinehart J, Miller C, Rao R, Gleicher N: Limited success using the "flare" protocol in poor responders in cycles with low basal follicle-stimulating hormone levels during in vitro fertilization. Fertil Steril 1997;67:900–903
10. Toth TL, Awward JT, Veeck LL, Jones HW, 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;41:321–326
11. Salat-Baroux J, Rotten D, Alvarez S, Antoine JM: Comparison of growth hormone responses to growth hormone-releasing factor and clonidine in women with normal or poor ovarian response to gonadotropin stimulation. Fertil Steril 1993;60:791–799
12. Olivennes F, Righini C, Fanchin R, Torrisi C, Hazout A, Glissant M, Fernandez H, Frydman F: A protocol using a low dose of gonadotropin-releasing hormone agonist might be the best protocol for patients with high follicle-stimulating hormone concentrations on day 3. Hum Reprod 1996;11:1169–1172
13. Broekmans FJ, Scheffer GJ, Bancsi LFJMM, Dorland M, Blankenstein MA, te Velde ER: Ovarian reserve tests in infertility practice and normal fertile women. Maturitas 1998;30:205–214
14. Sharara FD, Scott RT, Jr, Seifer DB: The detection of diminished ovarian reserve in infertile women. Am J Obstet Gynecol 1998;179:804–812
15. Lindheim SR, Barad DH, Witt BR, Ditkoff E, Sauer MV: Short-term gonadotropin suppression with oral contraceptives benefits poor responders prior to controlled ovarian hyperstimulation. J Assist Reprod Genet 1996;13:745–747
16. Kovacs P, Barg PE, Witt BR: Hypothalamic-pituitary suppression with oral contraceptive pills does not improve outcome in poor responder patients undergoing in vitro

fertilization–embryo transfer cycles. J Assist Reprod Genet 2001;18:391–394
17. Schoolcraft W, Schlenker T, Gee M, Stevens J, Wagley L: Improved controlled ovarian hyperstimulation in poor responder in vitro fertilization patients with a microdose follicle-stimulating hormone flare, growth hormone protocol. Fertil Steril 1997;67:93–97
18. Loumaye E, Vankrieken L, Depreester S, Psalti I, de Cooman S, Thomas K: Hormonal changes induced by short-term administration of a gonadotropin-releasing hormone agonist during ovarian hyperstimulation for in vitro fertilization and their consequences for embryo development. Fertil Steril 1989;51:105–111

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