“Co-Flare” Stimulation in the Poor Responder Patient: Predictive Value of the Flare Response¹

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Purpose: In this study we review our experience with a day 2 start, “Co-Flare” protocol analyzing the flare response as a predictor of outcome in patients with a history of a poor response.

Methods: This study was conducted at a university based IVF Center. A total of 564 patient cycles over a 2.5-year time frame in patients that had either elevated FSH levels or a previous poor response to conventional leuprolide acetate down-regulated stimulation were retrospectively analyzed. These patients were treated with our co-flare protocol (1.0 mg of leuprolide acetate administered on day 2 and decreased on day 5 to 0.5 mg; gonadotropins initiated on day 3). We analyzed the initial flare response and the outcome for these patients.

Results: A total of 564 patients attempted to initiate an IVF cycle. The patients mean age was 39.4 ± 3.7 years. Of the 450 cycles that started, the outcomes were as follows: 24% (108/450) cancellation, 20.4% (92/450) clinical pregnancy per initiated cycle, and a 14.0% delivery rate per initiated cycle. Patients with an estradiol flare that doubled were less likely to have cycles cancelled (13.6% vs. 35.6%, $P < 0.01$), but no differences were noted in pregnancy outcome if the cycle was not cancelled once the patient made it to retrieval.

Conclusions: We have demonstrated an overall 14.0% delivery rate per initiated cycle in these “poor prognosis” patients. While the initial flare response (as indicated by a doubling of the estradiol by the second day of stimulation) was indicative

of a better stimulation, no difference in pregnancy outcome was seen if the patient underwent retrieval.

KEY WORDS: co-flare stimulation; IVF; poor responders.

INTRODUCTION

In vitro fertilization success has dramatically increased over the past decade; patients with a poor response to stimulation present a particularly difficult problem. These patients often have a diminished ovarian reserve as demonstrated by an elevated FSH and estradiol levels as well as diminished inhibin-B production on menstrual cycle day 3. FSH elevations in the early follicular phase signal declining ovarian reserve; this occurs even when the patient continues to have regular menses (1). In previous studies, we have shown that patients with abnormal day 3 testing have a poor prognosis when undergoing IVF (2). Although intercycle fluctuation in basal gonadotropin levels may be seen, once a woman is found to have an elevated basal FSH, the ovarian response is diminished even in subsequent treatment cycles in which her basal FSH is normal (3). Pellicer *et al.* have shown that granulosa-luteal cells obtained from older women at follicular aspiration for IVF have significantly reduced ability to secrete immunoreactive-inhibin in vitro (4). This relative lack of inhibin production permits the rise of FSH levels despite normal estradiol production. Recently, it was reported that women with low serum day 3 Inhibin-B levels demonstrate a poorer response to ovulation induction and are less likely to conceive a clinical pregnancy through ART relative to women with high day 3 inhibin-B (5).

Often these patients are referred to oocyte donation programs. However, not all patients wish to abandon attempts of conception with their own eggs and

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therefore, more “aggressive” protocols have been developed. Several approaches have been advocated in treating the low responder. These have included increasing the dosage of gonadotropins, decreasing the luteal leuprolide dosage, clomiphene citrate based protocols and flare-up protocols. Winslow *et al.* further described a GnRH-a Stimulation Test (GAST) (6). In this test, the day 3 estradiol response following the subcutaneous administration of 1 mg of leuprolide acetate on day 2 was ascertained. In this study, gonadotropins were administered on day 4. The estradiol increase was an indicator of ovarian response to stimulation in flare-up cycles. There were significant correlations between estradiol and peak estradiol levels, number of mature oocytes retrieved, and pregnancy rates. When there was no estradiol rise after the leuprolide administration, the response was significantly lower and the pregnancy rate was zero (6).

In this study, we will present our experience with our co-flare stimulation protocol in patients with a previous poor response to conventional luteal leuprolide and/or abnormal ovarian reserve testing. We have altered the flare protocol in that we begin the gonadotropins earlier on cycle day 3. Specifically, we wish to analyze the flare response to the initial dose of leuprolide acetate as a predictor of outcome.

MATERIALS AND METHODS

A total of 564 consecutive cycles of patients utilizing the co-flare protocol from March 1996 to September 1998 were retrospectively analyzed. All patients had had a previous poor response to traditional IVF stimulations or were found to have abnormal day 3 hormonal testing (FSH ≥ 20 mIU/mL and/or estradiol > 75 pg/mL). All of the cycles were conducted at a single institution (The New York Presbyterian Hospital-Cornell Medical Center).

Stimulation was initiated on day 2 of a spontaneous cycle. All patients were evaluated with a day 2 hormone profile (FSH, LH, and estradiol) and sonogram. In general, patients with elevated FSH (FSH ≥ 20) or estradiol levels (E2 > 75) did not start. Furthermore, patients with baseline follicular cysts greater than 20 mm did not initiate an IVF cycle. Stimulation was then started on day 2. One milligram of leuprolide acetate was administered on days 2–4, and the dose was then decreased to 0.5 mg on cycle day 5. Gonadotropins were administered on cycle day 3. Patients were generally stimulated with six ampules of gonadotropins (either six ampules of FSH or a

combination of four ampules of FSH and two ampules of HMG). The leuprolide acetate and gonadotropins were continued until the day of hCG administration.

Patients were stimulated in accordance with our general protocols as previously described (7). Gonadotropins were decreased in a “step-down” fashion. Leuprolide acetate was continued until the day of hCG administration. hCG was administered when two follicles were at least 17 mm in diameter. In general, cycles were cancelled when there were fewer than three follicles or estradiol levels had not risen by cycle day 7. Intracytoplasmic sperm injection and assisted hatching were utilized when deemed appropriate. Embryo transfer was performed on day 3. In general, patients under the age of 34 years received three embryos, patients 34–39 years of age received four embryos, and patients over the age of 40 years received five embryos. A clinical pregnancy is defined as the presence of a fetal heart at a 7-week sonogram.

Estradiol and FSH serum concentrations were determined by commercial RIA kits (Pantax, Santa Monica, CA, and FSH Quant; Leeco Diagnostics, Southfield, MI, respectively). Interassay and intra-assay coefficients of variation for estradiol and FSH were 5.7 and 5.8, and 4.8 and 7.9, respectively.

In this study we describe the overall outcomes of this protocol. We also analyze the flare response (day 3 estradiol/day 2 estradiol) as a means to predict the outcome. Statistical analysis included Mann–Whitney U test, chi-square analysis, and nonparametric *t* tests where appropriate. $P < 0.05$ was considered significant. Data is presented as mean $\pm$ standard deviation.

RESULTS

Over the 2 1/2-year study period, 564 cycles were evaluated. Of these, 114 patients (114/564, 20.2%) did not start an IVF cycle because of abnormal hormone levels or large ovarian cysts on cycle day 2. The patients that initiated a cycle were on average 39.4 (± 3.5) years old. Two hundred and forty-two (53.8%) of the patients were over 40 years old.

Table I presents the outcome by initiated cycle. One hundred and six (106/450, 23.6%) of the initiated cycles were cancelled prior to retrieval. Of the multiple pregnancies per initiated cycle, only 8 (1.8%) were found to be triplets or more at the 7-week sonogram. Table II presents the outcome per cycle undergoing retrieval.

We then analyzed patient's outcome based on the estradiol flare response from day 2 to day 3 after the

Table I. Outcome by Initiated Cycle ($n = 450$)

	Age < 40 years ($n = 208$)	Age $\geq$ 40 years ($n = 242$)	All patients ($n = 450$)
Cancelled cycle	24.0	23.1	23.6
No transfer	6.7	6.2	6.4
Clinical pregnancy	24.0	17.4	20.4
Multiple pregnancy	7.2	2.5	4.7
Delivery	18.8	9.9	14

Note. Data presented as percent of all initiated cycles.

1.0 mg dose of leuprolide acetate. Table III illustrates outcome differences between patients whose estradiol levels doubled as compared to those whose levels did not. (Not all patients had blood studies done on day 3.) Patients whose estradiol levels did not double were more likely to have their cycles cancelled prior to undergoing retrieval. However, if the cycles were not cancelled prior to retrieval, the clinical pregnancy rate was not different from that of those who underwent retrieval and manifested a doubling of their estradiol from cycle day 2 to cycle day 3. On the other hand, when evaluating outcome by cycle initiation, those patients with less than a doubling of the flare response were less likely to deliver a child.

DISCUSSION

Poor responders to conventional stimulation represent a difficult group of IVF–ET patients. While many stimulation techniques have been attempted, none have proven clearly superior. In this paper we describe our results with a co-flare protocol initiating leuprolide acetate on day 2 and gonadotropins on day 3.

Table II. Outcome by Retrieval ($n = 344$)

	Age < 40 years ($n = 158$)	Age $\geq$ 40 years ($n = 186$)	All patients ($n = 344$)
Peak estradiol ^a	1450 $\pm$ 1062	1296 $\pm$ 922	1168 $\pm$ 975
# Oocytes	6.9 $\pm$ 4.3	6.0 $\pm$ 3.6	6.3 $\pm$ 4.0
# 2PN	4.1 $\pm$ 3.3	3.5 $\pm$ 2.4	3.8 $\pm$ 2.6
# ET	2.9 $\pm$ 1.4	3.0 $\pm$ 1.7	3.0 $\pm$ 1.4
No transfer (%)	8.9 (14/158)	8.1 (15/186)	8.4 (29/344)
Implantation rate (%)	15.2	7.3	10.9
Clinical pregnancy (%)	31.6 (50/158)	22.6 (42/186)	26.7 (92/344)
Delivery (%)	26.7	12.9	18.3
Multiple pregnancy (%)	9.5 (15/158)	3.2 (6/186)	6.1 (21/344)

^aData presented as mean ($\pm$ SD) measured in pg/mL.

The benefit of such a protocol is that it allows the practitioner to assess baseline day 2 FSH and estradiol levels before initiating a cycle. If these baseline levels are normal and the patient has an appropriate rise in estradiol, then an IVF cycle can be initiated. If not, then an IVF cycle may be cancelled with minimal involvement (financially and emotionally) for the patient.

We have clearly shown that these patients represent a poor prognostic group. The overall no start rate because of abnormal ovarian testing was just over 20%. In addition, the cancellation rate is high, with an approximately 23% cancellation rate for stimulation cycles. In short, almost one-third (170/564) of these women attempting IVF never made it to retrieval. In addition, the overall delivery rate was low (14% per initiated cycle). This is important information for guiding the patient and might lead her to instead opt for oocyte donation. Of note, Cramer *et al.* found that flare protocol cycles were associated with a decrease in the number of oocytes and clinical pregnancy rates when compared to luteal suppression protocols (8). They adjusted their results for age, but not for ovarian reserve, which may have significantly affected the outcomes reported. We will consider a flare protocol in these patients with diminished ovarian reserve as demonstrated either by abnormal baseline day 3 testing or because of previously attempted stimulation with poor results. We therefore expect these patients to have a lower pregnancy rate than that of our typical luteal suppression patients. Another study, also not controlling for ovarian reserve, found lower pregnancy rates with flare-up protocols in comparison to down-regulated, long protocols (9).

It is important to emphasize the significantly worse prognosis that is predicted in this group of patients based on their diminished ovarian reserve. When we compared this group of patients to good prognostic patients under the age of 40 years in previously reported work from our center, a doubling of the clinical pregnancy rate is seen (10). This highlights the importance of interpreting IVF results not just by age, but by other factors including ovarian reserve.

The “co-flare” protocol for poor prognostic patients is appealing for several reasons. First, it permits the clinician to analyze the day 2 hormone levels prior to stimulation. Abnormal levels may lead a physician to guide the patient to consider alternative treatments. A single abnormal ovarian reserve test indicates a poor prognosis despite subsequent normal tests. However, after multiple abnormal ovarian reserve tests the prognosis does worsen. Second, the physician can

Table III. Outcome as Determined by the Day 2 to Day 3 Estradiol Flare Response

	E ₂ flare $\geq$ doubled (<i>n</i> = 235)	E ₂ flare < doubled (<i>n</i> = 146)	<i>P</i> -value
Cancelled cycles (%)	13.6	35.6	<0.01
Mean age	39.2 $\pm$ 3.6	39.5 $\pm$ 3.4	ns
Peak estradiol	1543 $\pm$ 853	1011 $\pm$ 471	<0.001
Mature oocytes	5.8 $\pm$ 3.8	3.9 $\pm$ 2.3	<0.001
# ET	3.2 $\pm$ 1.5	2.4 $\pm$ 1.5	0.0002
Clinical pregnancy/retrieval (%)	25.6	23.4	ns
Clinical pregnancy/transfer (%)	26.9	26.5	ns
Delivery/initiated cycle (%)	16.2	8.9	0.04
Delivery/retrieval (%)	18.6	13.7	ns

Note. Not all patients had day 3 bloods determined.

utilize the day 2 to day 3 estradiol flare response as a guide in determining whether to proceed with a cycle. A patient could cancel an IVF cycle after a single dose of leuprolide acetate if her estradiol response is not reassuring. This can result in a significantly lower cost and emotional burden. We have demonstrated that a patient who doesn't exhibit at least a doubling of her estradiol will have the cycle cancelled prior to retrieval over one-third of the time. All of the information gained by determining the response to the first dose of leuprolide appears useful to the practitioner in determining a patient's cycle prognosis. This utility of the estradiol response to the flare concurs with published work by Winslow *et al.* (6).

This study consisted mostly of women over 40 years of age. The combination of age and poor ovarian reserve connotes a poor prognosis. Only 13% of all retrieved patients 40 and over successfully delivered a child after their IVF cycle. Fewer than 10% of all patients over 40 years of age who attempted to initiate a cycle successfully delivered a child. Understanding this prognosis is very important for the psychological well being of the patient.

Other studies have evaluated the utility of flare lupron in poor responder patients (11–13). One study combined birth control pills, microdose lupron, and human growth hormone in a group of 32 poor responder patients (11). This group suggested that the flare protocol was an improvement over the standard luteal phase down regulated stimulation, which had previously been attempted. An impressive number of 10 oocytes per retrieval and a 50% clinical pregnancy rate was obtained in this small group (*n* = 32) of patients. This improvement in outcome is in agreement with earlier work by Scott and Navot (12). However, not all studies have found so pronounced an improvement in outcome. The work by Karande *et al.* found an improved cycle response but with very low

implantation rates (13). These studies, combined with our work, highlight the marked heterogeneity of poor responder patients including the definition of what comprises a "poor responder." Low ovarian reserve is a prominent factor in determining the etiology of failed IVF attempts.

Age and ovarian reserve are clearly the most important predictors of IVF outcome. However, additional tests have been described and are utilized by many programs including the clomid challenge test, pre-antral follicle number on day 3, ovarian volume, and ovarian stromal blood flow tests. These ancillary techniques were not utilized for this study.

In conclusion, we have demonstrated that the overall prognosis for these patients is poor. The initial flare response appears to yield useful information about the risk of cycle cancellation. However, if the cycle is not cancelled, pregnancy rates were no different based on the flare response of the initial dose of the GnRH-agonist.

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