CLINICAL ASSISTED REPRODUCTION

In Vitro Fertilization with Low-Dose Clomiphene Citrate Stimulation in Women Who Respond Poorly to Superovulation

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Submitted: March 20, 1997

Accepted: July 18, 1997

Purpose: Our experience with IVF using low-dose clomiphene citrate for stimulation in "non-" and "poor" responders was reviewed and the treatment outcomes with the previous controlled ovarian stimulation cycles in which hMG and GnRH agonist were used were compared.

Methods: The treatment outcome in 11 non- and 20 poor responders having 30 and 53 clomiphene citrate IVF treatment cycles, respectively, were compared with the treatment outcome in the previous long-protocol buserelin/hMG cycles.

Results: The clinical pregnancy rates per oocyte collection achieved in the first clomiphene citrate cycle in non (9.1%) and poor (10%) responders were comparable to those achieved by poor responders (11.9%) who had buserelin/hMG using the long protocol. Although the numbers were small, a similar pregnancy rate could still be achieved in poor responders up to the third attempt using clomiphene citrate.

Conclusions: IVF using long-protocol buserelin/hMG is more successful than using clomiphene citrate stimulation. However, this advantage may not be significant in those women with a previous poor response to buserelin/hMG. It is suggested that for such poor responders, three attempts of IVF in a clomiphene citrate cycle may offer a viable therapeutic alternative before reverting to more stressful, expensive, and time-consuming treatment.

KEY WORDS: clomiphene citrate; in vitro fertilization; poor responders.

INTRODUCTION

The outcome of treatment following in vitro fertilization has continued to improve since the birth of the first baby conceived by this procedure in 1977. This is due mainly to progressively more successful ovarian stimulation regimens, leading to an increased number of oocytes being collected and usually more embryos transferred. Despite the improvement in ovarian stimulation regimens, a small group of patients continues to have responses characterized by poor follicular recruitment and inadequate estradiol levels. In these patients, stimulation cycles are often canceled, as continuation of treatment usually results in fewer oocytes and embryos and a lower pregnancy rate compared to "good" responders (1).

Several empirical treatments have been proposed for use in "poor" responders, and although the ovarian response may improve (2), the cancellation rate remains high and no treatment has been shown clearly to increase the low pregnancy rate associated with this group of patients (3,4). Among the various options we discuss with our patients who fail to respond to gonadotrophin superovulation is IVF using low-dose clomiphene citrate. Various authors have reported on the use of clomiphene stimulation for IVF (5,6), but only Lindheim *et al.* (7) addressed the specific question of whether this drug could be of benefit in poor responders.

In the present study, we report our experience with the use of clomiphene citrate for ovarian stimulation in IVF for these difficult patients over a 5-year period. The objective was to determine whether the fewer

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oocytes produced with this drug can give a reasonable probability of pregnancy compared with the previous controlled ovarian stimulation cycles in which hMG and GnRH agonist were used.

MATERIALS AND METHODS

Between January 1991 and December 1995, 1616 standard IVF cycles were performed at the Midland Fertility Services using buserelin/hMG in the long protocol. These patients started hMG on a Thursday and returned for a transvaginal scan the following Friday (day 9), when the number and the mean diameter of follicles >10 mm (recruited follicles) were noted. Assuming that recruited follicles grow at 2 mm per day, 10,000 IU hCG was given when it was estimated that the patient would have two or more follicles >17 mm. Oocyte collection was then booked for the following Monday through Friday. The median numbers of follicles >10 mm on day 9 of superovulation and oocytes collected in these patients were six and nine, respectively. Ten percent of our population had one or no follicles >10 mm on day 9, and the corresponding value for oocytes was three. For the purpose of this study, "poor" responders were defined as patients whose cycles were canceled after buserelin/hMG stimulation because of an inadequate response (one or no follicles >10 mm on day 9 of superovulation) or in whom three or fewer oocytes were collected.

Two hundred sixty patients having 260 cycles were classified as poor responders according to our definition. Of these, 72 had no follicles >10 mm on day 9 of superovulation ("non"-responders) and had their cycles canceled. Of the remaining 188 poor responders, 29 (15.4%) decided to cancel their treatment, while 159 (84.6%) went on to oocyte collection. There were 19 (15 singletons and 4 twins) clinical pregnancies, giving a 10.1 and 11.9 % clinical pregnancy rate per cycle and per oocyte recovery, respectively. Seventeen (10.7%) delivered live babies, 14 singletons and 3 twins. One fetus from a set of twins vanished spontaneously. There were two miscarriages, at 10 and 12 weeks' gestation in a 38- and a 43-year-old woman, respectively. Of the remaining 243 (260 - 17) patients, 11 non- and 20 poor responders returned to have IVF using low-dose clomiphene citrate for ovulation induction. These patients took clomiphene citrate, 50 mg daily for 5 days, starting on day 1 of their cycle. Monitoring of the cycle was by endovaginal ultrasound scanning starting on day 10. Serial scanning was instituted as necessary until the leading follicle reached a

diameter of >16 mm. Prospective monitoring of E_2 or LH was not used to minimize costs and eliminate the need for repeated phlebotomy (5). hCG (5000 IU) was given intramuscularly, and transvaginal ultrasound-directed follicle aspiration performed 34 hr later.

All aspirations were performed under intravenous sedation with pethidine and midazolam. All follicles 10 mm in diameter or larger were aspirated. Semen preparation, insemination of oocyte(s), and embryo culture were performed using standard procedures. Fertilization status (number of pronuclei) was established by light microscopy 16 to 18 hrs after insemination. A maximum of three embryos was transferred when available 2 days after oocyte collection.

Normally distributed data were summarized using means and SD, while the median (range) was used for other continuous data. Categorical data were summarized by proportions. Comparisons between groups (such as patients who did and those who did not get pregnant) were performed with Student's *t* test for means and the Wilcoxon rank sum test for medians. Chi-square test and two-tailed Fisher's exact test were used for proportions. Statistical significance was set at $P < 0.05$.

RESULTS

Comparison of the response to stimulation in "poor" responders having their first clomiphene citrate cycles with the preceding buserelin/hMG cycles showed that significantly more follicles were recruited and more oocytes were collected, fertilized, and transferred in the controlled ovarian stimulation cycles. However, the implantation rate per embryo transfer, clinical pregnancy, and live-birth rate were similar in both groups Table I. All 72 non-responders had their controlled ovarian stimulation cycle canceled after having had a total [mean (SD)] of 26.9 (11.3) ampoules of hMG for 9.2 (0.8) days.

Eleven non- and 20 poor responders had a total of 30 and 53 clomiphene citrate-stimulated IVF treatment cycles, respectively. Patients who became pregnant did so within the first three treatment cycles, although a further two non-, and five poor responders had an additional two to eight and one to seven cycles, respectively. There were four successful pregnancies (7.5% per oocyte collection and 20% per patient) among poor responders (three singletons and one twin in four patients), while the only clinical pregnancy (3.3% per egg collection and 9.1% per patient) that occurred in the non-responders ended in a miscarriage at 8 weeks

Table I. Results of the First Clomiphene Citrate Cycle Compared to the Previous Buserelin/hMG Cycle in "Poor" Responders

	Clomiphene citrate (n = 20)	Buserelin/hMG (n = 188)
Days on hMG	—	10.2 (1.2)
Dose hMG (ampoules)	—	31.9 (13.9)
No. of follicles	2.1 (1.0) [2, 1–4]	4.5 (2.9) [4, 1–26] ^a
No. of oocytes	1.3 (1.0) [1, 0–4]	2.1 (0.9) [2, 0–3] ^b
No. of oocytes fertilized	0.9 (0.8) [1, 0–3]	1.3 (1.0) [1, 0–3] ^c
No. of embryos transferred	0.9 (0.8) [1, 0–3]	1.8 (0.8) [2, 0–3] ^a
Clinical pregnancies per OC	2/20 (10%)	19/159 (11.9%)
Implantation rate per ET	3/17 (17.6%)	23/197 (11.6%)
Live-birth rate per OC	2/20 (10%)	20/159 (12.6%)

Note. OC, oocyte collection. Values are the mean (SD) [median, range].

^a $P = 0.00001$.

^b $P = 0.0003$.

^c $P = 0.04$.

(Table II). Although the clinical pregnancy rate overall did not differ significantly between non- and poor responders, there was a trend toward a better implantation and clinical pregnancy rate in poor responders (Table III).

Among patients who had clomiphene citrate-stimulated IVF, those who became pregnant were further compared with those who did not, irrespective of whether they were non- or poor responders. Patients who became pregnant were significantly younger than those who did not, and although their mean basal FSH was higher (four nonpregnant and one pregnant patients did not have a baseline FSH done), this difference was not significant (Table IV). Although the median (range) number of follicles recruited per cycle

Table II. Pregnancy Rates in Clomiphene Citrate-Stimulated IVF

Attempts	Number of clinical pregnancies per patient	
	"Non"-responders	"Poor" responders
1	1/11 (9.1%)	2/20 (10%)
2	0/5	1/9 (11.1%)
3	0/4	1/8 (12.5%)
4	0/2	0/5
5	0/2	0/3
6	0/1	0/3
7	0/1	0/2
8	0/1	0/1
9	0/1	0/1
10	0/1	0/1
11	0/1	

Table III. Demographic Variables and Outcome in "Non"- and "Poor" Responders Undergoing IVF Using Clomiphene Citrate Stimulation

	Nonresponders (11 patients; 30 cycles)	Poor responders (20 patients; 53 cycles)
Patient's age (years) ^a	38.2 (1.99)	37.3 (4.2)
Infertility duration (years)	8.4 (4.8)	7.1 (4.6)
Day 3 FSH (IU/L) ^a	13.4 (5.4)	9.1 (3.5) ^b
Follicles aspirated	2 [1–3]	2 [1–4]
Oocytes collected (OC)	1 [0–3]	1 [0–4]
Oocytes collection rate	32/52 (61.5%)	57/102 (55.9%)
Oocytes fertilized	1 [1–3]	1 [1–3]
Fertilization rate	29/32 (90.6%)	36/57 (63.1%)
Embryo(s) transferred	1 [1–3]	1 [1–3]
Cumulative embryo score	4 [0–32]	6 [0–16]
Implantation rate per OC	1/29 (3.4%)	5/36 (13.9%)
Clinical pregnancy rate per OC	1/30 (3.3%)	4/53 (7.5%)

Note. Values are the median [range].

^a Values are the mean (SD).

^b $P = 0.02$.

did not differ, the median (range) numbers of oocyte(s) collected and fertilized and embryo(s) transferred were significantly higher in those who got pregnant compared to those who did not (Table V). Other parameters such as parity, gravidity, etiology, and duration of subfertility, number of previous attempts at IVF, and the size of the leading follicle were comparable in the two groups (Table IV).

DISCUSSION

The management of "non-" or "poor" responders poses a therapeutic challenge in assisted reproductive technology. The relatively low pregnancy rate associated with these groups of patients may be due to poor

Table IV. Demographic Variables in Pregnant and Nonpregnant Patients Using Clomiphene Citrate Stimulation for IVF

	Pregnant (n = 5)	Not pregnant (n = 26)
Age (years)	34.4 (4.1) ^a	38.2 (3.2) ^a
Partner's age (years)	36.4 (4.5)	38.8 (6.8)
Basal FSH (IU/L)	13.7 (3.8)	10.2 (4.8)
Duration of subfertility (years)	10.4 (5.7), 4–18	7.0 (4.3), 2–20

Note. Values are the mean (SD), range.

^a $P = 0.03$.

Table V. Response to Clomiphene Citrate Stimulation in Pregnant and Nonpregnant Cycles

	Pregnant (n = 5)	Not pregnant (n = 78)
Size of lead follicle (mm)	~18.2 (1.8)	~18.7 (3.0)
Follicles aspirated	2.2 (1.1) [2, 1-4]	1.8 (0.9) [2, 1-4]
Oocytes collected	1.8 (0.4) [2, 1-2]	1.0 (0.8) [1, 0-4] ^a
Oocytes fertilized	1.4 (0.5) [1, 1-2]	0.7 (0.8) [1, 0-3] ^b
Embryo(s) transferred	1.4 (0.5) [1, 1-2]	0.7 (0.7) [1, 0-3] ^c

Note. Values are the mean (SD) [median, range]. (~) Estimated follicular size on the day of hCG.

^a P = 0.008.

^b P = 0.04.

^c P = 0.03.

follicular recruitment, leading to fewer oocytes being collected (8), a lower fertilization (9), and slower cleavage rates (1). Several empirical treatments have been tried in these patients, including doubling the dose of hMG during the course of an IVF-ET treatment cycle (10); increasing the dose of hMG in subsequent cycle (1); adding of GnRH agonist in the long (11), short (3,12), or ultrashort (13) protocols; and using minidose GnRH agonist (14) and cotreatment with growth hormone (4,15). However, there is no agreement as to which protocol is better.

One of the difficulties with analyzing previous reports on poor responders is that different criteria are used to characterize the poor responder. In this study, we have defined poor responders as patients whose cycles were canceled after buserelin/hMG stimulation because of an inadequate response (one or no follicles >10 mm on day 9 of superovulation) or in whom three or fewer oocytes were collected at oocyte recovery. Those who recruited no follicle >10 mm on day 9 of superovulation and who had their cycle canceled were further categorized as nonresponders. These patients represent the lower 10th centile of our IVF population in terms of response to standard doses of hMG as judged by age, previous response, and baseline FSH levels.

Given that the optimal ovarian stimulation protocol for this group of patients remains unclear, one wonders whether the marginal benefit in the number of oocytes recovered and the pregnancy rates achieved in this group of patients, even with an increased dose of hMG (1), justifies the psychological, physical, and financial commitment involved. Assisted hatching may help (16), especially in a laboratory competent to perform the procedure. However, the potential for embryo damage at the time of the procedure and during embryo

transfer (17) is a cause for concern. Oocyte donation represents the most effective treatment option for non- and poor responders (18). Oocyte donors are, however, difficult to recruit, and while waiting for donor oocytes to become available, there is the need for a treatment that is cheap and simple and that offers little patient discomfort without disrupting lifestyle. This could be of immense benefit to poor responders provided that the clinical pregnancy rates are not significantly reduced.

We have shown that the clinical pregnancy rates per oocyte collection achieved with long protocol GnRHa/hMG in poor responders (11.9%), though slightly higher, is not significantly different from that achieved with clomiphene stimulation in non (9.1%)- and poor (10.0%) responders, respectively. This is despite the better response in the buserelin/hMG cycles in terms of follicles recruited, oocytes collected and fertilized, and embryo(s) transferred. These results, coupled with the lower but not significantly reduced implantation rate suggest a decrease in endometrial receptivity in the buserelin/hMG cycles.

There was no significant difference between the cycle cancellation rate in the buserelin/hMG (101/260; 38.8%) and that prior to the first clomiphene citrate-stimulated IVF (18/49; 37.6%) cycles. The 36.7% cycle cancellation rate with clomiphene citrate use in our patients, all of whom were non- or poor responders, is higher than the 15% reported by Steinkampf *et al.* (5) but comparable to the 35% reported by others (21). Our result is in agreement with Steinkampf *et al.* (5) that, with ultrasound monitoring alone, the cancellation rate was not increased with this protocol.

The clinical pregnancy rate overall did not differ significantly between non- and poor responders having ovulation induction with clomiphene citrate. However, there was a trend toward a better clinical pregnancy rate in poor responders. The two most important determinants of success were age and whether some response was noted in the previous buserelin/hMG stimulation cycle even though poor. Patients who got pregnant were <35 years (except one patient who was 41 years old), which is very similar to the findings of others (5,20). As with superovulated cycles, a greater number of oocytes were collected and fertilized with more embryos transferred in those cycles in which pregnancy occurred. Other demographic parameters did not appear to distinguish those poor responders who will and those who will not get pregnant.

Various authors have reported on their experience with natural-cycle IVF with (5,6) or without clomiphene (7,19-21). Lindheim *et al.* (7) said that poor

responders to ovarian hyperstimulation may benefit from an attempt at natural cycle oocyte retrieval. Although this may be true for non-responders, the experience of our unit suggests that poor responders may be offered up to three clomiphene citrate cycles before reverting to other forms of treatment.

We suggest that IVF in a clomiphene citrate cycle may reasonably be offered to poor responders versus non-responders for up to a maximum of three completed cycles before reverting to more stressful, often expensive, and time-consuming treatment or while waiting for oocyte donation.

ACKNOWLEDGMENTS

We acknowledge the contributions of the laboratory and scientific staff of the Midland Fertility Services. We are grateful to Anna Kavanagh, Peter Bromwich, and Andy Walker for their review of the manuscript.

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