Does the addition of clomiphene citrate or letrozole to gonadotropin treatment enhance the oocyte yield in poor responders undergoing IVF?

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Abstract

Purpose To evaluate the effect of adjunctive letrozole or clomiphene in IVF stimulation protocols.

Methods A retrospective analysis of high dose GnRH antagonist IVF cycles (450–600 IU of gonadotropins) that have met poor responder criteria. Selected consecutive cycles in same patients differed solely in presence or absence of adjunctive clomiphene or letrozole.

Results Supplementation with clomiphene citrate in poor responders showed significant improvements ($p < 0.05$) in estradiol levels (1506 vs. 1044 pg/ml), number of dominant follicles (5.6 vs. 3.9), oocytes retrieved (5.2 vs. 3.4) and number of transferred embryos (1.7 vs. 1.1). It significantly improved biochemical pregnancy rates (18.1% vs. 5.9%) while reducing cycle cancellations (11.7% vs. 32.6%). Letrozole supplementation showed similar effects.

Conclusion Both Clomiphene and Letrozole performed similarly and showed significant effects. However, despite increasing oocyte yield and embryo transfer rates, the

overall clinical and live birth rate in this population remained low and showed no measurable increase.

Keywords IVF · Poor responders · Clomiphene citrate · Aromatase inhibitors · Letrozole

Introduction

Women who receive high doses of gonadotropins for in vitro fertilization (IVF), and develop only a few follicles, or have their treatment cycles cancelled because of an inadequate response, have been labeled “poor responders.” Although the prognosis for these women is guarded, leading some to consider other options such as egg donation or adoption, various adjunctive measures have been suggested to improve the ovarian response to gonadotropins. Since there is little evidence to support gonadotropin doses over 450–600 IU/day [1], current approaches have included the addition of oral agents such as clomiphene citrate [2, 3] or letrozole [4–9], the administration of GnRH antagonists instead of agonists [10–12], microdose GnRH agonist “flare” [6, 13] and other agents such as growth hormone (GH) [14] and most recently the suggestion that DHEA may be helpful [15].

At our center we have generally used an antagonist protocol for poor responders, and have either prescribed maximal doses of gonadotropins (450–600 IU/day) alone or with the addition of either clomiphene citrate (CC) or letrozole (LZ). There are sparse data available using CC with high doses of gonadotropins. In initial reports, CC was used in combination with an antagonist, but with a focus on testing smaller and less frequent antagonist doses [3] or as an alternative in patients with exaggerated responses in the

Capsule Clomiphene Citrate and Letrozole significantly improve characteristics of antagonist IVF cycles in poor responders. The effect on clinical pregnancy outcomes could not be validated.

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agonist protocol [16]. CC was also used with gonadotropins in a “novel” antagonist protocol in poor responders in comparison with a standard long cycle agonist protocol, but the focus here was on delaying the start of the antagonist [2]. To our knowledge no study has addressed the efficacy of the addition of CC to high dose gonadotropins in poor responders. Despite the relatively recent introduction of LZ in the repertoire of agents for ovulation induction [17], LZ has been used increasingly for different types of ovulation induction and has gained acceptance for use in poor responders [4–9, 18]. A pilot study from 2005 showed that LZ supplemented antagonist cycles showed a significantly higher number of oocytes retrieved and increased implantation rates [5]. This was consistent with an earlier report that compared a high dose gonadotropin agonist protocol with low dose LZ with gonadotropin in a small number of poor responders and found cost saving effects with the use of LZ, but with no differences in cycle outcomes [7]. In another prospective trial, in direct comparison with antagonist gonadotropin cycles, the addition of LZ significantly reduced cycle cancellation rates due to poor responses and total gonadotropin utilization reduced overall costs [8]. A small meta-analysis however could not demonstrate the superiority of LZ addition over gonadotropins alone [19]. In comparison to other reports where different regimens including agonists have been used, we chose a design where each patient served as her own control, and patients underwent consecutive antagonist cycles with the same dose of gonadotropins with the only difference in addition of either CC or LZ.

Materials and methods

We screened 4859 IVF cycles between 2005 and 2009 at our infertility clinic to identify those patients undergoing IVF who had a poor response, defined as retrieval of 3 or less oocytes or cancellation in at least one of their stimulation cycles and stimulation with a high dose of gonadotropins (450 or 600 IU per day) in antagonist cycles. Other non-ovarian causes of cycle failure were excluded (i.e. medication error, voluntary cycle cancellations etc.).

Our final data analysis was further narrowed to patients who had undergone at least 2 different IVF cycles using the same gonadotropin dose (either 450 IU or 600 IU) but with one of the cycles utilizing CC or LZ as an adjunctive agent. We only utilized cycles within a 6 months' time period in order to minimize the effect of ovarian aging on outcome analysis.

Similar to a crossover study, each patient served as her own control for the different treatment regimens. If a patient had multiple cycles with various gonadotropin doses, only cycles at the same dose were compared to each other and used for analysis. If a patient had more than one cycle with an identical dose and same adjunctive medica-

tion (LZ or CC), the cycles were averaged into a “single cycle” that was then compared to the other treatment modality. Out of 519 antagonist cycles that were supplemented with either LZ ($n=189$) or CC ($n=330$) in our data set, 233 cycles from 85 patients met all the requirements for such comparisons. The average age and average day 2 FSH levels for the group were calculated for each comparison.

Endpoints for our assessment included estradiol levels on cycle day 2, day 5/6 and cycle maximum attained; endometrial stripe thickness, the number of dominant follicles on ultrasonography, the number of oocytes retrieved, fertilized and the number of embryos transferred. The outcomes evaluated were the cancellation rate, biochemical pregnancy rate, implantation rate, clinical pregnancy rate and live birth rate.

The patients were allowed to initiate an IVF cycle on cycle day 2 if serum FSH was less than 15 mIU/mL, estradiol levels were less than 70 pg/mL and ovarian ultrasound showed no significant follicular activity. Gonadotropin injections were initiated at doses 450–600 IU/day on day 2. The types of preparations used were 4–6 ampules/day of recombinant FSH and 2 ampules/day of highly purified menotropins. In the adjunctive CC or LZ cycles, CC 100 mg daily or LZ 5 mg daily was ingested on D2 and continued for 5 days. Ganarelix acetate 250 mcg per day was begun only when a lead follicle reached 13 mm or greater in diameter, and was continued until HCG administration. When at least 3 follicles reached a maximum diameter of 18 mm, ovulation was triggered with 10,000 IU of hCG and oocyte retrieval was performed 34–36 h later. Cycles were cancelled if there were less than 3 dominant follicles on ultrasonography. The vast majority of the patients underwent IVF with conventional insemination without ICSI. The few patients that underwent ICSI were only considered in our analysis if ICSI was utilized in the matched cycle as well. In this way all compared cycles of the same patient were either fertilized by conventional insemination or ICSI assuring that the method of fertilization did not account for differences in our comparisons.

An IRB exemption was requested and obtained following its review by the IRB committee at Columbia University for retrospective nonidentifying data analyses based upon our research aims and protocol. Outcomes were analysed by a paired *T*-Test in 3 subsections to seek differences among treatment modalities utilizing SPSS 13.0 statistical software.

Results

Addition of CC to high dose gonadotropins

A total of 125 cycles from 48 patients were analyzed in this comparison (Table 1). Estradiol levels on day 5/6 and

Table 1 A total of 233 cycles were identified for direct comparison. Three different comparisons among 3 different treatment protocols were evaluated: Gonadotropins alone (Gn) vs. Gonadotropins and Clomiphene Citrate (Gn+CC); Gonadotropins alone (Gn) vs. Gonadotropins and Letrozole (Gn+LZ) and lastly Gonadotropins and Clomiphene Citrate

(Gn+CC) vs. Gonadotropins and Letrozole (Gn+LZ). The table provides information about the number of patients in each comparison, average age, day 2 FSH levels and number of cycles utilized. The number of biochemical pregnancies, clinical pregnancies and live births are provided for each group

Comparison group	Patient group	# of Patients	Age	Day 2 FSH (mg/dl)	# of Cycles	Biochemical pregnancies	Clinical pregnancies	Live births
(Gn) vs. (Gn+CC)	(Gn)	48	41.2 ± 1.9	8.9 ± 2.3	55	4 (2.9) ^a	2	0
	(Gn+CC)				60	8 (7.0) ^a	5	2
(Gn) vs. (Gn+LZ)	(Gn)	15	41.1 ± 1.9	9.5 ± 2.9	15	5 (4.5) ^a	2	1
	(Gn+LZ)				18	4 (3.0) ^a	4 (3.0) ^a	1
(Gn+CC) vs. (Gn+LZ)	(Gn+CC)	29	42.1 ± 2.2	8.9 ± 1.3	40	2 (0.8) ^a	0	0
	(Gn+LZ)				45	4 (2.7) ^a	2 (1.3) ^a	1

All data presented as mean ± standard error of the mean

^a denotes the corrected average number of biochemical/clinical pregnancies in brackets in cases when a single patient had multiple cycles with different outcomes on the same treatment protocol

maximum cycle estradiol levels were significantly higher levels in the CC supplemented group (Gn+CC) compared to high dose gonadotropins alone (Gn) (413±30 pg/ml vs. 294±30 pg/ml and up to 1506±108 pg/ml vs. 1044±87 pg/ml, $p<0.01$; See Table 2). CC did not affect endometrial thickness and was comparable (9.5±0.3 mm vs. 9.9±0.3 mm) in the 2 groups. The number of dominant follicles on sonography (5.6±0.4 vs 3.9±0.4) and the number of retrieved follicles (5.2±0.5 vs. 3.4±0.5) was significantly higher ($p<0.01$) in the (Gn+CC) group compared to (Gn). There were not any statistically significant differences in the number of fertilized oocytes or fertilization rates. The number of transferred embryos was significantly higher in the (Gn+CC) group (1.7±0.2 vs. 1.1±0.2, $p<0.05$) as well as the biochemical pregnancy rate (18.1±5.2% vs. 5.9±3.1%, $p<0.05$). There were also significantly less cancellations in the CC supplemented group (Gn+CC) (11.7%±4.5% vs. 32.6%±6.8%, $p<0.05$). There were 2 clinical pregnancies in the (Gn) group and 5 clinical pregnancies in the (Gn+CC) group. This resulted in 2 live births in the (Gn+CC) group. Figure 1 depicts the cycle outcome with CC with and without Gn.

Addition of LZ to high dose gonadotropins

For the effect of addition of LZ to high dose Gonadotropins, we were able to identify 15 patients with 33 cycles for pair wise analysis (Table 1). There was no difference in starting day 2 estradiol levels, however levels on day 5/6 were significantly lower in the LZ supplemented (Gn+LZ) group (102±18 pg/ml vs. 269±58 pg/ml, $p<0.01$). The endometrial stripe thickness was not significantly different between the two groups (9.5±0.3 mm vs. 9.9±0.3 mm).

The number of dominant follicles on sonography appeared higher in the (Gn+LZ) group than when using high dose gonadotropins alone (Gn) (4.6±0.7 vs. 3.5±0.5) and the (Gn+LZ) group appeared to have higher numbers of oocytes retrieved (4.1±0.7 vs. 3.0±0.7), however these data did not reach statistical significance. There was no statistically significant difference in the number of oocytes fertilized, the fertilization rate or in the biochemical pregnancy rate. The biochemical pregnancy rate appeared unusually high (21.4±10.1%) in the (Gn+LZ) group as well as in the (Gn) group (30±11%) but showed no statistically significant difference. The cancellation rate, however, reached statistical significance and was reduced in the (Gn+LZ) group (16.7±9.3 vs. 43.3±12.7%, $p<0.05$). Only one live birth resulted in the (Gn+LZ) group.

High dose gonadotropins with CC vs. high dose gonadotropins with LZ

For the direct comparison of the adjuncts CC/Gonadotropins vs. LZ/Gonadotropins we identified 29 patients with a total of 85 cycles suitable for analysis (Table 1). The initial day 2 estradiol levels were not statistically different, however the estradiol levels on day 5/6 (333±33 vs. 84±11, $p<0.01$) and maximum estradiol levels (1045±102 vs. 736±94, $p<0.01$) were significantly higher in the CC group (Gn+CC) than in the LZ group (Gn+LZ) (Table 2). On ultrasonography both groups showed similar endometrial thickness (8.2±0.4 mm vs. 8.2±0.4 mm) and the number of dominant follicles trended higher in the (Gn+LZ) group, however was not statistically significant. The LZ supplemented group (Gn+LZ) had a statistically significant increase in number of oocytes retrieved (4.0±0.5

Table 2 A detailed analysis of cycles provides cycle estradiol levels, endometrial stripe thickness and number of oocytes retrieved and fertilized. For each group the fertilization, biochemical pregnancy and cancellation rate was calculated. A p-value was calculated using pair wise analysis utilizing the advantage that the cycles compared originated from the same patients

Patient group	Estradiol levels (pg/ml)				Ultrasoundography			Outcomes				
	Day 2	Day 5/6	Maximum	Endometrial stripe (mm)	# of dominant follicles	# of retrieved oocytes	# of fertilized oocytes	# of transferred embryos	Fertilization rate (%)	Biochemical pregnancy rate (%)	Cancellation rate (%)	
(Gn)	32 ± 1.6	294 ± 30	1044 ± 87	9.9 ± 0.3	3.9 ± 0.4	3.4 ± 0.5	2.4 ± 0.2	1.1 ± 0.2	54 ± 6	5.9 ± 3.1	32.6 ± 6.8	
(Gn+CC)	33 ± 1.8	413 ± 30	1506 ± 108	9.5 ± 0.3	5.6 ± 0.4	5.2 ± 0.5	2.8 ± 0.3	1.7 ± 0.2	51 ± 4	18.1 ± 5.2	11.7 ± 4.5	
Sig.	ns	$p<0.01$	$p<0.01$	ns	$p<0.01$	$p<0.01$	ns	$p<0.05$	ns	$p<0.05$	$p<0.05$	
(Gn)	34 ± 3.7	269 ± 58	961 ± 161	9.8 ± 0.4	3.5 ± 0.5	3.0 ± 0.7	3.1 ± 0.5	0.8 ± 0.3	66 ± 10	30 ± 11	43.3 ± 12.7	
(Gn+LZ)	43 ± 4.3	102 ± 18	685 ± 107	9.0 ± 0.4	4.6 ± 0.7	4.1 ± 0.7	2.8 ± 0.5	1.7 ± 0.4	56 ± 7	21.4 ± 10.1	16.7 ± 9.3	
Sig.	ns	$p<0.01$	ns	ns	ns	ns	ns	ns	ns	ns	$p<0.05$	
(Gn+CC)	37 ± 3	333 ± 33	1045 ± 102	8.2 ± 0.4	3.5 ± 0.3	2.5 ± 0.4	2.0 ± 0.3	1.0 ± 0.2	57.6 ± 8.1	2.8 ± 2.0	39.8 ± 8.5	
(Gn+LZ)	40 ± 3	84 ± 11	736 ± 94	8.9 ± 0.4	4.2 ± 0.4	4.0 ± 0.5	2.8 ± 0.3	1.5 ± 0.2	64.4 ± 6.1	9.7 ± 4.9	24.8 ± 7.6	
Sig.	ns	$p<0.01$	$p<0.01$	ns	ns	$p<0.01$	ns	ns	ns	ns	ns	

All data presented as mean ± standard error of the mean; ns non significant; for group description see Table 1

Fig. 1 Cycle outcome data from the gonadotropin only (Gn) group compared with the gonadotropin plus clomiphene supplemented (Gn + CC) group. Presented are fertilization (Fert), biochemical pregnancy (Preg), implantation (Impl. Rate) and cancellation (cancellation) rates. * sig. at $p<0.05$; ** sig. at $p<0.01$

vs. 2.5 ± 0.4 , $p<0.01$) but showed no statistically significant difference in number of fertilized oocytes or in the fertilization rate. The number of transferred embryos trended higher in the (Gn+LZ) group but did not reach statistical significance. The biochemical pregnancy rate was low in both groups without a statistical difference ($9.7 \pm 4.9\%$ in the Gn+LZ group and $2.8 \pm 2.0\%$ in the Gn+CC group). The cancellation rate was not statistically different in the 2 groups ($24.8 \pm 7.6\%$ vs. $39.8 \pm 8.5\%$). There was only one live birth resulting from Gn+LZ.

Overall pregnancy rates

In a total of 233 initiated IVF cycles included in our analysis, there were 27 biochemical and 14 clinical pregnancies that corresponded to a biochemical pregnancy rate of 11.6% and clinical pregnancy rate of 6%. The live birth rate comprising 5 births was 2.1%. The data for clinical pregnancy and live births for different treatment modalities in these poor responders was too low to allow for meaningful statistical analysis.

Discussion

While most clinicians have abandoned the more routine “long” GnRH agonist protocol in this setting, the agonist flare protocol is preferred by some, but may be similar to using the antagonist protocol [20]. As stated earlier, in our practice we have routinely used an antagonist protocol in poor responders. However, we have also used oral adjuncts, namely CC and LZ, but without evidence for additional efficacy. Accordingly we carried out this step wise paired

analysis of our own data to provide guidance for clinical management and to plan more rigorous trials in the future.

An obvious weakness in this study is its retrospective nature, which builds in unavoidable biases. However, we have carefully screened a large cycle database to identify only those cycles that could be appropriately matched for control data, and according to inclusion and exclusion criteria we set up, to provide statistical guidance as to the efficacy of the adjunctive use of CC or LZ using pair wise comparisons. Although the selection of an adjunct in our practice has been relatively arbitrary, CC has been clearly favored, which explains the higher number of patients and cycles using this comparison.

The strength of this study is that we were able to use the same patients in both treatment and control groups and therefore show significant differences in these patient groups. By this design we were able to make a pair wise comparison and the effect of the adjunctive CC or LZ could be evaluated in subsequent cycles in the same patient. It is important to note again that in this selection process of cases for matching, different patients were included in the 3 different comparisons. An example would be a patient who had only undergone CC/gonadotropins and LZ/gonadotropins cycles could not be included in the comparisons of CC/gonadotropins versus gonadotropins alone. This explains the discrepancy in baseline data among patients treated with the same medication across the 3 different comparisons groups.

Our most robust data was with the adjunctive use of CC, where we had a greater number of treatment cycles. The response with CC/gonadotropins was clearly superior to that of gonadotropins alone, looking at several endpoints as noted above: higher estradiol, a significantly higher number of retrieved oocytes, a higher number of embryos transferred, and a higher number of pregnancies. The cancellation rate was also significantly lower. These data are unique in that these positive findings have not been studied previously in poor responders using antagonist cycles to our knowledge.

The evidence for LZ in comparison to high dose gonadotropins alone lacks some rigor in that there were fewer cycles. We were able to show that cancellation rates in the LZ group were also significantly lower than with high dose gonadotropins alone. This is consistent with several other reports suggesting the efficacy of adjunctive LZ in poor responders [21].

The direct comparisons of CC/gonadotropins and LZ/gonadotropins slightly favored the addition of LZ in that more oocytes were obtained (Table 2), but the data does not allow us to suggest that LZ is superior to CC as an adjunct.

In conclusion, we were able to confirm the beneficial effect of using LZ and provide new evidence for using CC as and adjunctive to high dose gonadotropins in antagonist

cycles of poor responders undergoing IVF. Although the added benefit is not marked, the effect is clinically measurable, leading to less cycle cancellations and potentially an increased rate of pregnancy. Nevertheless, the effect on overall live birth rates is not improved according to the data at hand. It is our hope and expectation that in the future well designed prospective randomized trials will be able to confirm our findings and perhaps help to determine the superiority of one adjunct over another.

Conflict of Interest None to disclose

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