ORIGINAL ARTICLE: ASSISTED REPRODUCTION

A novel "delayed start" protocol with gonadotropin-releasing hormone antagonist improves outcomes in poor responders

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Objective: To investigate whether delaying the start of ovarian stimulation with GnRH antagonist improves ovarian response in poor responders.
Design: Retrospective study.
Setting: Academic medical center.
Patient(s): Thirty patients, who responded poorly and did not get pregnant with conventional estrogen priming antagonist IVF protocol.
Intervention(s): Delayed-start antagonist protocol (estrogen priming followed by early follicular-phase GnRH antagonist treatment for 7 days before ovarian stimulation).
Main Outcome Measure(s): Number of dominant follicles and mature oocytes retrieved, mature oocyte yield, and fertilization rate.
Result(s): The number of patients who met the criteria to proceed to oocyte retrieval was significantly higher in the delayed-start protocol [21/30 (70%)] compared with the previous conventional estrogen priming antagonist cycle [11/30 (36.7%)]. The number of dominant follicles was significantly higher in the delayed-start (4.2 ± 2.7) compared with conventional (2.4 ± 1.3) protocol. In patients who had oocyte retrieval after both protocols (n = 9), the delayed start resulted in shorter ovarian stimulation (9.4 ± 1.4 days vs. 11.1 ± 2.0 days), higher number of mature oocytes retrieved (4.9 ± 2.0 vs. 2.2 ± 1.1), and a trend toward increased fertilization rates with intracytoplasmic sperm injection (ICSI; 86 ± 17% vs. 69 ± 21%) compared with conventional protocol. After delayed start, the average number of embryos transferred was 2.8 ± 1.4 with implantation rate of 9.8% and clinical pregnancy rate of 23.8%.
Conclusion(s): The delayed-start protocol improves ovarian response in poor responders by promoting and synchronizing follicle development without impairing oocyte developmental competence. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Delayed start, poor responder, controlled ovarian stimulation

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The goal of controlled ovarian stimulation (COS) in in vitro fertilization (IVF) cycles is to recruit multiple follicles in an effort to compensate for the inefficiencies of embryo culture systems and to increase the chance of creating euploid embryos and subsequent viable pregnancies (1). The prevalence of poor responders varies from 5.6% to 35.1% depending on the definition of poor response (2, 3). Regardless of the definition, a poor response to COS potentially results in high cancellation rates, reduced numbers of oocytes retrieved, decreased numbers of embryos available for transfer, and lower pregnancy rates compared with normal responders (3–5). Treatment of this common condition remains a major challenge in assisted reproduction technology. Although many protocols have been proposed to increase ovarian response, there is presently insufficient evidence to support the routine use of any particular intervention either for pituitary down-regulation or for ovarian

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stimulation or adjuvant therapy in the management of poor responders (6).

Various factors, including decreased ovarian reserve, have been associated with a poor response. However, alterations in intraovarian factors or gonadotropin receptor regulation could also contribute to suboptimal response (7, 8). Additionally, poor responses may result in part from a shortened follicular phase with limited ability to recruit a sizable cohort, or from differential sensitivity of early antral follicles to FSH (9, 10).

The mechanisms underlying the heterogeneity of antral follicle responsiveness to gonadotropins during the early follicular phase remain unclear. A possible explanation for this phenomenon involves follicles being at different developmental stages with various FSH receptor levels due to recruitment of these follicles at different time points. Another major reason for the variable response to COS is interference due to the actions of endogenous gonadotropins. During the last days of the menstrual cycle, paralleling the breakdown of the corpus luteum, FSH concentration increases progressively to preserve antral follicles from atresia and ensure their subsequent growth (11). Depending on their inherent sensitivity to FSH, it is possible that some antral follicles are able to respond to the lower amounts of FSH better than others, and therefore to start their development during the late luteal phase, accentuating size discrepancies observed during the first days of the subsequent cycle and leading to asynchronous growth with COS (12).

COS protocols for poor responders are designed to minimize early follicle selection in the luteal phase and optimize the follicular hormonal milieu and antral follicle responsiveness. One of the reasons behind using GnRH agonist or birth control pills in the late luteal phase is to suppress FSH rise and subsequent premature dominant follicle selection. However, for poor responders, down-regulation protocol with GnRH agonist or birth control pills before the antagonist protocol may cause oversuppression of ovarian function, leading to low oocyte yield (13). As a result, incorporating E_2 pretreatment to the GnRH antagonist protocol gained attention to lower endogenous luteal FSH secretion without suppressing the ovarian response. In earlier studies, E_2 pretreatment was shown to improve follicle synchronization, and eventually resulted in more coordinated follicular development, leading to the recovery of more mature oocytes (14, 15). However, a substantial number of patients still suffer from asynchronous follicle growth with this protocol, likely owing to higher early follicular-phase FSH levels compared with down-regulated protocols (16, 17).

In the present study, we hypothesized that by delaying the start of COS with GnRH antagonist pretreatment for 7 days after estrogen priming, there would be further suppression of endogenous FSH during the early follicular phase, resulting in more FSH-responsive follicles and thus improving synchronous follicular development. To test this hypothesis, we compared the COS outcomes of delayed-start antagonist protocol with the same poor responder patient's previous failed conventional estrogen priming antagonist cycle.

MATERIALS AND METHODS

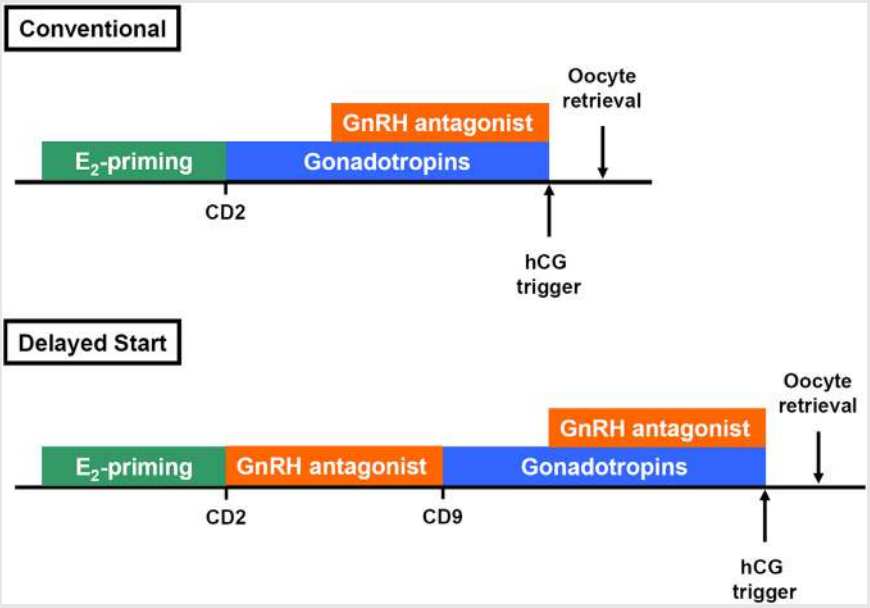
Study Population

This study received Institutional Board Review approval from the Committee for Human Research at the University of California, San Francisco (UCSF). The patients selected for inclusion were identified after review of all estrogen-priming GnRH antagonist IVF cycles performed at the UCSF Center for Reproductive Health from June 2011 to April 2013. Patients who met the Bologna poor responder criteria (18) and had an unsuccessful estrogen-priming GnRH antagonist IVF cycle (conventional) followed by a delayed-start protocol were included to the study for analysis. For patients with more than one such IVF cycles, only the first delayed-start antagonist protocol and the last conventional cycle, preceding the delayed-start protocol, were included for analysis to avoid repeated-measures bias.

Ovarian Stimulation Protocols

Before both conventional and delayed-start protocols, all patients received estrogen priming (E_2 patch or tablet) starting a week after LH surge until menses. Baseline ultrasounds on cycle day 2 and after the completion of GnRH antagonist pretreatment in the delayed-start protocol were performed to document absence of ovarian cyst or lead follicle >10 mm. In conventional protocol (standard antagonist protocol), ovarian stimulation with gonadotropins was started on cycle day 2 of menstrual cycle. In the delayed-start protocol, ovarian stimulation was started after 7 days of GnRH antagonist pretreatment (0.25 mg ganirelix acetate; Organon; Fig. 1). In both protocols, 300 IU FSH (Follistim; Merck; or Gonal-F; EMD-Serono) and 150 IU hMG (Menopur; Ferring) were used for ovarian stimulation. The patients used the same FSH preparation (Follistim or Gonal-F) in both conventional and delayed-start protocols. The gonadotropin doses were maintained fixed throughout the whole stimulation period. GnRH antagonist (0.25 mg ganirelix acetate; Organon) was added to prevent premature ovulation when the lead follicle measured ≥ 12 mm and was continued until the hCG trigger. Final oocyte maturation was triggered with 10,000 IU hCG (Pregnyl; Schering Plough) when the largest two follicles attained a mean diameter of 18 mm with a general cohort of follicles >13 mm. Patients were allowed to proceed to oocyte retrieval if three or more follicles were in the dominant range (≥ 13 mm in diameter). In case of fewer than three dominant follicles, the cycle was canceled and intrauterine insemination was performed. If there were three or more dominant follicles, oocyte retrieval was performed under transvaginal ultrasound guidance 36 hours after hCG administration. After stripping the cumulus cells, intracytoplasmic sperm injection (ICSI) was performed with ejaculated sperm to mature (metaphase II [MII]) oocytes in all cycles. None of the male partners had any history of infertility. ICSI was performed in all cases to prevent infrequent cases of fertilization failures with conventional IVF. All of the embryos were transferred after fertilization day 2 or 3 owing to the limited numbers of embryos.

FIGURE 1



Outline of conventional estrogen priming and delayed start antagonist controlled ovarian stimulation protocols. CD = cycle day.

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Outcome Measures

The main outcome measure was the number of mature (MII) oocytes collected after conventional versus delayed-start ovarian stimulation protocol. Secondary outcome measures included the number of dominant follicles (≥13 mm) on the day of hCG trigger, total number of oocytes retrieved, oocyte maturity rate (number of MII oocytes/total number of oocytes), oocyte yield (total number of oocytes retrieved/antral follicle count [AFC]), mature oocyte yield (number of mature oocytes retrieved/AFC), total dosage of gonadotropin (recombinant FSH and/or highly purified hMG) needed, number of days needed for ovarian stimulation, and fertilization rate (percentage of two-pronuclear [2PN] stage zygotes ~16 hours after ICSI treatment). Because the conventional-start cycle did not result in pregnancy by design, we were unable to compare pregnancy outcomes. However, for descriptive purposes the implantation rate was defined by the number of gestational sacs seen by transvaginal ultrasound divided by the number of embryos transferred. Clinical pregnancy rate was defined as presence of fetal heart motion by transvaginal ultrasound per embryo transfer.

Serum Assays

Serum E_2 assay was calibrated to known standards and validated by serial dilution. E_2 was quantified in batch and duplicate and was measured with commercially available automated chemiluminescent immunoassays on a DPC-Immulite 1000 (Diagnostic Products). Each test was run with three control subjects of low, medium, and high concentrations. Dilutions were performed before measurements of E_2 (1:1,000) depending on the calibration range. The intra-assay coefficient of variation for E_2 was 11.9%. High and low results were repeated with appropriate dilution.

Statistical Analysis

The statistical analyses for parametric data were performed with the use of paired *t* test. Nonparametric data were analyzed with the use of McNemar test with Yates correction for continuity. Stata 12.1 software (Statacorp) was used for analysis. Statistical significance was defined as $P<.05$.

RESULTS

Baseline characteristics of the patients included in the study are presented in Table 1. All of the study patients had an unsuccessful estrogen-priming GnRH antagonist IVF cycle (conventional) followed by a delayed-start protocol. The median time period between the two COS cycles was 4 months (range 2–12 months). The number of patients who met the criteria to proceed to oocyte retrieval (three or more follicles ≥13 mm in diameter) were significantly higher in the delayed-start protocol [21/30 (70%)] than in the previous conventional estrogen priming antagonist cycle [11/30 (36.7%); $P=.016$; Fig. 2]. Twelve patients who failed COS with previous conventional protocol owing to poor response and had rescue intrauterine insemination, met the criteria to proceed to oocyte retrieval with subsequent delayed-start protocol. In contrast, only two patients met the criteria for oocyte retrieval after conventional antagonist cycle but not with delayed-start protocol.

The number of dominant follicles (≥13 mm) was significantly higher in delayed-start COS (mean 4.2 ± 2.7) when compared with conventional protocol (2.4 ± 1.3;

TABLE 1

Demographics and baseline characteristics of patients (n = 30) who underwent an estrogen priming antagonist controlled ovarian stimulation (COS) cycle followed by the delayed-start protocol.

Age (y)	41.5 (34–44)
Body mass index (kg/m^2)	22.6 (18.9–29.0)
Ethnicity	
White	20 (66.7%)
Asian	10 (33.3%)
Previous pregnancy	15 (50%)
Previous live birth	5 (16.7%)
Antral follicle count	6 (3–11)
Length of infertility (mo)	23.5 (8–60)
Previous IVF cycle	9 (30%)
Number of previous IVF cycles	1 (1–2)
Time between two COS cycles (mo)	4 (2–12)

Note: Values are presented as median (range) or n (%).

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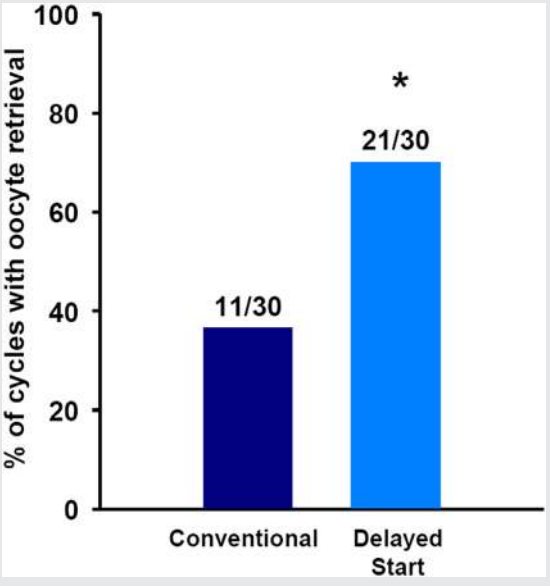
between-group difference 1.87, 95% confidence interval [CI] 1.01–2.72; $P<.001$). Twenty-one patients had higher, five had the same, and four had lower numbers of dominant follicles in delayed-start COS compared with conventional protocol. The patients who had fewer dominant follicles with delayed start had significantly lower AFC (3.25 ± 0.5) compared with the rest of the patients (6.5 ± 2.0; $P=.003$). Delaying ovarian stimulation with GnRH antagonist resulted in increased serum E_2 levels on ovarian stimulation day 6 (561 ± 286 vs. 212 ± 147 pg/mL), day 8 (903 ± 409 vs. 479 ± 280 pg/mL), and the day of hCG trigger (1,589 ± 488 vs. 1,175 ± 466 pg/mL) compared with conventional estrogen priming antagonist protocol.

FIGURE 2



Comparison of percentage of cycles meeting the criteria to proceed to oocyte retrieval in conventional and delayed-start controlled ovarian stimulation cycles. *$P=.016$.

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Because only nine patients met the criteria to proceed to oocyte retrieval in both conventional and delayed-start protocols, the rest of the comparative analysis was performed in only those nine patients. In delayed-start antagonist protocol, shorter ovarian stimulation duration (9.4 ± 1.4 vs. 11.1 ± 2.0 days), lower total gonadotropin dose (4,250 ± 641 vs. 5,000 ± 884 IU), higher number of total (6.6 ± 2.6 vs. 4.3 ± 1.8) and mature (MII) (4.9 ± 2.0 vs. 2.2 ± 1.1) oocytes retrieved, higher oocyte maturity rate (MII/total oocytes) and mature oocyte yield (MII oocytes/AFC), with more day 2 or 3 viable embryos transferred (3.4 ± 1.6 vs. 1.6 ± 1.2), were observed compared with the preceding conventional estrogen priming antagonist COS cycle (Table 2). Although it was not statistically significant, there was a trend for higher fertilization rate in delayed-start cycles (Table 2).

When only the delayed-start COS cycles that resulted in oocyte retrieval (n = 21) were evaluated, the following results were obtained. The length of ovarian stimulation was 8.7 ± 1.4 days and total dose gonadotropins used was 3,696 ± 728 IU. On the day of hCG trigger, there were 5.5 ± 2.3 follicles ≥ 13 mm in diameter and serum E_2 level was 1,430 ± 505 pg/mL. An average of 6.1 ± 2.8 oocytes were retrieved, and 4.9 ± 2.2 of them showed nuclear maturity (MII). Oocyte maturity rate and mature oocyte yield were 0.82 ± 0.14 and 0.77 ± 0.31, respectively. Fertilization rate was 0.82 ± 0.20. Fifteen patients had day 2 embryo transfers, and six had day 3 embryo transfers. The average number of embryos transferred was 2.8 ± 1.4. The implantation and clinical pregnancy rates were 9.8% and 23.8% (5/21), respectively.

DISCUSSION

In poor responders, an increase in the number of oocytes and embryos is a critical aspect of a successful cycle, given the generally diminished oocyte quality in these patients (19). In the present study, we describe a novel protocol incorporating estrogen treatment in the preceding luteal phase and an immediate and short pituitary shutdown with GnRH antagonist in the early follicular phase, followed by COS. We demonstrated that pretreatment with GnRH antagonists for 7 consecutive days before the onset of ovarian stimulation resulted in more synchronous follicle growth, higher mature oocyte yield, and more embryos to transfer compared with conventional estrogen priming GnRH antagonist protocol. The results also showed that a significant number of women who had failed COS owing to poor response met the criteria to proceed to oocyte retrieval with this subsequent delayed-start protocol.

To date, few studies have evaluated whether a delayed start to ovarian stimulation improves COS outcomes. A recent randomized controlled trial among "normal" responders showed that early follicular phase GnRH antagonist pretreatment for 3 days resulted in a trend toward a higher number of retrieved oocytes but failed to yield significantly higher pregnancy rates (20). In another clinical trial performed among women with normal ovarian reserve, 3-day GnRH antagonist pretreatment before COS in an antagonist protocol improved oocyte maturity and fertilization rates, but did not change the pregnancy rates (21). In poor responders, delaying the ovarian

TABLE 2

Comparison of characteristics and outcomes of conventional and delayed-start controlled ovarian stimulation (COS) cycles.

	Conventional start (n = 9)	Delayed start (n = 9)	Between-group difference (95% CI)	*P* value
Days of ovarian stimulation	11.1 ± 2.0	9.4 ± 1.4	−1.7 (−3.1 to −0.3)	.024
Total dose of gonadotropins (IU)	5,000 ± 884	4,250 ± 641	−750 (−1,374 to −126)	.024
Endometrial thickness (mm)	9.5 ± 2.2	10.9 ± 2.5	1.4 (−0.2–3.1)	.082
Follicles ≥13 mm	3.9 ± 1.3	6.7 ± 2.2	2.8 (1.3–4.3)	.002
Oocytes retrieved	4.3 ± 1.8	6.6 ± 2.6	2.3 (0.13–4.3)	.04
MII oocytes retrieved	2.2 ± 1.1	4.9 ± 2.0	2.7 (1.1–4.2)	.004
MII/total oocytes ratio	0.53 ± 0.20	0.73 ± 0.10	0.20 (0.01–0.4)	.041
Oocytes/AFC ratio	0.76 ± 0.36	1.13 ± 0.21	0.37 (0.06–0.68)	.024
MII oocytes/AFC ratio	0.38 ± 0.19	0.86 ± 0.21	0.48 (0.24–0.72)	.003
Fertilization rate after ICSI (2PN/MII)	0.69 ± 0.21	0.86 ± 0.17	0.17 (−0.10–0.44)	.17
Day of transfer				
Day 2	8 (100%)[a]	6 (67%)		
Day 3	0	3 (33%)		
Embryos transferred	1.6 ± 1.2	3.4 ± 1.6	1.8 (0.5–3.0)	.013
Implantation rate	0	6.5%		
Clinical pregnancy rate	0	2 (22.2%)		

Note: 2PN = two pronuclei; AFC = antral follicle count; ICSI = intracytoplasmic sperm injection; MII = metaphase II.
[a] One patient did not have any viable oocytes at the time of retrieval.

Cakmak. Delayed-start ovarian stimulation. Fertil Steril 2014.

stimulation with a GnRH antagonist protocol was shown to improve numbers of oocytes retrieved and embryos transferred (22). In that proposed protocol, 8-day GnRH antagonist pretreatment was started on cycle day 5–8 and two doses of GnRH antagonist (3 mg cetrorelix acetate) 4 days apart with daily progestin was used to lengthen the oocyte recruitment interval (22). In our study, the length of GnRH antagonist pretreatment was similar (7-day course of 250 μg ganirelix daily). However, we initiated GnRH antagonist much earlier (on cycle day 2, before the dominant follicle was selected) after estrogen priming to suppress early follicular phase FSH rise.

In ovarian stimulation, follicles are required to grow coordinately in response to exogenous gonadotropins to accomplish simultaneous maturation. Marked follicular size discrepancies result in decreased odds of oocyte maturation and fertilization potential, which can limit the number of embryos created and the probability of conception (23). These follicular discrepancies may be more common in those with decreased ovarian reserve.

It has been postulated that suppression of endogenous FSH in early follicular phase results in improved follicular development (24). In a GnRH antagonist protocol, compared with a GnRH agonist down-regulation protocol, higher serum gonadotropin concentrations are found at the beginning of ovarian stimulation (16, 17). As a result, the unsuppressed FSH level at the start of a GnRH antagonist cycle allows the initial growth of a few leading follicles before the addition of exogenous FSH. The GnRH antagonist protocol with estrogen, birth control pills, and GnRH antagonist pretreatment in the preceding luteal phase offers simple alternatives to achieve endogenous gonadotropin suppression during the early follicular phase (15, 25, 26). In addition, GnRH antagonist administration in early follicular phase, resulting in a delayed start, also results in rapid and reversible suppression of FSH, which may contribute to the improvement in follicular development (21).

The heterogeneity in follicular growth may be due to differences in the follicular sensitivity to FSH within the cohort (12). There are two possible explanations of heterogeneous FSH responsiveness of the follicles in early follicular phase. First, FSH rise in the late luteal phase may cause premature dominant follicle selection, which can be partially prevented by estrogen priming in antagonist cycle. Recent studies indicate multiple waves of follicle recruitment within a single interovulatory period (27–29). Another explanation is that recruitment of the follicles at different time points may result in follicles at different developmental stages with various FSH receptor levels. We hypothesize that GnRH antagonist pretreatment in the early follicular phase before COS may temporarily halt follicular growth by suppressing the endogenous FSH and may provide a hormonal milieu for the follicles to express similar amounts of FSH receptors and consequently to respond to gonadotropins with synchronous growth.

The limitations of this study, as with most published trials of stimulation protocols for poor responders, are its retrospective nature and small sample size. Our best measure to judge the efficacy of the delayed start protocol was the historical control of the patients' previous estrogen priming antagonist cycle. Moreover, only the patients who failed in their estrogen priming antagonist protocol underwent delayed-start protocol, which results in selection bias. Nevertheless, because the daily doses of gonadotropins were fixed for the entire stimulation period, the differences observed between the estrogen levels and the follicular development of the two protocols compared are not biased. In addition, E_2 levels were not considered in deciding on hCG trigger administration. As a result, duration of stimulation reflects only follicular development characteristics.

Because each patient had a failed conventional estrogen priming antagonist cycle followed by the delayed-start antagonist protocol, it is possible that the initial poor response in the conventional antagonist cycle was idiosyncratic and

that the improved response on the study regimen was caused by selection bias. Although subsequent improvement in response to the same stimulation has been observed on an individual basis, it was previously demonstrated that when patients repeated the same ovarian stimulation strategy in consecutive cycles, no significant differences in COS outcomes were noted between the two cycles, suggesting internal consistency in response from cycle to cycle (30, 31). Other data showed that a change in protocol for the second cycle may affect outcomes in a positive or negative way regarding oocyte recovery and total number of mature oocytes/embryos (30, 32, 33). In our study, nine patients had more than one COS cycle before the delayed-start protocol, which all resulted in similar poor response. Therefore, we think that significant improvement in COS outcomes with this delayed-start protocol are not likely to be based solely on an idiosyncratic poor response.

In summary, the use of the delayed-start protocol appears to improve ovarian responsiveness during COS and may result in more uniform follicular development, more mature oocytes retrieved, transfer of higher numbers of embryos, and possibly improved pregnancy rates compared with a previous estrogen priming antagonist cycle in poor responders. Although this treatment protocol is longer and the total cost is higher, it gives new hope to poor-responder cases. Ultimately, however, prospective randomized controlled trials will be necessary to determine whether delayed-start protocol is superior compared with other protocols for poor responders.

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