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Minimal Stimulation Using Letrozole in Poor Responders

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Abstract

Background: Poor responders refer to women who undergo ovarian stimulation with suboptimal response and an optimal treatment for them has yet to be proven. Minimal stimulation which uses lower doses of gonadotropins than standard long protocols had been shown to yield fewer but higher quality embryos. Minimal stimulation had thus been proposed as an option for poor responders who do not benefit from longer and higher cost regimens. The aim of this retrospective study was to evaluate outcomes of poor responders who underwent minimal stimulation using letrozole and whether they are comparable to outcomes of conventional *in vitro* fertilization (IVF) in a tertiary center in Singapore.

Methods: This was a retrospective study of 43 poor responders who underwent minimal stimulation protocol in KK Women's and Children's Hospital IVF center between March 2011 and March 2014. The protocol involved sequential administration of letrozole followed by low-dose gonadotropins and a gonadotropin-releasing hormone (GnRH) antagonist. Primary outcomes included clinical pregnancy rate and live birth rate per patient. A subgroup analysis was done for women undergoing minimal stimulation protocol and having failed conventional IVF protocol in which their outcomes were compared.

Results: The average age at time of stimulation was 38.6 years old. Clinical pregnancies were achieved in nine women (20.9%), out of which eight (18.6%) were live births. In the subgroup analysis, cleavage rate obtained in the minimal stimulation protocol was significantly higher at 61.3% compared to the cleavage rate obtained in the conventional IVF cycle at 28.7%. There were no significant differences in the pregnancy and live birth rates of the two cycles.

Conclusion: Minimal stimulation protocol using letrozole produces a reasonable live birth rate in poor responders and potentially higher quality oocytes than conventional IVF protocol.

Keywords: Poor responder; Clomiphene citrate; *In vitro* fertilization; Letrozole; Minimal ovarian stimulation

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Introduction

Poor responders refer to the group of women who undergo ovarian stimulation with suboptimal response, which can be measured as the number of oocytes retrieved or pregnancy rates. Women with poor ovarian reserve may also be identified as poor responders. The incidence of poor response varies from 9% to 25% of patients undergoing assisted reproductive treatments [1]. Various treatment approaches have been proposed to improve ovarian response to controlled ovarian stimulation in poor responders but none has been adequately proven to be the optimal treatment for them [2]. Poor responders often have higher cancellation rates and are subject to longer and more expensive cycles. Besides financial burden from reduced working days and high treatment costs, they also face emotional burden from repeated failed cycles. Minimal stimulation, using low-dose gonadotropins or human menopausal gonadotropin in a gonadotropin-releasing hormone (GnRH) antagonist co-treatment cycle with or without usage of oral anti-estrogenic agents, has been proposed as a treatment for poor responders. High doses of gonadotropins have been observed to have detrimental effects on oocyte and embryo quality. Minimal stimulation, while yielding fewer oocytes, might improve embryo quality [3]. A randomized trial by Youssef et al in 2017 compared 195 poor responders who received minimal stimulation protocol to 199 poor responders who received conventional long agonist protocol with high-dose gonadotropins found no significant differences in ongoing pregnancy rates [4]. The duration of ovarian stimulation and the amount of gonadotropins used were significantly lower in the minimal stimulation protocol than in the conventional long agonist protocol. Studies comparing minimal stimulation with clomiphene citrate to high-dose gonadotropins stimulation protocol have also reported similar clinical pregnancy rates amongst the two groups [5, 6].

Aromatase inhibitors were introduced as a safe stimulation method for *in vitro* fertilization (IVF) in patients with breast cancer [7] but remained as an off-label use for ovarian stimulation. Compared to clomiphene citrate, there are fewer studies on the use of aromatase inhibitors in minimal stimulation protocols. Two small randomized controlled trials performed in poor responders by Mohsen et al in 2012 [8] and Bastu et

al in 2016 [9] found similar pregnancy rates between those who received minimal stimulation protocol with letrozole and those who received high-dose gonadotropins stimulation protocol. In another study on poor responders by Lazer et al in 2014, those who received minimal stimulation protocol with letrozole had higher clinical pregnancy and live birth rates than those who received high-dose stimulation protocol [10].

The aim of this retrospective study was to evaluate outcomes of poor responders who underwent minimal stimulation using letrozole and whether they were comparable to outcomes of conventional IVF in a tertiary center in Singapore.

Materials and Methods

This was a retrospective study conducted in the IVF center in KK Women's and Children's Hospital, a tertiary center in Singapore. The center's database was used to trace women who underwent fertility treatment from March 2011 to March 2014. Inclusion criteria were poor responders who underwent minimal stimulation protocol. A poor responder was defined as a woman who had either failed previous cycles of conventional IVF protocols or who had poor ovarian reserves defined as anti-Mullerian hormone (AMH) level of less than 1 ng/mL. Women were excluded if they had significant pelvic pathology such as hydrosalpinges or uterine anomalies, any endocrine disorders or severe male factors. This study was approved by the SingHealth Centralized Institutional Review Board. The study was conducted in compliance with the ethical standards of the responsible institution on human subjects as well as with the Helsinki Declaration.

The minimal stimulation protocol involved sequential administration of either 2.5 or 5 mg of letrozole for 5 days followed by 150 units of recombinant follicle stimulating hormone or 75 units of menotropins. Serial transvaginal scans were used to track follicular growth and dosages of gonadotropins or menotropins were adjusted according to ovarian response. A GnRH antagonist, 0.25 mg of cetrorelix or ganirelix, was initiated daily once a dominant follicle reached 14 mm. Ovulation was triggered by subcutaneous administration of human chorionic gonadotropin (hCG) once two or more leading follicles were at least 17 mm in diameter. Oocyte retrieval was performed 36 h after hCG administration. All oocytes were fertilized with intracytoplasmic sperm injection to reduce fertilization failure. A maximum of two embryos were transferred depending on quality of embryos. Luteal phase was supported by vaginal progesterone.

The primary outcomes were number of oocytes retrieved, fertilization rate, cleavage rate, clinical pregnancy rate and live birth rate per patient. A clinical pregnancy was defined as presence of an intrauterine gestational sac at 6 weeks of gestation. A live delivery at or after 24 weeks of gestation was counted as a live birth. A subgroup analysis was done for women undergoing minimal stimulation protocol and having failed conventional IVF protocol previously in which their outcomes in the minimal stimulation cycle were compared to their most recent failed IVF cycle. Fisher's exact test was used to compare nominal variables, while Student's *t*-test was used to compare continuous variables. Significance level was set at $P < 0.05$.

Table 1. Minimal Stimulation Protocol With Letrozole (n = 43)

Variable	
Mean age (years)	38.6 ± 3.5
Mean AMH (ng/mL)	0.4 ± 0.3
Cycle	
First (n)	14
Second or more (n)	29
Number of oocytes received per patient	2.5 ± 2.1
Fertilization rate per patient (%)	63.1
Cleavage rate per patient (%)	55.2
Outcome of cycle, n (%)	
No oocytes obtained	8 (18.6)
No embryos obtained	4 (9.3)
No pregnancy	22 (51.2)
Ectopic pregnancy	1 (2.3)
Live birth	8 (18.6)

AMH: anti-Mullerian hormone.

Results

A total of 43 women underwent minimal stimulation from March 2011 to March 2014. The average age at time of stimulation was 38.6 ± 3.5 years old. The average number of oocytes retrieved was 2.5, and fertilization and cleavage rate were 63.1 % and 55.2%, respectively (Table 1). No blastocysts were achieved. No oocytes were obtained in eight women. Fertilization of oocytes failed in four women due to poor quality of oocytes obtained. Clinical pregnancies were achieved in nine women (20.9%), in which eight (18.6%) were live births and one (2.3%) was an ectopic pregnancy. There were no hospital admissions for ovarian hyperstimulation syndrome.

In a subgroup analysis of women who underwent minimal stimulation protocol after a failed conventional IVF cycle (Table 2), there were no significant differences in number of oocytes retrieved per patient and fertilization rate. Cleavage rate obtained in the minimal stimulation protocol was significantly higher at 61.3% compared to the cleavage rate obtained in the conventional IVF cycle at 28.7%. There was no significant difference in the outcomes of the two cycles.

Discussion

Poor responders present a conundrum to IVF specialists. There has been so far insufficient evidence to recommend any particular IVF strategy to improve pregnancy rates in poor responders [2, 11]. A conventional protocol for a woman predicted to respond poorly is a long protocol with upward dose adjustments of gonadotropins as necessary to reduce cancellation rates. Land et al in 1996 [12] observed that poor responders

Table 2. Subgroup Analysis of Women (n = 25) Who Underwent Minimal Stimulation After a Previous Failed Conventional IVF Cycle

Variable	Minimal stimulation protocol	Conventional IVF protocol	P value
Number of oocytes received per patient	2.1 ± 1.6	2.7 ± 1.6	0.17
Fertilization rate per patient, %	66.8 ± 33.8	47.9 ± 35.7	0.09
Cleavage rate per patient, %	61.3 ± 31.9	28.7 ± 29.4	0.002*
Outcome of cycle, n (%)			0.13
No oocytes obtained	5 (20)	4 (16)	
No embryos obtained	2 (8)	27 (28)	
No pregnancy	14 (56)	14 (56)	
Miscarriage/ectopic/abortion	1 (4)	0	
Live birth	3 (12)	0	

*Statistically significant at P < 0.05. IVF: *in vitro* fertilization.

who received 450 IU of gonadotropins per day had more oocytes than those who received 225 IU of gonadotropins per day but their reproductive outcomes did not differ. High doses of gonadotropins may have negative impact on growing follicles and hence reduce embryo quality. On the other hand, minimal stimulation induces a more physiological response which may aid to select quality oocytes, resulting in better embryos and higher implantation rates [3]. In this study, cleavage rate in the minimal stimulation group was significantly higher than that in the conventional group. The minimal stimulation group also had higher albeit non-significant live birth rate. Poor responders may thus benefit more in a milder regime than in a high-dose regime.

A few large studies have been published on outcomes of clomiphene-based minimal stimulation protocols in unselected patients [13-15] which reported pregnancy rates of above 20%. One study by Revelli in 2014 [6] compared mild stimulation using clomiphene citrate to long protocol in 695 patients with expected poor ovarian response. The mild group used significantly lower amount of exogenous gonadotropins, and yielded fewer oocytes and embryos. However, both groups did not differ in ongoing pregnancy rate (17.8% versus 16.8%). In contrast, studies on letrozole-based stimulation protocol have been lacking. Mohsen et al in 2013 [8] randomized 30 patients to receive mild stimulation with letrozole and GnRH antagonist and 30 patients to microdose GnRH agonist flare up protocol. All the patients recruited had failed one or more previous IVF cycles using protocols with high-dose gonadotropins. The duration of stimulation and dose of gonadotropins used were significantly lower in the mild stimulation group. Clinical pregnancy rate in the mild stimulation group was 13.3%, which was not significantly different from that of microdose flare group at 16.6%. In a more recent study by Bastu et al in 2016 [9], 95 poor responders were randomized to three stimulation arms: 450 IU gonadotropins per day, 300 IU gonadotropins per day or 150 IU gonadotropins with 5 mg of letrozole per day. Clinical pregnancy rates were not significantly different at 13%, 16% and 15%, respectively. In a retrospective study by Lazer et al in 2014, minimal stimulation protocol with letrozole was compared to high-dose stimulation protocol in 70 and 71 poor responders, respectively. Both the clinical pregnancy rate (31.4% versus 12.7%) and the live birth rate (21.4% versus 7%) were higher in the minimal stimulation group than in the high stimulation group [10].

In our study, the ongoing pregnancy rate of 18.6% was comparable to Mohsen's and Bastu's. Letrozole blocks estrogen biosynthesis and hence reduces negative feedback to follicle-stimulating hormone (FSH) production by the pituitary gland. In a study by Yang et al [16] comparing letrozole combined with 150 - 225 IU of gonadotropin in an antagonist protocol to high-dose gonadotropins (300 - 450 IU) in an antagonist protocol in poor responders, adding letrozole significantly reduced the amount of gonadotropins used. The letrozole group also had higher live birth rates than the group without letrozole, although not statistically significant. While letrozole has been widely explored in the last decade for ovulation induction, such usage has remained off-labeled. Compared to clomiphene citrate, letrozole has no anti-estrogenic effect on the endometrium [17] and the increased intra-ovarian androgen levels sensitize antral follicles to FSH and thus improve recruitment [18]. However, there has yet to be convincing evidence that either letrozole or clomiphene is superior to the other when used in minimal stimulation in poor responders [19]. There is also lack of conclusive data on the optimal dosage of letrozole in stimulation protocols. Most studies have used letrozole at doses from 2.5 to 7.5 mg per day with varying clinical outcomes [16, 20].

In conclusion, this study shows that use of letrozole in minimal stimulation protocol produces a reasonable live birth rate in poor responders and potentially higher quality oocytes than conventional IVF protocol. The major limitation of this study was the small number of women analyzed. In addition, as this was a retrospective study, the minimal stimulation protocols used for the women recruited were not standardized. With reasonable success rates, minimal stimulation protocols offer an attractive alternative over more expensive and time-consuming IVF protocols.

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Conflict of Interest

The authors declare they have no conflict of interest.

Informed Consent

Informed consent was waived due to the retrospective nature of this study, as approved by the SingHealth Centralized Institutional Review Board.

Author Contributions

The authors are fully responsible for the conception, collection and analysis of data; drafting the article and revising it critically for important intellectual content; and the final approval of the version to be considered for publication.

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