

Cumulative outcomes of minimal stimulation IVF for severe diminished ovarian reserve women: A retrospective cohort study

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Abstract

Objective - To analyze the effectiveness of minimal stimulation protocol of IVF treatment for patients with severe diminished ovarian reserve (DOR), especially for whom failed with several cycles of conventional stimulation IVF protocols previously. **Design, setting and sample** – A single center retrospective cohort study of undergoing IVF during January 1st, 2005 to December 31st, 2021. A total of 1,111 conventional stimulation cycles and 14,139 cycles of minimal stimulation IVF cycles were analyzed. **Methods** - Women who had undergone minimal stimulation protocol for IVF treatment and who met the study design were admitted to the study. Patients who met the study design undergone conventional stimulation IVF cycles were also included as control. Basic characteristics and clinical outcomes were all recorded and matched for the analysis of the effectiveness of minimal stimulation IVF treatment. **Main Outcome Measures** – The number of oocytes retrieved, the number of embryos obtained and gonadotrophin stimulation dosage, stimulation days and clinical pregnancy rates were analyzed. **Results** - In 16 years, a total of 14,139 cycles of minimal stimulation IVF cycles were included into this study in order to compare the effectiveness of the minimal stimulation cycles and the conventional stimulation cycles. Although a smaller number of oocytes was retrieved in the minimal stimulation cycles compared with the conventional stimulation cycles (1.39 vs 2.26, $p < 0.05$), the similar pregnancy rates were obtained in both groups (16.93% vs 18.99%). There were also no differences in live birth and miscarriage rates in the minimal stimulation cycles compared with the conventional cycles. Further analysis indicates that the clinical outcomes were reduced significantly after two cycles of conventional stimulation cycles with high dosage of gonadotropins in those women with DOR. **Conclusion**- Cumulative minimal stimulation IVF treatment is an effective alternative for women with severe DOR. If a woman with DOR is seeking fertility treatment, it is wise to turn to minimal stimulation protocol after no more than two cycles of the conventional stimulation.

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Minimal stimulation IVF for severe DOR women

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Conclusion- Cumulative minimal stimulation IVF treatment is an effective alternative for women with severe DOR. If a woman with DOR is seeking fertility treatment, it is wise to turn to minimal stimulation protocol after no more than two cycles of the conventional stimulation.

Funding

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Keywords

DOR, minimal stimulation, conventional stimulation, IVF

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Introduction

Due to advanced reproductive age, genetic and, iatrogenic factors, diminished ovarian reserve (DOR) patients are becoming a large proportion of assisted reproductive technology (ART) practices. The prevalence of DOR increased from 19% to 26% during 2004 to 2011 in US ART Population^[1].

Ovarian stimulation has been undeniably one of the biggest breakthroughs in ART. The conventional ovarian stimulation protocol aims to provide the maximum number of oocytes retrieved for fertilization and thus several embryos for selection^[2]. In past 30 years, many attempts have been proposed to improve the IVF outcomes in DOR patients, however, the clinical prognosis of DOR patients remains poor regardless. We must realize that a good oocyte can merely be encountered occasionally and randomly for this group of patients with very poor ovarian reserve.

In this context, mild/minimal stimulation protocol has been raised and proven effective, but controversial. According to the proposed definition by the International Society for Mild Approaches to Assisted Reproduction (ISMAAR) in 2007, the term mild stimulation may apply in three scenarios: (i) when oral compounds (anti-estrogens or aromatase inhibitors) are used (alone or with gonadotropins)^[3] (ii) when stimulation is performed with low gonadotropin doses^[4] and (iii) in case of delay in the start of stimulation (shorter duration) in a GnRH antagonist cotreated cycle^[5]. The American Society for Reproductive Medicine recommended that in patients who are considered to be poor responders, ‘strong consideration’ should be given to a mild ovarian stimulation protocol ([?]150 IU FSH) due to lower costs and comparable pregnancy rates^[6] (ASRM, 2018). Montoya-Botero P et al. concluded that mild/minimal stimulation could be considered as an option in low prognosis poor responder patients, given that it results in similar fresh and cumulative live birth rate (CLBR) ^[7].

In this article, we aim to investigate the effectiveness of minimal stimulation protocol women in comparison with conventional ovarian stimulation protocol on severe DOR women. We compared the outcomes in women seeking minimal stimulation protocol according to the patient’s previous IVF/ICSI(below as IVF) history, we then stratified patients according to numbers of previous cycles done. We compared the clinical outcomes and propose that if a DOR patient is seeking fertility treatment, when is wise to turn to minimal stimulation protocol IVF after no more than two conventional stimulation cycles.

Method

Patients

In this study, women who had undergone minimal stimulation protocol in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) in the Clinical Center of Reproductive Medicine, First Affiliated Hospital of Nanjing Medical University during January 1st, 2005 to December 31st, 2021 were considered for inclusion.

The inclusion criteria include: (1) women diagnosed with DOR (2) women age between 20 to 45 years of age. Exclusion criteria include: (1) PGD/PGS cycles (2) patients with chromosomal/genetic disorders or autoimmune diseases (3) patients with untreated severe endometriosis or intrauterine adhesion. For the propose of this study, women with the same inclusion and exclusion criteria who and undergone conventional ovarian stimulation protocol were also included.

Study criteria for DOR

It is blur to distinguish DOR from poor ovarian responder (POR), that was given the similar definition in this study The Bologna ESHRE consensus defines women as “poor ovarian responders” when at least

two of the following three characteristics are present: (i) advanced maternal age (≥ 40 years) or any of the risk factors for POR, (ii) a previous poor ovarian response (≥ 3 oocytes with a conventional stimulation protocol), and (iii) an abnormal ovarian reserve test (i.e., antral follicular count (AFC) $< 5-7$ follicles or AMH $< 0.5-1.1$ ng/ml)^[3]. Consequently, a young infertile woman with markers of poor ovarian reserve who has never undergone ART does not meet the Bologna criteria.

In this study, we define DOR more as a state of poor ovarian reserve, belonging the POSEIDON group 3 or group 4 population, a state of extreme poor ovarian function, specifically a: (i) women with ≥ 40 years old or any of the risk factors for poor ovarian response and/or (ii) an abnormal ovarian reserve test: antral follicular count (AFC) $< 5-7$ follicles or anti-Müllerian hormone (AMH) < 1.1 ng/ml^[10].

Conventional stimulation IVF

Conventional stimulation IVF is defined as when 150IU or more gonadotrophin daily were administered, including when long term GnRH-a is used for pituitary down-regulation followed by high doses of stimulation (150 – 450 U) with rFSH (*Gonal-F*® , Ares Serono, Geneva, Switzerland or *Puregon*® , NV Organon, Oss, The Netherlands, randomly) or hMG (*Meropu*® , Ferring, Milan, Italy), or when GnRH agonist (*Cetrotide*® injection of 0.25 mg, Merck Biopharma Co., Ltd) is administered in a flare protocol with high doses of stimulation (150 – 450 U) with rFSH or hMG, or when GnRH antagonist is used with conventional doses of early start of rFSH or hMG. When at least one dominating follicle exceeded a mean diameter of 14–18 mm and serum estradiol was 800-1000pmol/L per follicle, 6,500IU HCG (*r-hCG Ovitrelle*® , 6500 IU, Merck, Rome, Italy) were given as trigger, and a transvaginal oocyte retrieval followed 36 hours later.

After in vitro fertilization or intracytoplasmic sperm injection according to the husbands' semen samples, embryo morphology was assessed on the third day after oocyte retrieval. All available embryos were transferred on day 3 after the retrieval. Remaining supernumerary embryos were vitrified, and no more than 2 embryos were transferred in subsequent naturally prepared frozen cycles.

Luteal phase support of progesterone (*Duphaston*® 10 mg; Abbott BV, Netherlands) or micronized progesterone (*Crinone*® 8% Wyeth-Ayerst Laboratories, Inc., Philadelphia, PA) or dydrogesterone (*Utrogestan*® 200 mg, Besins Healthcare) were used for 2 weeks until the determination of pregnancy.

Minimal stimulation

In this study, minimal stimulation protocol is defined as: an extended regimen (from day 3 until the day before triggering) of clomiphene (25-50 mg/d orally) in conjunction with gonadotropin injection starting on cycle days 3 with 75IU daily. No hypothalamic-pituitary suppression was performed, and the final maturation of oocytes was induced by a GnRH-a administration.

When at least one dominating follicle exceeded a mean diameter of 14–18 mm and serum estradiol was 800-1000pmol/L per follicle, 0.1mg of GnRH-a (*Triptorelin acetate* 0.10 mg, fertipeptil, Ferring, Milan, Italy) were given as trigger, and a transvaginal oocyte retrieval followed 36 hours later.

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Luteal phase support of progesterone or micronized progesterone or dydrogesterone were used for 2 weeks until the determination of pregnancy. The serum hCG level was measured by immunofluorescence assay 2 weeks after the embryo transfer.

Main outcomes

We recorded the following outcomes: the number of oocytes retrieved, the number of embryos obtained, the number of good quality embryos, clinical pregnancy rate (a gestational sac with fetal heartbeat, confirmed

by ultrasound is defined as clinical pregnancy), the number of live births (Live birth was defined as delivery of one or more living infants after 28 completed weeks of gestation), and the rate of miscarriage.

Statistical analysis

Results are presented either as the mean $\pm$ standard deviation (SD), percentages or absolute numbers. The continuous variables were expressed as mean $\pm$ SD. Whereas categorical variables (e.g., clinical outcomes and type of infertility) were presented as percentage (%) and absolute numbers (n). The analysis of all outcomes was on an intention-to-treat basis. To address potential confounding variables, multivariable logistic regression analysis was used to assess whether clinical results, particularly clinical pregnancy rate was affected by age, BMI, basal FSH level, AMH level and AFC count, as well as stimulation protocol. A probability value of <0.05 was considered statistically significant. SPSS software (version 22.0; SPSS Inc, Chicago, IL) was used to perform all statistical analyses.

Results

General patient information

In 16 years of clinical practice, between Jan 1st, 2005 and December 31st, 2021, more than 20,000 cycles of minimal stimulation IVF were done. Of which, a total of 14,139 of minimal stimulation IVF cycles that met the study design was eligible for inclusion. Of which, many cycles were the same patient undergoing a second or third IVF cycle or patients who turned to minimal stimulation after failed conventional stimulation cycles. These cycles were excluded for the purpose of statistical analysis. 6,108 cycles with the patients' first time undergoing conventional IVF and minimal stimulation IVF were included in this study. Indeed, these women ovarian reserve were significantly poor, significantly worse than average IVF population.

Minimal stimulation IVF clinical outcomes compared to conventional IVF

We first and foremost want to the answer the question: whether minimal stimulation has the same efficacy in treating DOR related infertility.

In this study, 4,997 cycles of minimal stimulation IVF(MS-IVF) and 1,111 cycles of conventional stimulation(C-IVF) were included as comparison. We first compared the clinical outcomes in minimal stimulation and conventional IVF.

As shown in Table1, in patients diagnosed with DOR who met the study design and receiving IVF treatment, there was no differences in BMI, basal FSH levels, years of infertility as well as the percentage of primary infertility. However, there was significant differences in patients age, AMH level and AFC counts. Women of conventional IVF group were significantly younger, with higher AFC count and AMH level. This is congruent with our IVF strategy, women with "better" prognosis would always turn to conventional IVF in hopes of harvesting more available oocytes and embryos.

Table1. Basic Clinical characteristics of Conventional and minimal stimulation IVF

	Conventional IVF N=1,111	Minimal stimulation IVF N=4,997	P value
Female Age (years)	34.99	37.9	$p<0.05$
BMI (kg/M ²)	22.08 $\pm$ 2.59	22.14 $\pm$ 2.88	$p<0.05$
Male age	35.36	37.10	$p<0.05$
AMH	0.86 $\pm$ 1.13	0.58 $\pm$ 0.29	$p<0.05$
AFC count	5.49 $\pm$ 3.73	4.80 $\pm$ 3.64	$p<0.05$
Basal FSH	10.15	10.78	$p<0.05$
Primary infertility	41.76%(464)	49.53%(2475)	$p<0.05$
Years of infertility	6.58	6.53	$p<0.05$

As shown in Table2, on average, only 75 IU/day of Gn were used in minimal stimulation IVF according to

our stimulation protocol and 280. 67 IU/day of Gn were used in conventional stimulation protocol, which was significantly different. The average oocytes retrieved in MS-IVF was 1.39 which was less than 2.26 oocytes in conventional IVF($p<0.05$), the average available embryo was 1.51 in conventional stimulation and 0.90 in MS-IVF($p<0.05$), the good quality embryo was 1.01 in conventional stimulation and 0.63 in MS-IVF($p<0.05$). Even though there were statistical differences in average oocytes, the average embryos available and the good quality embryos obtained between C-IVF and MS-IVF, though slightly higher, there were no statistical differences in clinical pregnancy rate (18.99% in C-IVF vs 16.93% in MS-IVF, $p>0.05$) in the first fresh transfer cycle. There were also significant differences in the gonadotrophin stimulation days between groups, the time interval between initiation of stimulation and trigger day was significantly shorter in MS-IVF.

Table2. Clinical outcomes of Conventional and minimal stimulation IVF

	C-IVF N=1,111	MS-IVF N=4,997	P value
Total Gn stimulation days	7.46	6.32	$p<0.05$
Gn stimulation dosage per day	280.67	75	$p<0.05$
Average oocytes retrieved	2.26	1.39	$p<0.05$
Average available embryos	1.51	0.90	$p<0.05$
Average good quality embryos	1.01	0.63	$p<0.05$
Clinical pregnancy rate	18.99(211)	16.93%(846)	$p<0.05$
miscarriage rate	2.01%(23)	2.52%(126)	$p<0.05$
Live birth rate	16.92%(188)	14.40%(720)	$p<0.05$
Multiple pregnancy rate	2.43%(26)	0.001%(5)	$p<0.05$

C-IVF: Conventional stimulation IVF; MS-IVF: Minimal stimulation IVF

There were also no significant differences in the live birth rate and miscarriage rate between two groups. As minimal stimulation on average has only one dominating follicle each cycle and on average only one embryo transferred subsequently, the multiple pregnancy rate in MS-IVF was significantly low, only 0.001%.

To address potential confounding variables, multivariable logistic regression analysis was used to assess whether clinical results, particularly clinical pregnancy rate was affected by age, BMI, basal FSH level, AMH level and AFC count, as well as stimulation protocol. As a result, it showed that clinical pregnancy rate was only affected by age, AMH level and AFC count.

Previous conventional stimulation has negative effect on following minimal stimulation

To further certify whether previous conventional IVF ovarian stimulation has effects on the following IVF treatments, all patients undergoing minimal stimulation in vitro fertilization (IVF) during January 1st, 2005 to December 31st, 2021 were considered inclusion into this study. Patients with no IVF treatment history was assigned to the First-time minimal stimulation group (FT group), consisting 6,177 cycles, 2,576 patients. Patients who had undergone previous conventional stimulation protocol IVF was assigned to the Previous-Conventional stimulation group (PC group), this group had a total of 8,022 cycles and 2,871 patients.

Table3. Basic characteristics in previous C-IVF group and First time minimal stimulation group

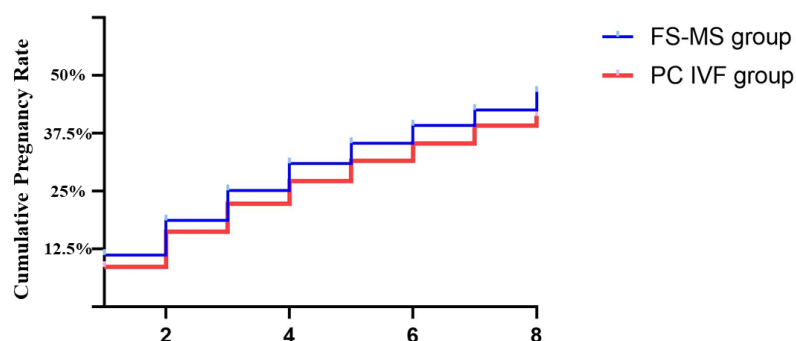
	Previous Conventional MS-group	First-time MS-group	P value
	N= 8,022	N= 6,117	
Age	36.10±5.00	36.61±5.58	$p<0.001$
BMI	21.98±2.93	22.08±4.11	$p<0.05$
AMH	0.73±1.64	0.60±1.46	$p<0.001$

	Previous Conventional MS-group	First-time MS-group	P value
AFC	5.73±4.25	4.63±3.99	p<0.001
Basal FSH	9.90±4.96	10.54±10.18	p<0.05

Among these two groups, there was significant differences in the ovarian reserve(Table3). In the PC- group, the patients showed a younger age, higher AMH level and higher AFC count.

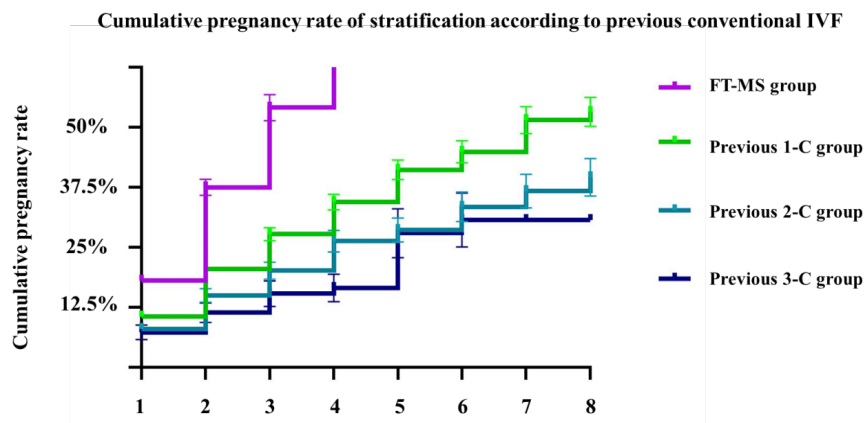
Even though the PC-group showed a slight better ovarian reserve, the results for IVF treatment did not improve. As shown in Graph1, the FT-IVF group showed a significantly higher cumulative pregnancy rate according cycles.

Cumulative Pregnancy Rate in FT-MS group and PC-IVF group



Graph1. Cumulative pregnancy rate in PC-IVF and FT- IVF group

Further stratification according previous cycles of conventional IVF was done to determine how many conventional IVF stimulation is ‘too much’ for one patient, or is “more is really better”. Cumulative pregnancy rate is calculated. As shown in Grpah2, it is obvious that when 3 or more cycles has been performed, the clinical outcomes are significantly worsened.

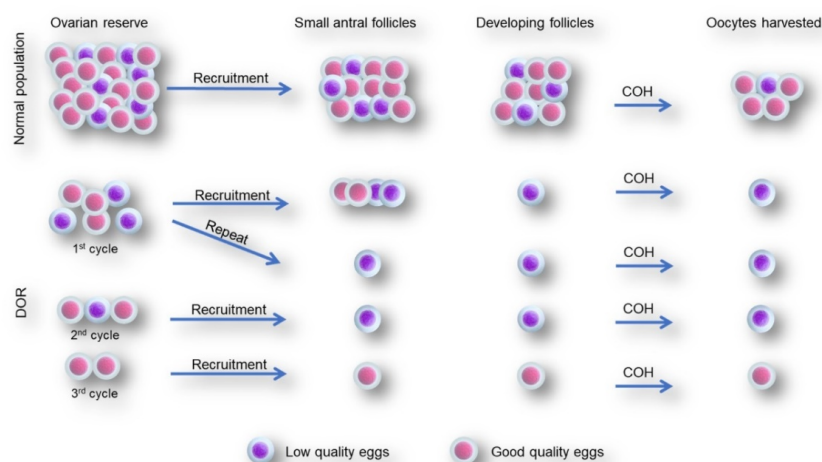


Graph2. Cumulative Pregnancy Rate according to previous C-IVF cycles

Discussion

Waiting for a good oocyte

Diminished ovarian reserve (DOR) is characterized by poor fertility outcomes even when assisted reproductive techniques (ART) are used and represents a major challenge in reproductive medicine. Although consensus exists on the concept of DOR, its definition remains blurry^[8]. As a most popular theory on follicle recruitment and development, a foundation of ovarian stimulation is that FSH threshold determined number of antral follicles growing to mature follicle, by increasing the dosage of gonadotrophin, the recruit window of follicles has been enlarged^[11], allowing more oocytes to be harvested in one cycle. But this may not be the case in DOR women because there are very few antral follicles to begin with, even less ones with good quality oocytes, the recruitment of a good quality oocyte in DOR patient is inconsistent, random and occasional. If heavy ovarian stimulation still can't achieve expected number of follicles, down low the gonadotropins might be same. Keeping retrieval with minimal stimulation for several cycles, “waiting for a good oocyte (Graph 3)” strategy is acceptable.



Graph3.

waiting for a good oocyte

The controversy in conventional stimulation in DOR

It is a challenge for DOR patients to achieve pregnancy, no matter which protocol applied to IVF. A continuing interest has been vested in DOR research worldwide and many attempts have been made to improve IVF outcomes. Athanasios Papathanasiou et al^[12] reports that in a comprehensive review undertaken of RCTs on ‘poor responders/DOR’ published in the last 15 years, The most popular stimulation protocols investigated in such patients are the antagonist protocol, the micro dose flare protocol and the long down-regulation protocol. However, RCTs on popular protocols for poor responders or DOR have reported conflicting results with regard to oocyte yields and reproductive outcomes.

There has been controversy on whether increasing gonadotrophins stimulation doses would improve IVF outcomes. Many studies showed that large gonadotrophins doses do not increase total number of eggs harvested and the number of mature eggs, and there were also no differences clinical pregnancy rates and live birth rates in women with DOR^[13]. Several large RCT found incremental doses of FSH to have a direct correlation with the number of oocytes recovered, but with no increment in the number of good-quality blastocysts^[14]. Furthermore, the blastocyst/oocyte ratio and fertilization rate demonstrated an inverse relationship with the dose of stimulation^[15-16]. Federica Di Guardo concluded that in DOR context,

natural cycle/modified natural cycle–in vitro fertilization, as a ‘milder’ approach, could be a reasonable alternative to high-dose/conventional ovarian stimulation in poor ovarian responders, with the aim to retrieve a single oocyte with transferred to a more receptive endometrium^[17]. However, even today, many clinicians still believe that higher stimulation doses improve outcomes in poor responders, despite much recent data challenging this view.

Studies show that the concentration of oxidative stress parameters significantly rose after ovarian stimulation. Superoxide generated in the ovary induces apoptosis of granulosa cells to break down follicular walls leading a worsened oocyte quality^[18]. Some researches on oxidative stress also indicated that controlled ovarian hyper-stimulation can lead to disruption of oxidative-antioxidative balance in follicle cells and oocytes, which is similar as the aging related changes.

The minimal stimulation protocols for DOR

Mild/minimal stimulation protocols have been implemented to be offered to sub-fertile patients who respond poorly to ovarian stimulation or suffer from DOR. Mild/minimal stimulation protocols using low doses of gonadotrophins have been implemented in clinical practice, demonstrating significant advantages, including cost effectiveness. Currently, most opposing opinions for minimal stimulating protocol were based on its longer time to achieve pregnancy, its higher cancellation rate and its lower pregnancy rate. However, in a DOR setting, the cancellation rate is high regardless of the stimulation protocol. In these women, based on our “waiting for a good oocyte” strategy, the rationale of mild/minimal stimulation is that its benefits through lower gonadotrophin doses, the lower costs that accompanies the treatment, together with its equality in terms of effectiveness to the conventionally used protocols for ovarian stimulation. If patients with DOR just spend 1/10 of money to obtain the same 1-2 oocyte comparing with hyper-stimulation protocol, why not?

The minimal stimulation vs. conventional stimulation for DOR patients

To the best our knowledge, this is the largest study discussing minimal stimulation, our study included more than 1,000 cycles of conventional stimulation and 14,000 cycles minimal stimulation IVF in our 16 years of clinical practices. Our results suggests that in women with similar ovarian reserve, minimal stimulation IVF showed same success rate as conventional IVF in terms of oocytes retrieved, available embryos per cycle, pregnancy rate and live birth rate. Many studies have come to this same conclusion. Minimal stimulation is more cost-effective considering less gonadotrophins are used per cycle and if a similar success can be achieved, it can be considered a good alternative stimulation protocol for conventional IVF.

Furthermore, the cumulative pregnancy rate show a less steep curve after two or three cycles, but for the minimal stimulation protocol, the cumulative pregnancy rate slowly increases after each cycle with the same speed, which indicates its beneficial to continue the IVF treatments if possible and fits our hypothesis of waiting for a good oocyte.

The impact of previous conventional stimulation in a DOR setting

In clinical practices, it is difficult to tell DOR patients they may never achieve pregnancy despite aggressive treatment. Many DOR patients would go through conventional IVF first in hopes to obtained oocytes as more as possible, receiving a ‘heavy’ or a ‘conventional’ stimulation first. We further discuss if previous conventional IVF has influences on the outcomes of consequent minimal stimulation IVF.

Minimal stimulation cycles were divided into two groups according to whether the patient had gone through previous conventional IVF cycles. We discovered that in patients who had gone through conventional stimulation IVF, less oocytes were retrieved and poor oocyte quality was observed in next cycles. So, it is possible that conventional stimulation would in contrast cause adverse IVF results and is not very suitable for DOR population. The underlying mechanism is well discussed, most studies report increased oocyte aneuploidy, embryo mortality, fetal growth retardation, and congenital abnormalities have been studied at higher-dose stimulations, and these adverse effects seem to be cumulative, increasing by each stimulation cycle^[19].

Patients in our study were further stratified by previous C-IVF cycles. We calculated the cumulative pregnancy rate according to previous cycles. After three cycles of previous IVF cycles, there is a significant drop in terms of pregnancy rate, indicating that more than two previous cycles may bring significantly adverse results in DOR woman. Even though COH has been the most crucial part of IVF treatment, more studies have proven the advantages of MS-IVF, including higher safety profile (lowering OHSS rate ^[20]), higher singleton birth weight ^[21], higher patient satisfaction and lower financial cost. The aim of MS-IVF is to achieve “quality” and not “quantity” in terms of oocytes and embryos in the stimulated cycle.

Indeed, minimal stimulation IVF has been proven to be a safer, better tolerated, more woman-friendly and affordable way of conducting ovarian stimulation in IVF cycles.

Disclosure

No conflicts of interest of all authors. This original work has not been present at any meeting.

Contribution to authorship

Nan Lu was responsible for writing of this article. Yan Gao, Feiyang Diao were responsible for accumulation of data, Yanqiu Hu was responsible for technical and laboratory support, Chunyan Jiang was responsible for statistical analysis. Jiayin Liu was responsible for the idea and design of this study,

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Conclusion

Minimal stimulation IVF is an alternative and effective treatment for DOR patients based on our strategy of “waiting for a good oocyte”, suggesting that it is wise to switch to a minimal stimulation protocol after no more than two cycles of conventional stimulation.

Tables and Figures

Table1. Basic Clinical characteristics of Conventional and minimal stimulation IVF

	Conventional IVF N=1,111	Minimal stimulation IVF N=4,997	P value
Female Age (years)	34.99	37.9	p<0.05
BMI (kg/M ²)	22.08±2.59	22.14±2.88	p<0.05
Male age	35.36	37.10	p<0.05
AMH	0.86±1.13	0.58±0.29	p<0.05
AFC count	5.49±3.73	4.80±3.64	p<0.05
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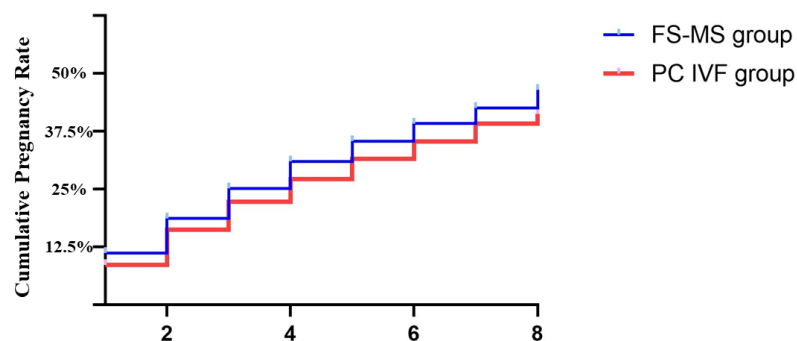
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Clinical pregnancy rate	18.99(211)	16.93%(846)	p<0.05
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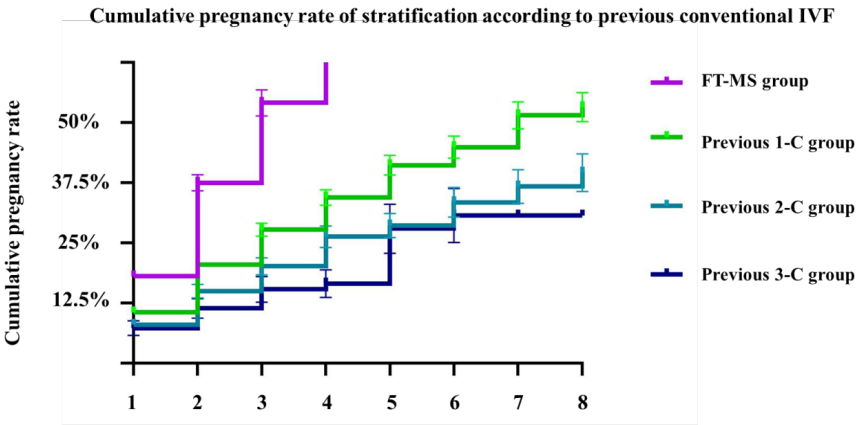
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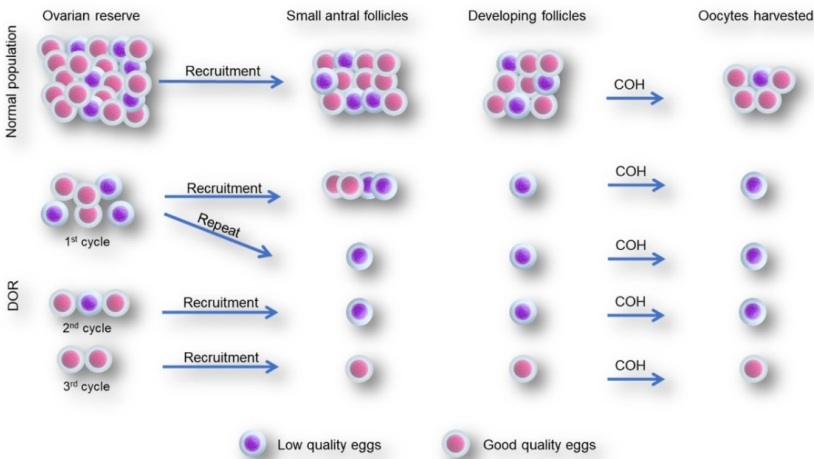
Cumulative Pregnancy Rate in FT-MS group and PC-IVF group



Graph1. Cumulative pregnancy rate in PC-IVF and FT- IVF group



Graph2. Cumulative Pregnancy Rate according to previous C-IVF cycles



Graph3.

waiting for a good oocyte

Patient's consent

All patients gave consent to the collection, analyze and publication of their clinical information to this study.

Funding

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Ethics approval

This is a retrospective, observational study. Consent was given at the beginning of IVF treatment for each patient. No ethics approval was needed.

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Cumulative Pregnancy Rate in FT-MS group and PC-IVF group

