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Freely Available Online

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JOURNAL OF FERTILITY BIOMARKERS
ISSN NO: 2576-2818

RESEARCH ARTICLE
DOI : 10.14302/issn.2576-2818.jfb-17-1812

Mild Ovarian Stimulation has similar Live Birth Rates as Compared with Hyper Stimulation for Treatment of Poor Responding IVF Patients of Advanced Maternal Age

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Abstract

The number of patients with poor ovarian response (POR) for in vitro fertilization (IVF) varies from 9 to 25%, especially high in patients of advanced maternal age. Although various stimulation protocols have been developed to improve clinical outcomes in patients with POR, a typical and effective protocol remains improvement. Some physicians prefer a mild stimulation protocol, while others like hyper stimulation protocol to obtain more eggs. This study was designed to compare the efficiency of a mild stimulation protocol with hyper stimulation protocol in patients with POR, particularly focused on live birth rate after IVF. Data were collected from 30 poor responders (over 39 years old). Patients were assigned to 2 protocols at the start of ovarian stimulation: Patients in group A were treated with a hyper stimulation (GnRH-antagonist) protocol and patients in group B were treated with a mild stimulation protocol. The ovarian stimulation characteristics, gonadotropin doses, number of eggs collected, number of high quality embryos, clinical pregnancy rates and live birth rates were compared between two groups. Although number of eggs, number of high quality embryos, clinical pregnancy rates were significantly higher in group A than in group B, miscarriage rate was also higher in group A than group B, which eventually resulted in a similar live birth rate (6.7%) in both groups. However, dosages of gonadotropins were smaller and stimulation days were shorter in group B than in group A. When poorly responding patients were treated for IVF, similar live birth rates were observed with mild stimulation protocol and hyper-stimulation protocol. After considering the higher dosages of gonadotropins and longer stimulation days in patients with hyper-stimulation protocol, it is suggested that poor responders may benefit with the mild stimulation protocol for IVF.

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Running head: Ovarian stimulation for infertility treatment

Keywords: Mild stimulation, Infertility, IVF, Poor ovarian response

Received : Oct 13, 2017; **Accepted** : Nov 12, 2017; **Published** : Nov 20 , 2017

Introduction

The number of women over 40 years old requiring assisted reproductive technology to get pregnancy has increased significantly in the past decades [1, 2]. It has been reported that clinical pregnancy rates are 10~15% in these patients after in vitro fertilization (IVF) [3]. Poor response to ovarian stimulation is one of the main reasons for the low pregnancy rate. In addition to patient's age, poor ovarian response (POR) can also be related to other causes, such as endometriosis, genetic factors, ovarian surgery, and iatrogenic factors. Although there is a lack of uniform definition of POR [4], the most common criterion used for diagnosis of POR is small number of oocytes collected after ovarian stimulation [5].

Recently, The European Society for Human Reproduction and Embryology consensus conference [6] published the "Bologna criteria" to define POR as the presence of two of the following three features: (i) advanced maternal age (≥40 years) or any other risk factor for POR; (ii) a previously characterized POR cycle (≤3 oocytes with a conventional stimulation protocol); (iii) an abnormal ovarian reserve test (<5-7 antral follicle count or <0.5-1.1 ng/ml anti-mullerian hormone).

Some controlled ovarian hyper stimulation strategies have been used for treating poor responders, but currently, there are no clear guidelines for treating these patients. Many clinicians use high doses of gonadotropin for stimulation (up to 450–600 IU/day), but there is no data to indicate that hyper stimulation with high doses of gonadotropins can increase clinical pregnancy or live birth rates [7,8]. It has been found that application of gonadotropin releasing hormone (GnRH) antagonist protocol may result in a better clinical outcomes for the poor responders by its effects on immediate suppression of luteinizing hormone. However, Fasouliotis et al. did not find higher embryo implantation and clinical pregnancy rates after using a GnRH antagonist protocol in poor responders [9]. Other studies found a higher cancellation rate [10] and reduced numbers of oocytes [11] after use of GnRH antagonist protocol.

On the other hand, a mild stimulation protocol was suggested for patients with POR [12]. During the mild stimulation, lower doses of gonadotropins are administered and stimulation durations are usually shorter than traditional hyper stimulation. However, the mild stimulation usually leads to the retrieval of fewer oocytes. It has been originally proposed for young, and good responding patients to use mild stimulation protocol [13, 14], but some physicians tried it in older patients [15-17]. It has been found that mild stimulation works for both good and poor responding patients [13-17]. However, a direct comparison on live birth rate between a mild stimulation protocol and regular hyper stimulation protocol has not been reported in patients with POR.

Therefore, the present study was designed to compare two different protocols in poor responders in terms of ovarian stimulation characteristics, gonadotropin doses, number of eggs collected, number of high quality embryos, clinical pregnancy rates and live birth rates.

Materials and Methods

Cases

A total of 30 infertile women with 55 IVF cycles in 2015 were included in the study, GnRH antagonist protocol was used in 20 IVF cycles from 15 patients (group A), and mild stimulation protocol was used in 35 IVF cycles from another 15 patients (group B).

Ovarian Stimulation Protocols

GnRH-Antagonist Group (Group A)

The patients received i.m. injections of 450-600 IU/day gonadotropins (follicle-stimulating hormone and/or human menopausal gonadotropin) for 5 days from the 2nd or 3rd day of the menstrual period, and then the gonadotropin dose was adjusted according to the ovarian response and blood estradiol level. From the 4-5th day of gonadotropin injection, 0.25 mg/day cetrorelix acetate (Baxter Oncology GmbH, Westfalen, Germany) was subcutaneously injected until the day of human chorionic gonadotropin (hCG) administration. When two dominant follicles reached a diameter of 18 mm or one dominant follicle exceeded 20 mm in diameter, 250 μg hCG was injected to trigger the final oocyte maturation.

Mild Stimulation Group (Group B)

The patients for mild stimulation protocol received 100 mg/day clomiphene citrate (Serophene, Merck-Serono, Switzerland) for 5 days from the 2nd to

6th day of the menstrual cycle, and then 150-225 IU/ day gonadotropins (Meropur, Ferring, Germany or Pergoveris, Merck-Serono, Switzerland) from the 5th day until the day of hCG administration. When two dominant follicles reached a diameter of 18 mm or one mature dominant follicle exceeded 20 mm in diameter, 250 μg hCG was injected to trigger the final oocyte maturation.

Oocyte Retrieval, Embryo Transfer, Pregnancy Detection and Live Birth Verification

Oocyte retrieval was performed 35-36 hours after hCG administration. Matured oocytes were inseminated 3-5 hours after retrieval. In group A, embryos were cultured to the blastocyst stage and the blastocysts were transferred or frozen for subsequent frozen embryo transfer (FET). In group B, embryos were cultured to day 3 and high quality embryos were transferred or frozen for FET.

Endometrial preparation was performed in a natural cycle, a stimulation cycle or a hormone replacement therapy (HRT) cycle. For endometrial preparation in the HRT cycle, oral 4-6 mg/day Estrogen (Bayer Pharma AG, Berlin, Germany) was given from cycle day 2-3. Once the endometrial lining thickness reached ≥8 mm, 60 mg/ day progesterone oil was administered i.m. until day 14 after embryo transfer, and embryo transfer was carried out on day 4 or 6 after progesterone injection. The maximum number of transferred embryos was three (only one patient). Pregnant patients continued to receive progesterone until 8-10 weeks of gestation.

Pregnancy was examined on day 14 after embryo transfer by measuring blood beta-hCG level and further verified on day 35 by transvaginal ultrasound. Live birth was verified in the ongoing patients after delivery of healthy babies.

Statistical Analysis

Main outcome measures were biochemical pregnancy, clinical pregnancy and live birth rates per cycle, per patient and per embryo transfer. Patients' age, body mass index (BMI), duration of stimulation, total gonadotropin doses, number of oocytes retrieved and fertilized, number of available embryos, good quality embryos, cancellation rate and mean number of embryos transferred were also assessed. The statistical analysis was performed using the Statistics Package for Social Sciences version 12.0 (SPSS, SPSS Inc., Chicago). The Chi square ($\chi 2$) test and Fisher's exact test were used to analyze nominal variables in the form of frequency tables. Normally distributed (Kolmogorov-Smirnov test) parametric variables were tested by independent Student's-t test. Normally distributed metric variables were analyzed by Mann-Whitney U test. A value of $P<0.05$ was considered statistically significant. Values were expressed as mean ± standard deviation (SD) unless otherwise stated.

Results

As shown in Table 1, there were no statistical differences between the two groups in terms of base follicle-stimulating hormone, patients' ages, and BMI. However, significantly ($P< 0.05$ at least) more total gonadotropin doses, longer stimulation days, more eggs retrieved, fertilized and developed to embryos were observed in group A than those in group B. Cancellation rate was significantly ($P<0.001$) higher in group B than in group A because no egg was collected or there was poor fertilization or embryo development in some cases from group B.

All 30 patients had embryo transfer after one or more stimulation cycles. In group A, one patient had three transfers and all others had only one transfer each, resulting in a total of 17 transfers. In group B, one patient had two transfers and remaining had one transfer each, resulting in a total of 16 transfers. As shown in Table 1, significantly ($P<0.001$) more embryos were transferred in group A than in group B. Higher pregnancy rate, clinical pregnancy rate and implantation rate were observed in group A than in group B.

As shown in Table 2, although the biochemical pregnancy rates, clinical pregnancy rates, and the embryo implantation rates per cycle, patient or transfer were significantly ($P<0.05$) higher in group A than those in group B, 6 out of 7 pregnant women in group A had a miscarriage during the subsequent gestation, which resulted in a same live birth rate (per cycle, patient or transfer) between the two groups. Only one patient in each group had term delivery.

Discussion

Woman's age is the most important factor affecting IVF success rate. Although pregnancy and live birth rates are low in women aged ≥40 years who use autologous

Table 1. Comparison between hyper and mild stimulations for IVF in poor responding patients (categories before embryo transfer)

Categories examined	Group A	Group B	P value
Number of patients	15	15	NA
Base FSH	9.13±1.86	9.24±1.91	>0.05
No. of cycles	20	35	NA
Age of woman (Mean ± SD)	41.45±1.79	42.94±2.75	>0.05
BMI of woman(kg/m²)	23.80±2.39	23.81±3.86	>0.05
Total FSH/HMG dose (IU)	6153.75±1517.25	1030.71±735.66	0.003
Duration of stimulation (days)	12.61±2.38	8.01±3.01	0.047
No. of oocytes retrieved	4.9±1.89	2.2±1.28	0.008
No. of fertilized eggs	3.6 ±1.76	1.54±1.15	0.024
No. of available embryos	3.5±1.7	0.83±0.75	0.001
No. of good quality embryos	1.4±1.19	0.6±0.6	0.001
No. of cancellation (%)	2/20 (10.0)	17/35 (48.6)	0.001
No. of patients had ET	15	15	NA
No. of total ET	17*	16**	NA
Mean No. of embryos transferred	1.38±0.71	0.91±0.8	0.001

*One patient had three embryo transfers; **One patient had two embryo transfers.

Table 2. Comparison between hyper and mild stimulations for IVF in poor responding patients (categories after embryo transfer)

Categories examined	Group A	Group B	P value
Pregnancy rate (by beta hCG) per cycle (%)	8/20 (40.0)	3/35 (7.9)	<0.05
Pregnancy rate (by beta hCG) per Transfer (%)	8/17 (47.1)	3/16 (18.8)	<0.05
Pregnancy rate (by beta hCG) per patient (%)	8/15 (53.3)	3/15 (20.0)	<0.05
Clinical pregnancy rate per cycle (%)	7/20 (35.0)	2/35 (5.7)	<0.05
Clinical pregnancy rate per transfer (%)	7/17 (41.2)	2/16 (12.5)	<0.05
Clinical pregnancy rate per patient (%)	7/15 (46.7)	2/15 (13.3)	<0.05
Implantation rate (%)	7/34 (20.6)	2/27 (7.4)	<0.05
No. of miscarriage (%)	6 (85.7)	1 (50.0)	<0.05
No. of live birth delivery per cycle (%)	1/20 (5.0)	1/35 (2.8)	>0.05
No. of live birth delivery per transfer (%)	1/17 (5.9)	1/16 (6.3)	>0.05
No. of live birth delivery per patient (%)	1/15 (6.7)	1/15 (6.7)	>0.05

IVF: in vitro fertilization; hCG: human chorionic gonadotropin

oocytes, the number of these infertile women asking for IVF treatment is increasing, particularly in countries where egg donation is not allowed.

A previous study with 2386 IVF cycles in women aged ≥40 years showed that overall clinical pregnancy rate was 13.4% and live birth rate was 6.7% per cycle [18]. They also found that the cancellation rate was 16% per cycle, and the cancellation rate increased significantly in patients at 45 years old and above. In our study, we also obtained a similar live birth rate (6.7%) in patients with two different stimulation protocols. However, we found that cancelation rate was 10% in group A, which was significantly lower than that (48.6%) in group B. This may be due to high dose of gonadotropins that initialized more follicles to grow in patients of group A.

It is still a challenge for clinicians to choose the suitable stimulation protocols for older patients (more than 40 years old) with a diminished ovarian reserve. Several studies have compared the efficiency of different kinds of protocols in the past few years. In one retrospective study, it was found that there was no significant difference in fertilization rates, or embryo development rates among standard long protocol, short protocol and GnRH-antagonist protocol [19]. Several meta-analysis and Cochrane reviews also tried to examine different treatment protocols in PORs, but so far none of these attempts has drawn any conclusion [20-22]. Compared to a GnRH-antagonist protocol with high dose of gonadotropins, mild stimulation with a low dose of gonadotropins is an interesting alternative for patients with poor ovarian reserve, which may produce more high quality embryos, and result in better implantation and pregnancy rates when these embryos were transferred [23].

In the present study, patients in group A produced more high quality (based on morphology assessment) embryos, more patients were pregnant and more embryos implanted; however, most women got miscarriage during the first trimester. It has been reported that more embryos from poor responders (especial old patients) are aneuploidy, which leads to early miscarriage and loss of pregnancy during the first trimester [24]. Some laboratory procedures may be beneficial to the poor responders, such as preimplantation genetic screening. However, due to the limited number of eggs and embryos, cancellation rates are very high in patients with advanced maternal ages with or without poor response after preimplantation genetic screening procedure [25]. It is still unclear whether high doses of gonadotropins could cause more chromosomal anomalies in old women, but it has been found that mild stimulation and natural cycles provide better clinical outcomes in patients with POR [26]. However, a direct comparison with more patients between mild and hyper stimulations may be necessary to draw a solid conclusion of whether mild stimulation is similar to or better than hyper stimulation for POR.

Our present study indicates that mild stimulation has a similar livebirth rate as hyper stimulation in PORs. There data were similar with previous studies with more patient population [26, 27] in which ongoing pregnancy rates were reported between two groups. Our current study further provided the evidence that mild stimulation and hyper stimulation have the similar live birth rate. Although more eggs/embryos are produced in patients with hyper stimulation protocol, miscarriage was observed in most patients during the first trimester of pregnancy, suggesting that the embryos resulting from hyper stimulation may be aneuploidy. However, further studies remain necessary to test this hypothesis with more patients involved. After considering the outcomes from these studies (our current study and previous studies), and the quantity of gonadotropins dose used and long stimulation days in hyper stimulated patients, it is suggested that mild stimulation may be more appropriate (cost-effective) for these patients.

Conclusions

When poorly responding patients were treated for IVF, similar live birth rates were observed between mild stimulation protocol and hyper stimulation protocol although more eggs can be retrieved and more embryos can be produced for transfer in patients receiving hyper stimulation. After considering the higher dosage of gonadotropins and longer stimulation days in patients with hyper stimulation protocol, it is suggested that poor responders may benefit with the mild stimulation protocol for IVF. A limitation of our study was that these data was based on small patient numbers, therefore, it remains necessary to have more patients to be involved in the study so that a solid and reliable conclusion can be drawn.

Abbreviations

IVF: in vitro fertilization

hCG: human chorionic gonadotropin

GnRH: gonadotropin releasing hormone

FET: frozen embryo transfer

HRT: hormone replacement therapy

POR poor ovarian response

BMI: body mass index

Ethics Approval and Consent to Participate

The Institutional Review Board and Ethics Committee at the Changsha Hospital for Maternal and Children Health Care approved this retrospective cohort study.

Competing Interests

The author(s) declare that they have no competing interests.

Funding

No external funding was received for this study.

Authors' Contributions

PY, JL and WW carried out study design and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

No Applicable

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