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*Original Article*

# Comparison of different ovarian hyperstimulation protocols efficacy in poor ovarian responders according to the Bologna criteria

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**Abstract:** Many protocols have been proposed to improve IVF outcomes for poor ovarian responders. The aim of this study was to explore the relationship between age, ovarian hyperstimulation protocol and IVF/ICSI outcomes in poor ovarian responder (POR) according to the Bologna criteria, and to compare the efficacy of different protocols used in PORs undergoing IVF/ICSI. We retrospectively analyzed clinical data of 4875 IVF/ICSI cycles, including 592 cycles of women diagnosed with POR according to Bologna criteria. We explored the association of age, different types of ovarian hyperstimulation protocols and prevalence of POR, IVF/ICSI outcomes. Age, basic FSH, AFC, and as well as ovarian hyperstimulation protocols, were all associated with POR. Irrespective of age, PORs in different ovarian hyperstimulation protocol groups had similar AFC, basic sex hormones, number of retrieved oocyte, implantation rate and clinical pregnancy rate as well. However, PORs treated with mild stimulation protocol used least doses of gonadotropins and shortest days of stimulation compared with those treated with other protocols (P<.05). The current study has shown that age, basic FSH, AFC and ovarian stimulation protocols patients used are all significantly associated with POR according to the Bologna criteria. It seems that there is no difference in clinical outcomes such as clinical pregnancy rate, implantation rate and spontaneous abortion rate between different protocols.

**Keywords:** Ovarian hyperstimulation protocol, poor ovarian responder, Bologna criteria

## Introduction

The ultimate goal for an in vitro fertilization-embryo transfer (IVF-ET)/Intracytoplasmic sperm injection (ICSI) treatment is the birth of a healthy infant, which depends on many factors. Among those factors, ovarian stimulation is considered to be the cornerstone of IVF treatment, since it includes the development and maturation of several follicles to increase the likelihood of conception. However, not all women undergoing IVF can gain the benefits related to ovarian hyperstimulation, because some of them do not response well to treatment.

As early as 1983, poor ovarian responder (POR) was first reported and defined as decreased follicular response and low E2 levels to ovarian stimulation by FSH/HMG, resulting in few oocytes being retrieved and few transferred embryos [1]. Early publications report that the incidence of POR is 9%-24%, and clinical pregnancy rate is very low [2, 3]. At present, even though several interventions have been used to try to improve the outcomes in poor responders, there is insufficient evidence to identify which one is most effective [4-6].

The main reason for this controversy is that there is no uniform definition of POR for universal use [7]. In order to estimate the accurate incidence of POR, and most importantly, to compare the protocols proposed, the European Society for Human Reproduction and Embryology performed the first consistent definition of POR in 2011 (Bologna criteria) [8]. Retrospectively analyzing information of 4875 IVF/ICSI cycles, here we are trying to explore the association of age, ovarian hyperstimulation protocols and POR using Bologna criteria of poor response, and to compare the several most commonly used protocols for PORs.

## Materials and methods

A retrospective study of 4875 IVF/ICSI cycles, including 592 cycles diagnosed with POR, from August 2009 to December 2011 in the Reproductive Medical Center, First Affiliated Hospital of Zhengzhou University was undertaken. The definition of POR was in accordance with the Bologna criteria: If at least two of the following three features are present, POR can be diagnosed: 1) Advanced maternal age (≥40 years) or any other risk factor for POR; 2) A previous POR (≤3 oocytes with a conventional stimulation protocol); 3) An abnormal ovarian reserve test (i.e. AFC<5-7 follicles or AMH<0.5-1.1 ng/ml). This study was approved by the Institutional Review Board of the First Affiliated Hospital of Zhengzhou University.

*Controlled ovarian hyperstimulation protocols*

At present, several ovarian hyperstimulation protocols are available for patients undergoing IVF/ICSI treatment. Reasons for protocols vary in different patients are from our own experiences. Standard long protocol, one of the most commonly used regimes, is mainly for those young (age <35 years) women with normal ovarian reserve. However, for the "expected PORs (age >38 years, diminished ovarian reserve or undergoing their ≥2 cycles of IVF/ICSI)", short protocol, modified super long protocol or mild stimulation protocol are used. Other than that, super long protocol is mainly used in women with advanced endometriosis [9].

The details for these protocols are listed as below:

*Long protocol:* Patients in the long protocol group were administered s.c. GnRH agonist 0.1 mg triptorelin (Arvekap, Ipsen, France) daily. When desensitization was achieved (about 14 days after the initiation of GnRH agonists), as evidenced by plasma E2 levels of ≤30 pg/ml, the absence of ovarian follicles and endometrial thickness ≤5 mm on transvaginal ultrasound examination, daily s.c. injection of recombinant FSH (rFSH, Puregon, Organon, The Netherlands) at a dosage of 150-300 IU daily was commenced. The dose of GnRH agonist was decreased on that day to 0.05 mg/day and continued until and including the day of triggering of final oocyte maturation.

*Short protocol:* GnRH agonist 0.1 mg was administered on day 2 of menstruation, and rFSH was given on day 3 of menstruation at a dosage of 150-300 IU per day.

*Super long protocol:* GnRH agonist 3.75 mg was injected intramuscularly on day 2 of menstruation twice and HMG was started 20 days later after the second GnRH agonist injection.

*Modified super long protocol:* GnRH agonist 1.875 mg was injected intramuscularly in mid-luteal phase twice and HMG was started 16 days later after the second GnRH agonist injection.

*Mild stimulation protocol:* Patients in this group were given Letrozol or human menopausal gonadotropin (HMG) on day 3 of menstruation.

When at least three follicles had reached 17 mm, human chorionic gonadotrophin (hCG, 10 000 IU; Serono) was injected. Oocyte retrieval was scheduled approximately 36 hours after hCG injection. Progesterone in oil was used for luteal support at a dose of 60 mg per day. Pregnancy was diagnosed by serum β-hCG 14 days after embryo transfer. Clinical pregnancy was defined as identification of a gestational sac 2-3 weeks after the positive pregnancy test. The implantation rate was defined as the number of gestational sacs per embryo transferred.

*Main outcomes measures*

Basic clinical data include age, body mass index (BMI), basic sex hormone, AFC, Gn-dosage and E2 concentration on day HCG. Main outcome measures include number of cancelled cycles, number of oocyte retrieved, number of embryo transferred, clinical pregnancy rate, implantation rate and early spontaneous abortion rate.

*Statistical analysis*

Data were expressed as mean ± SD and analyzed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Means were analyzed using the two-tailed t-test for parametric data, and proportions were compared using the Chi-square test. In all cases, statistical significance was set at $P<0.05$.

## Results

In order to explore the factors associated with POR, we used logistic model to evaluate the

**Table 1.** Logistic analysis for evaluating factors associated with POR

| | Beta | Exp (B) | 95% C.I | P |
|---|---|---|---|---|
| Age | 0.080 | 1.083 | 1.058-1.108 | <0.001 |
| Basic FSH | 0.155 | 1.167 | 1.133-1.203 | <0.001 |
| AFC | -0.525 | 0.767 | 0.737-0.797 | <0.001 |
| Ovarian Hyperstimulation protocols | 0.295 | 1.343 | 1.219-1.479 | <0.001 |

**Table 2.** Relationship between ovarian hyperstimulation protocols and POR

| | Long protocol | Short protocol | Super long protocol | Modified super long protocol | P |
|---|---|---|---|---|---|
| No. of cycles | 4124 | 59 | 234 | 187 | |
| Age (year) | 31.0 ± 4.7 | 38.0 ± 4.4* | 32.5 ± 4.3# | 34.9 ± 4.5*,#,Δ | <0.001 |
| No. of previous cycles | 1.2 ± 0.5 | 1.9 ± 0.9* | 1.5 ± 0.9*,# | 2.3 ± 1.0*,# | <0.001 |
| Basic FSH level (IU/L) | 7.8 ± 2.8 | 11.5 ± 7.8* | 8.3 ± 5.3# | 8.2 ± 3.7# | <0.001 |
| AFC | 9.6 ± 4.3 | 4.2 ± 2.4* | 7.0 ± 3.8*,# | 6.5 ± 3.6*,# | <0.001 |
| Incidence of POR (%) | 8.8 | 66.1* | 21.4*,# | 24.8*,# | <0.001 |
| Clinical pregnancy rate per ET cycle (%) | 47.3 | 25.5* | 44.2# | 35.7*,# | <0.01 |

*Compared with long protocol, P<0.05; #Compared with short protocol, P<0.05; ΔCompared with super long protocol, P<0.05.

relationship between age, basic FSH level, AFC, ovarian hyperstimulation protocols and POR. It was found that, not only age, FSH and AFC, but ovarian hyperstimulation protocols was also significantly associated with POR (**Table 1**). As expected, both numbers of previous cycles and basic FSH level in the ≥40 year olds were significantly higher than those in the younger groups. However, AFC and clinical pregnancy rate in the ≥40 year olds were significantly lower (P<0.001). In addition, the incidence of POR in the ≥40 year olds was 39.1%, and it was 8.1% in the <35 year olds.

*Patient's characteristic in different ovarian protocol groups*

According to ovarian hyperstimulation protocols, all the IVF/ICSI cycles were divided into: long protocol, 4124 cycles; short protocol, 59 cycles; super long protocol, 234 cycles; modified super long protocol, 187 cycles. The average age of women undergoing IVF/ICSI in long protocol group and super long protocol group were 31.0 and 32.5, respectively, which were significantly lower than that in other protocol groups. However, patients in short protocol group, with the average age being 38.0, were the oldest in these four groups (P<0.01).

Basic FSH levels of patients in short protocol group were significantly higher than that in the other three groups, while them were comparable in long protocol group, super long protocol group and modified super long protocol group. In addition, AFC of patients in long protocol group and short protocol group were significantly higher and lower than those in the other three groups, respectively (P<0.01).

In regard to the incidence of POR, it was 8.8% in long protocol group, 66.1% in short protocol group, 21.4% in super long protocol group and 24.8% in modified super long protocol group. The differences in these four groups were statistically significant.

As shown in **Table 2**, clinical pregnancy rates per ET cycle in these four groups varied from 25.5% (short protocol group) to 47.3% (long protocol group). The differences were also statistically significant.

*IVF/ICSI outcomes according to age and ovarian hyperstimulation protocol*

**Tables 3** and **4** show clinical characteristics and IVF/ICSI outcomes of patients with different ages in the five ovarian hyperstimulation protocol groups. In regardless of ovarian hyperstimulation protocol, those younger ones tended to use less gonadotrophin, yet had better results, such as higher clinical pregnancy rate, less cancellation rate and less spontaneous abortion rate. Overall, PORs using long protocol had least number of previous IVF/ICSI cycles,

**Table 3.** Clinical characteristics of PORs according to different ovarian hyperstimulation protocols and age

| | <35 years | | | | | ≥35 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Long | Short | Super long | Modified super long | Mild | Long | Short | Super long | Modified super long | Mild |
| No. of cycles | 180 | 10 | 29 | 19 | 25 | 183 | 27 | 21 | 27 | 71 |
| Age (year) | 30.9 ± 2.7 | 31.6 ± 2.0 | 30.5 ± 2.5 | 31.8 ± 1.8 | 31.9 ± 2.0 | 38.4 ± 2.8 | 38.9 ± 2.7 | 38.0 ± 1.9 | 38.3 ± 2.7 | 39.2 ± 3.2 |
| No. of previous cycles | 1.2 ± 0.4 | 1.8 ± 0.8$^{*}$ | 1.2 ± 0.5 | 1.5 ± 0.5 | 2.5 ± 1.3$^{*,\Delta,\&}$ | 1.2 ± 0.6 | 1.8 ± 0.9 | 1.6 ± 1.1 | 2.0 ± 1.0$^{*}$ | 2.9 ± 1.6$^{*,\#,\Delta,\&}$ |
| BMI (kg/cm$^2$) | 21.5 ± 2.4 | 21.6 ± 2.7 | 22.1 ± 3.6 | 20.6 ± 1.7 | 22.3 ± 2.6 | 22.9 ± 2.5 | 22.6 ± 2.2 | 22.9 ± 3.0 | 22.5 ± 3.3 | 22.8 ± 2.5 |
| AFC | 5.6 ± 2.6 | 4.4 ± 1.8 | 4.4 ± 2.3 | 5.0 ± 2.1 | 3.7 ± 2.5 | 4.6 ± 2.5 | 3.5 ± 1.7 | 3.9 ± 1.9 | 4.7 ± 2.4 | 2.5 ± 1.6$^{*}$ |
| Basic FSH (IU/L) | 9.9 ± 4.4 | 12.6 ± 7.4 | 8.8 ± 4.3 | 12.4 ± 6.7 | 11.7 ± 5.3 | 10.5 ± 4.7 | 13.6 ± 9.8 | 10.1 ± 6.3 | 8.7 ± 3.7 | 12.7 ± 5.6 |
| Basic LH (IU/L) | 5.4 ± 3.6 | 6.1 ± 3.2 | 5.5 ± 3.8 | 4.3 ± 2.1 | 5.7 ± 3.8 | 5.5 ± 2.8 | 7.9 ± 4.6 | 4.2 ± 3.7 | 4.8 ± 3.2 | 6.7 ± 4.2 |
| Basic E2 (ng/l) | 71.5 ± 101.5 | 67.8 ± 49.5 | 64.1 ± 68.4 | 62.3 ± 29.0 | 52.4 ± 33.1 | 68.7 ± 80.7 | 80.5 ± 92.5 | 69.1 ± 109.6 | 64.4 ± 56.0 | 70.7 ± 92.0 |

$^{*}$Compared with long protocol, P<0.05; $^{\#}$Compared with short protocol, P<0.05; $^{\Delta}$Compared with super long protocol, P<0.05; $^{\&}$Compared with modified super long protocol, P<0.05.

**Table 4.** IVF/ICSI outcomes of PORs according to different ovarian hyperstimulation protocols and age

| | <35 years | | | | | ≥35 years | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Long | Short | Super long | Modified super long | Mild | Long | Short | Super long | Modified super long | Mild |
| No. of cycles | 180 | 10 | 29 | 19 | 25 | 183 | 27 | 21 | 27 | 71 |
| No. of cycles with embryos transferred | 139 | 6 | 23 | 15 | 14 | 135 | 20 | 17 | 21 | 42 |
| Duration of Gn | 11.6 ± 2.1 | 12.3 ± 1.9 | 13.3 ± 2.2$^{*}$ | 13.6 ± 1.7$^{*}$ | 6.5 ± 1.8$^{*,\#,\Delta,\&}$ | 11.6 ± 2.3 | 10.7 ± 2.4 | 12.5 ± 2.6 | 12.6 ± 3.5 | 6.4 ± 2.5$^{*,\#,\Delta,\&}$ |
| Dose of Gn (IU) | 2873.9 ± 1045.1 | 3982.5 ± 1290.1$^{*}$ | 3732.6 ± 1192.6$^{*}$ | 3728.6 ± 859.2$^{*}$ | 854.0 ± 340.5$^{*,\#,\Delta,\&}$ | 3496.2 ± 975.7 | 3732.3 ± 1223.1 | 3500.0 ± 773.3$^{*}$ | 3637.5 ± 1178.1$^{*}$ | 1032.2 ± 1190.5$^{*,\#,\Delta,\&}$ |
| Oocytes retrieved | 2.3 ± 0.7 | 1.9 ± 0.9 | 2.16 ± 0.90 | 2.2 ± 0.8 | 1.9 ± 0.8 | 2.2 ± 0.8 | 2.0 ± 0.8 | 2.21 ± 0.92 | 2.3 ± 0.7 | 1.8 ± 0.7 |
| Embryos transferred | 1.6 ± 0.5 | 1.3 ± 0.8 | 1.5 ± 0.7 | 2.0 ± 0.7 | 1.4 ± 0.6 | 1.6 ± 0.7 | 1.6 ± 0.7 | 1.7 ± 0.8 | 1.5 ± 0.5 | 1.4 ± 0.5 |
| E2 level on day HCG (ng/l) | 1976.2 ± 1140.7 | 1556.8 ± 985.6 | 1367.9 ± 894.3 | 1432.1 ± 1073.2 | 891.6 ± 732.8$^{*,\#,\Delta,\&}$ | 1530.3 ± 956.7 | 918.7 ± 842.1 | 1074.8 ± 921.4 | 1289.7 ± 973.2 | 663.7 ± 589.2 |
| Cancellation rate (%) | 22.8 | 40.0 | 20.7 | 21.1 | 44.0$^{*}$ | 35.6 | 25.9 | 19.0 | 22.2 | 40.8 |
| Implantation rate (%) | 22.0 | 25.0 | 29.4 | 23.6 | 15.3 | 17.1 | 6.3 | 24.1 | 19.1 | 15.0 |
| Clinical pregnancy rate (%) | 28.1 | 33.3 | 39.1 | 34.3 | 21.4 | 25.2 | 10.0 | 35.3 | 29.7 | 19.0 |

$^{*}$Compared with long protocol, P<0.05; $^{\#}$Compared with short protocol, P<0.05; $^{\Delta}$Compared with super long protocol, P<0.05; $^{\&}$Compared with modified super long protocol, P<0.05.

whereas those using mild protocol had the highest. In the <35 year olds, patients in mild protocol group underwent 2.5 IVF/ICSI cycles, which was significantly higher compared with patients in long protocol, super long protocol and modified super long protocol groups. What is more, in the ≥35 year olds, patients in mild protocol group underwent 2.9 IVF/ICSI cycles, which was the highest in all the five groups (P<.05). In addition, the ≥35 year olds in mild protocol group had significantly less AFC when compared with PORs using long stimulation protocol. Other than that, clinical characteristics in PORs with different age using these five ovarian hyperstimulation protocols did not vary.

Irrespective of age, gonadotrophins dosage and duration in PORs using mild ovarian hyperstimulation were less than those in PORs using other protocols. However, for the <35 year olds, E2 levels on day HCG of PORs using mild protocol were the least compared with other four groups, and the cancellation rate was the highest when compared with poor responders in long protocol group. On the other hand, regardless of age, PORs in the five groups were statistically similar with regard to IVF/ICSI outcomes such as implantation rate and clinical pregnancy rate.

## Discussion

In order to determine whether there is a superior treatment regimen for POR, scholars have conducted several studies to compare the efficacy of different kinds of protocols in the past few years. In one retrospective study, it was found that, in PORs, there was no significant difference in fertilization rate, or utilization rate between standard long protocol, short protocol and GnRH-ant protocol [10]. Another recent study also suggested that the application of short, long, miniflare and antagonist protocols in PORs had similar efficacy in improving clinical outcomes such as implantation, pregnancy rates [11]. Other than that, prospective studies also showed similar results [12, 13]. As early as 2001, Akman et al. compared GnRH agonist flare protocol with multiple doses GnRH antagonist protocol on 48 PORs and showed similar clinical outcomes. Later than that, another study randomly divided 66 PORs into GnRH antagonist group and long GnRH agonist group and found that there was no significant differences in the cycle cancellation rates, duration of stimulation, consumption of gonadotrophins, oocytes and embryos obtained. Meanwhile, implantation rates and pregnancy rates were also similar in the two groups [14]. Other than that, several Meta analysis and Cochrane reviews also tried to examine different regimes of treatment in PORs. Unfortunately, so far none of these attempts have drawn any firm conclusions on which protocol is better [15-17].

Indeed, in the past 20 years, hundreds of studies, including more than 40 randomized trials, have been published comparing different treatment protocols to improve IVF/ICSI outcomes in the PORs. However, according to a survey conducted by Nikolaos et al., among the 47 randomized trials, there were 41 different definitions for women with poor ovarian response [18]. What is worse, even studies performed by the same centers used different definitions for these patients. The wide diversity in the definitions used to specify patients with impaired response to treatment makes us difficult to draw any conclusions on which protocol is best for this group of people. Recently, a definition has been proposed by the ESHRE Working Group on Poor Ovarian Response Definition in order to homogenize this patient population. The Bologna criteria may be helpful for us to make progress in the proper management of these patients.

The current large size retrospective study is the first one to explore factors associated with poor ovarian response and to compare the several commonly used protocols in PORs selected according to the Bologna criteria. According to our results, the total incidence of POR according to Bologna criteria is 12.1%. Age, basic FSH level, AFC and ovarian hyperstimulation protocols are all significantly associated with POR. Meanwhile, we have shown no difference in the clinical pregnancy rate/transferred cycle irrespective of age and the ovarian hyperstimulation protocols used in POR.

As is known, there is wide number of parameters, such as age, AFC, sex hormone, and number of oocytes retrieved to describe the patient with impaired ovarian response. The Bologna criteria incorporates age, number of oocytes retrieved, risk factors for poor ovarian response, AMH and AFC for the definition of a uniform group of patients. Thus, it is not surprising to

find that age, basic FSH level, and AFC are significantly associated with POR. Interestingly, our study also showed the significant relationship between POR and ovarian stimulation protocols. The incidence of POR in standard long protocol group was 8.8%, which was significantly lower than that in the other three groups. Meanwhile, the pregnancy rate per transferred cycle in long protocol group was also the highest. However, we should not just simply consider the standard long protocol being "superior to other protocols, because as we stated before, standard long protocol group always has the "high quality" patients. Some expected poor responders, especially those who underwent their second or third IVF/ICSI cycles, are more likely to use short protocol, super long protocol and mild stimulation protocol. It is those "tough" and "old" women that make the outcome in other protocol groups seem worse than that in standard long protocol group.

Age is usually considered to be the fundamental factor associated with adverse artificial reproductive technology outcomes, since other fertility associated parameters, such as basic FSH, AFC, AMH, etc, changed with increasing age. In our study, we also admit that a patient's age affects the ovarian hyperstimulation protocol she used. Thus, in order to control the bias caused by age, we divided all the PORs into two groups: <35 year olds (young PORs) and ≥35 year olds (old PORs). After controlling age, our study showed that, for all the young PORs, basic characteristics were comparable in the five protocol groups, except for previous IVF/ICSI cycles, which was significantly higher in mild stimulation group. What is more, the situation was also similar in the old PORs. After dividing PORs according to age, clinical pregnancy rate, implantation rate and spontaneous abortion rate were comparable in the five protocol groups.

As expected, PORs in mild stimulation group used less gonadotrophins compared with patients in other groups. However, we should also note that, nearly 50% of the patients using this friendly, economical regimen had to cancel their treatment due to advance unexpected ovulation.

A limitation of the current study is its retrospective design. However, after dividing the 592 PORs into two groups according to age, basic characteristics of the patients were comparable in the two groups. This considerably decreases the main bias caused by age.

In our large retrospective study of PORs diagnosed according to the Bologna criteria, we have demonstrated that age, basic FSH, AFC and ovarian stimulation protocols patients used are all significantly associated with POR. It seems that there is no difference in clinical outcomes such as clinical pregnancy rate, implantation rate and spontaneous abortion rate between different protocols. However, due to the limitations we mentioned above, we strongly propose that, in order to get a firm conclusion, large sample size, prospective and randomized controlled trials should be conducted using the definition for PORs as described by the Bologna criteria.

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## Disclosure of conflict of interest

None.

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