Cumulative Live Birth Rate after Three Ovarian Stimulation IVF Cycles for Poor Ovarian Responders According to the Bologna Criteria[*]

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Summary: This study explored the cumulative live birth rate after three ovarian stimulation *in vitro* fertilization (IVF) cycles for poor ovarian responders according to the Bologna criteria. In this retrospective cohort study, 479 poor ovarian responders according to the Bologna criteria in the first ovarian stimulation IVF cycle between July 2006 and January 2012 in our IVF centre were included. The cumulative live birth rate was calculated by optimistic and pessimistic methods. The cumulative live birth rate after three ovarian stimulation IVF cycles for poor ovarian responders according to the Bologna criteria was 12.7%—20.5%. The three-cycle cumulative live birth rate was 18.5%—24.5%, 13.2%—27.4% and 8.6%—14.9% for poor responders aged ≤35 years, 36—39 years and ≥40 years, respectively. In conclusion, poor responders according to the Bologna criteria can receive an acceptable cumulative live birth rate after three ovarian stimulation IVF cycles, especially poor responders aged <40 years.
Key words: ovarian stimulation; *in vitro* fertilization; Bologna criteria; poor responders; cumulative live birth

Controlled ovarian hyperstimulation (COH) was introduced into the *in vitro* fertilization (IVF) treatment for the availability of multiple oocytes retrieved in an effort to improve the pregnancy rates[1, 2]. However, poor response to COH indicates only few oocytes at retrieval, resulting in a reduced number of embryos available for transfer and then a poor pregnancy rate[3]. In IVF treatment, a considerable proportion of women respond poorly to ovarian stimulation, with the prevalence of poor ovarian responders being reported to vary between 5.6% and 35.1%, depending on various definitions of poor ovarian response[4-9].

The prospective identification and then the management of poor response to ovarian stimulation remain significant challenges in the current IVF treatment. Numerous studies have been performed to explore the effects of various treatment modalities on women with impaired ovarian response to stimulation during the past decades. However, there is not an internationally accepted uniform definition for poor ovarian response for a long time, so it is difficult to draw any reliable conclusion by comparing results among heterogeneous populations. Therefore, the ESHRE Working Group recently reached a consensus on the definition of poor ovarian response: the Bologna criteria, in order to include a homogeneous population with poor response in future researches[10]. However, the practical applicability of the Bologna criteria still needs to be examined in future clinical trails[11].

Recently, one trial has demonstrated that poor responders according to the Bologna criteria have a very poor pregnancy prognosis and do not appear to experience substantial benefits with natural cycle IVF, regardless of patient's age[12]. Whereas, it remains unclear whether poor responders according to the Bologna criteria can benefit from ovarian stimulation IVF. In addition, whether poor responders as described by the Bologna criteria are a homogeneous group of patients with the same poor pregnancy prognosis in ovarian stimulation IVF is still unknown. In Chinese mainland, the cost of ovarian stimulation IVF is expensive and all patients have to pay for the cost by themselves. Therefore, precognition of the pregnancy prognosis of Bologna criteria poor responders in ovarian stimulation IVF can help clinicians to counsel this group of patients whether they are worthwhile to continue with future expensive treatment.

The aim of the present study was to explore the cumulative live birth rate after three ovarian stimulation IVF cycles for poor ovarian responders according to the Bologna criteria.

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1 MATERIALS AND METHODS

1.1 Patients

Definition of poor ovarian responder in this study was based on the ESHRE consensus on the definition of poor ovarian response: the Bologna criteria[10]. At least two of the following three features must be present: (1) Advanced maternal age (≥40 years) or any other risk factor for poor ovarian response; (2) Poor ovarian response (≤3 oocytes with a conventional stimulation protocol); (3) An abnormal ovarian reserve test [i.e. antral follicle count (AFC) <7 follicles or anti-Mullerian hormone (AMH) <1.1 ng/mL].

Conventional stimulation protocol was defined as stimulation protocol of at least 150 IU gonadotrophin dose per day.

Between July 2006 and January 2012, 479 poor ovarian responders according to the Bologna criteria in the first ovarian stimulation IVF cycle were included in the present study.

This study was approved by our institutional review board.

1.2 Ovarian Stimulation Protocol

The ovarian stimulation protocols included in the present study were long gonadotrophin-releasing hormone (GnRH) agonist protocol, flare-up GnRH agonist protocol, GnRH antagonist protocol, ultra-long GnRH agonist protocol and Gn/human chorionic gonadotrophin (HCG) protocol.

Long GnRH agonist protocol: pituitary down-regulation was started in the midluteal phase of previous cycle by the administration of GnRH analogue. Ovarian stimulation was started after complete down-regulation. Flare-up GnRH agonist protocol: the administration of GnRH analogue was initiated on the day 2 of menstruation. Ovarian stimulation was started on the day 3 of menstruation. Ultra-long GnRH agonist protocol: long-acting GnRH agonist was administered every 28 days for three times before ovarian stimulation. The first injection was administered during the early follicular phase. Ovarian stimulation was performed 30 to 45 days after the third GnRH agonist injection. GnRH antagonist protocol: ovarian stimulation was commenced on the day 2 or 3 of the menstrual cycle with recombinant follicle-stimulating hormone (FSH) or human menopausal gonadotropins (HMG). Co-treatment with GnRH antagonist was initiated when at least one of the following criteria was fulfilled: (1) the presence of at least one follicle measuring ≥14 mm, (2) serum estradiol (E_2) level ≥600 pg/mL or (3) serum luteinizing hormone (LH) level ≥10 IU/L[13], and continued until and including the day of trigger. Gn/HCG protocol: the administration of Gn was initiated on the day 3—5 of menstruation.

Ovarian stimulation was performed with recombinant FSH, highly purified FSH or HMG. The starting dose of ovarian stimulation was determined according to patients' age, body mass index (BMI), AFC and previous ovarian response. Subsequent adjustment depended on follicular development and serum E_2 level. When at least three follicles reached 17 mm or one follicle reached 18 mm in diameter, HCG was administered for ovulation triggering. Oocyte retrieval was performed 36 h later. Two or three embryos were transferred on the day 3 after oocyte retrieval. Luteal phase support was performed with progesterone, E_2 or HCG supplements.

1.3 Outcome Measures

The main outcome measures of this study included clinical pregnancy rate, live birth rate, three-cycle cumulative pregnancy and live birth rates in ovarian stimulation IVF. In addition, the outcomes of poor responders were respectively analyzed according to the female age. The age thresholds adopted in this study were ≤35, 36—39 and ≥40 years. Patients who did not achieve clinical pregnancy or live birth and discontinued treatment before three cycles were considered as drop-outs, and dropped out of subsequent stimulation cycles due to their own choice. A clinical pregnancy was defined as at least one gestational sac detected on transvaginal ultrasound scan.

1.4 Statistical Methods

The data were analyzed with commercial software (SPSS 16.0; SPSS, USA). The χ^2 test was used for comparing quantitative variables. Continuous variables were compared with one-way ANOVA. A P value of <0.05 was considered statistically significant. The cumulative pregnancy and live birth rates were calculated by two methods proposed by several researchers[14, 15]. In the optimistic method, we assumed that patients dropped out of subsequent stimulation cycles had the same chance of pregnancy and live birth as those continued. However, patients discontinuing treatments were assumed to have no chance of pregnancy and live birth in subsequent cycles in the pessimistic method. The realistic cumulative pregnancy and live birth rates were between the pessimistic and optimistic cumulative rates.

2 RESULTS

2.1 Patients' Characteristics of "Bologna Criteria"-Poor Responders

The patients' characteristics of poor responders according to the Bologna criteria were presented in table 1. The mean age of poor responders according to the Bologna criteria in this study was 37.61±4.93 years. The mean AFC of these poor responders was 4.72±1.77. The mean basal FSH level of "Bologna criteria"-poor responders was 11.42±9.19 U/L. The mean duration of infertility of these poor responders was 6.56±4.54 years. The proportion of "Bologna criteria"-poor responders with primary infertility was 31.3%.

2.2 Cycle Characteristics in the First Cycle of "Bologna Criteria"-Poor Responders

The mean total dose of Gn and duration of stimulation were 3837±2512 IU and 9.72±2.87 days, respectively (table 2). The mean number of oocytes retrieved and available embryos was 2.79±2.31 and 1.60±1.36, respectively (table 2). The cycle cancellation rate of "Bologna criteria"-poor responders was 22.3% (table 2).

2.3 Pregnancy Outcomes of "Bologna Criteria"-Poor Responders

The pregnancy outcomes of poor responders according to the Bologna criteria were presented in table 3. There was no significant difference in clinical pregnancy rate and live birth rate per treatment cycle of poor responders among three stimulation cycles (P=0.069 and P=0.158). The clinical pregnancy rate and live birth rate per treatment cycle in ovarian stimulation IVF were 13.4% and 8.3%, respectively. The three-cycle cumulative clinical pregnancy and live birth rates for poor responders according to the Bologna criteria were

19.8%―34.2% and 12.7%―20.5%, respectively.

Table 1 Patients' characteristics of poor responders according to the Bologna criteria

Variables	
Patients, n	479
Age (years)	37.61±4.93
BMI (kg/m^2)	21.79±2.71
AFC	4.72±1.77
Basal FSH (IU/L)	11.42±9.19
Duration of infertility (years)	6.56±4.54
Primary infertility	150 (31.3)
Cause of infertility	
Tubal factor	132 (27.6)
Endometriosis	11 (2.3)
Male factor	28 (5.8)
Mixed	305 (63.7)
Unexplained	3 (0.6)

Results are expressed as number (percentage) or $\bar{x}\pm s$.

Table 2 Cycle characteristics in the first cycle for poor responders according to the Bologna criteria

Variables	
Cycles, n	479
Stimulation protocol	
Long	112 (23.4)
Flare-up	73 (15.2)
Antagonist	200 (41.8)
Ultra-long	14 (2.9)
Gn/HCG	80 (16.7)
Total Gn dose (IU)	3837±2512
Duration of stimulation (days)	9.72±2.87
Oocytes retrieved	2.79±2.31
Available embryos	1.60±1.36
Cycle cancellation rate	107 (22.3)

Results are expressed as number (percentage) or $\bar{x}\pm s$.

Table 3 Pregnancy outcomes in ovarian stimulation IVF for poor responders according to the Bologna criteria

Variables	Cycle 1	Cycle 2	Cycle 3
Patients, n	479	196	62
Clinical pregnancy per treatment cycle	59 (12.3)	35 (17.9)	5 (8.1)
Clinical pregnancy per transfer cycle	59 (15.9)	35 (20.3)	5 (9.4)
Drop-outs (clinical pregnancy)	236	97	−
Cumulative clinical pregnancy per treatment cycle			
Optimistic	−	132 (27.6)	164 (34.2)
Pessimistic	−	90 (18.8)	95 (19.8)
Live birth per treatment cycle	38 (8.0)	21 (10.7)	2 (3.2)
Live birth per transfer cycle	38 (10.2)	21 (12.2)	2 (3.8)
Drop-outs (live birth)	245	113	−
Cumulative live birth per treatment cycle			
Optimistic	−	85 (17.7)	98 (20.5)
Pessimistic	−	59 (12.3)	61 (12.7)

Results are expressed as number (percentage).

2.4 Patients' Characteristics among Age Subgroups of Poor Responders

The BMI of poor responders aged 36―39 years and aged ≥40 years was significantly higher than that aged ≤35 years (P=0.000) (table 4). The basal FSH level in poor responders aged ≥40 years was significantly lower than that in those aged ≤35 years (P=0.006) (table 4). There was no significant difference in AFC among three age categories (P=0.810) (table 4).

Table 4 Characteristics for age subgroups of poor responders according to the Bologna criteria

Variables	≤35 years	36―39 years	≥40 years	P
Patients, n	151	106	222	
Age (years)	31.46±2.70	37.73±1.32[*]	41.75±1.90[*]	0.000
BMI (kg/m^2)	20.92±2.57	22.22±2.99[*]	22.21±2.52[*]	0.000
AFC	4.67±1.43	4.67±1.63	4.77±2.03	0.810
Basal FSH (IU/L)	13.04±13.44	11.64±8.31	10.22±4.92[*]	0.021

Results are expressed as $\bar{x}\pm s$.
[*]P<0.05 *vs.* patients ≤35 years

2.5 Pregnancy Outcomes among Age Subgroups of Poor Responders

The pregnancy outcomes among age subgroups of poor responders were presented in table 5. Poor responders aged ≥40 years had significantly lower clinical pregnancy and live birth rates per treatment cycle than those aged ≤35 years (P=0.001 and P=0.008). The three-cycle cumulative clinical pregnancy rate was 28.5%―41.1%, 18.9%―37.7% and 14.4%―27.0% for poor responders aged ≤35 years, 36―39 years and ≥40 years, respectively. The cumulative live birth rate after three cycles was 18.5%―24.5%, 13.2%―27.4% and 8.6%―14.9% for three age categories of poor responders, respectively.

Table 5 Pregnancy outcomes in ovarian stimulation IVF for age subgroups of poor responders according to the Bologna criteria

Variables	Total	≤35 years	36—39 years	≥40 years
Patients, *n*	479	151	106	222
Cycles, *n*	737	235	168	334
Clinical pregnancy per cycle	99 (13.4)	46 (19.6)	21 (12.5)	32 (9.6)*
Three-cycle cumulative clinical pregnancy per patient				
Optimistic	164 (34.2)	62 (41.1)	40 (37.7)	60 (27.0)
Pessimistic	95 (19.8)	43 (28.5)	20 (18.9)	32 (14.4)
Live birth per cycle	61 (8.3)	28 (11.9)	14 (8.3)	19 (5.7)*
Three-cycle cumulative live birth per patient				
Optimistic	98 (20.5)	37 (24.5)	29 (27.4)	33 (14.9)
Pessimistic	61 (12.7)	28 (18.5)	14 (13.2)	19 (8.6)

Results are expressed as number (percentage).
*$P<0.05$ *vs.* ≤35 years

3 DISCUSSION

The present study showed that the clinical pregnancy rate per treatment cycle of poor ovarian responders according to the Bologna criteria in the first ovarian stimulation cycle was 12.3%. It has been reported previously that poor responders have a pregnancy rate varying from 7.6% to 17.5%, depending on different definitions of poor responders[4, 5, 8, 16-18]. Although the Bologna criteria of poor ovarian response are stricter than many other definitions, poor responders according to the Bologna criteria still have reasonable pregnancy rate in ovarian stimulation IVF.

The overall live birth rate per treatment cycle in ovarian stimulation IVF for poor ovarian responders according to the Bologna criteria was 8.3%. Polyzos *et al* recently explored the effect of natural cycle IVF in women with poor ovarian response according to the Bologna criteria[12]. Their retrospective cohort trial included 136 poor ovarian responders according to the Bologna criteria (390 natural cycles) and 28 normal ovarian responders (79 natural cycles). The results in their study showed that the overall live birth rate in natural cycle IVF for poor ovarian responders according to the Bologna criteria was prominently lower than that in normal ovarian responders: live birth rate per treatment cycle (2.6% *vs.* 8.9%, $P=0.006$) and live birth rate per patient (7.4% *vs.* 25%, $P=0.005$). Although natural cycle IVF is an easy and cheap treatment modality, the pregnancy prognosis of poor responders according to the Bologna criteria in natural cycle IVF is extremely poor. Poor responders according to the Bologna criteria can achieve more favorable live birth rate in ovarian stimulation IVF than in the natural cycle IVF. In our opinion, ovarian stimulation IVF should be a better option for poor responders according to the Bologna criteria than natural cycle IVF.

Although poor ovarian responders according to the Bologna criteria have a low live birth rate in the first stimulation IVF cycle, these patients can achieve an acceptable cumulative live birth rate after three stimulation cycles (12.7%—20.5%). Likewise, one previous study reported that the cumulative ongoing pregnancy rate of poor responders according to the Bologna criteria was 29.5% after five cycles and 36.4% after seven cycles[19]. The mean age of Bologna criteria-poor responders in this study was 38.5±5.2 years. Even poor responders with all the treatment cycles defined as poor response cycles, can achieve a maximal cumulative pregnancy rate (27.8%) after five treatment cycles. If poor responders according to the Bologna criteria can not receive a live birth in the first stimulation cycle, it is worthwhile for these patients to continue with subsequent stimulation cycles.

Several studies regarding the effect of female age on the pregnancy prognosis in women with poor ovarian response showed a tendency that older patients have lower pregnancy rates than younger patients[3, 5, 18, 20, 21]. The results in our study were absolutely in accordance with this conclusion. Bologna criteria-poor responders aged ≤35 years had prominently more favorable pregnancy prospects than those aged ≥40 years, even if Bologna criteria-poor responders aged ≥40 years in this study had significantly lower basal FSH level than those aged ≤35 years. It can be concluded that poor responders as described by the Bologna criteria are not a homogeneous group of patients with regard to pregnancy prospects according to female age in ovarian stimulation IVF. In our opinion, poor responders according to the Bologna criteria should be encouraged to attempt further ovarian stimulation IVF as soon as possible because their pregnancy rate will decrease with advancing age. Bologna criteria-poor responders aged ≥40 years should be appropriately informed the poor pregnancy prognosis in ovarian stimulation IVF and decide by themselves whether to continue with further treatments.

In conclusion, poor responders according to the Bologna criteria in the first stimulation cycle can achieve an acceptable cumulative pregnancy prospects after three ovarian stimulation IVF cycles, especially poor responders aged <40 years.

REFERENCES

1　Healy DL, Okamato S, Morrow L, *et al*. Contributions of *in vitro* fertilization to knowledge of the reproductive endocrinology of the menstrual cycle. Baillieres Clin Endocrinol Metab, 1987,1(1):133-152

2　Jennings JC, Moreland K, Peterson CM. *In vitro* fertilisation. A review of drug therapy and clinical management. Drugs, 1996,52(3):313-343

3　Ulug U, Ben-Shlomo I, Turan E, *et al*. Conception rates following assisted reproduction in poor responder patients: a retrospective study in 300 consecutive cycles. Reprod Biomed Online, 2003,6(4):439-443

4   Biljan MM, Buckett WM, Dean N, *et al*. The outcome of
     IVF-embryo transfer treatment in patients who develop
     three follicles or less. Hum Reprod, 2000,15(10):
     2140-2144
5   De Sutter P, Dhont M. Poor response after hormonal
     stimulation for *in vitro* fertilization is not related to ovar-
     ian aging. Fertil Steril, 2003,79(6):1294-1298
6   Inge GB, Brinsden PR, Elder KT. Oocyte number per live
     birth in IVF: were Steptoe and Edwards less wasteful?
     Hum Reprod, 2005,20(3):588-592
7   Veleva Z, Jarvela IY, Nuojua-Huttunen S, *et al*. An initial
     low response predicts poor outcome in *in vitro* fertiliza-
     tion/intracytoplasmic sperm injection despite improved
     ovarian response in consecutive cycles. Fertil Steril,
     2005,83(5):1384-1390
8   Hendriks DJ, te Velde ER, Looman CW, *et al*. Expected
     poor ovarian response in predicting cumulative preg-
     nancy rates: a powerful tool. Reprod Biomed Online,
     2008,17(5):727-736
9   Orvieto R, Meltcer S, Nahum R, *et al*. The influence of
     body mass index on *in vitro* fertilization outcome. Int J
     Gynaecol Obstet, 2009,104(1):53-55
10   Ferraretti AP, La Marca A, Fauser BC, *et al*. ESHRE
     consensus on the definition of 'poor response' to ovarian
     stimulation for *in vitro* fertilization: the Bologna criteria.
     Hum Reprod, 2011,26(7):1616-1624
11   Polyzos NP, Devroey P. A systematic review of random-
     ized trials for the treatment of poor ovarian responders: is
     there any light at the end of the tunnel? Fertil Steril,
     2011,96(5):1058-1061
12   Polyzos NP, Blockeel C, Verpoest W, *et al*. Live birth
     rates following natural cycle IVF in women with poor
     ovarian response according to the Bologna criteria. Hum
     Reprod, 2012,27(12):3481-3486
13   Lainas TG, Sfontouris IA, Zorzovilis IZ, *et al*. Flexible

     GnRH antagonist protocol versus GnRH agonist long
     protocol in patients with polycystic ovary syndrome
     treated for IVF: a prospective randomised controlled trial
     (RCT). Hum Reprod, 2010,25(3):683-689
14   Stolwijk AM, Hamilton CJ, Hollanders JM, *et al*. A more
     realistic approach to the cumulative pregnancy rate after
     in-vitro fertilization. Hum Reprod, 1996,11(3):660-663
15   Olivius K, Friden B, Lundin K, *et al*. Cumulative prob-
     ability of live birth after three *in vitro* fertiliza-
     tion/intracytoplasmic sperm injection cycles. Fertil Steril,
     2002,77(3):505-510
16   Saldeen P, Kallen K, Sundstrom P. The probability of
     successful IVF outcome after poor ovarian response. Acta
     Obstet Gynecol Scand, 2007,86(4):457-461
17   Timeva T, Milachich T, Antonova I, *et al*. Correlation
     between number of retrieved oocytes and pregnancy rate
     after *in vitro* fertilization/intracytoplasmic sperm infec-
     tion. Sci World J, 2006,6:686-690
18   Zhen XM, Qiao J, Li R, *et al*. The clinical analysis of
     poor ovarian response in *in-vitro*-fertilization em-
     bryo-transfer among Chinese couples. J Assist Reprod
     Genet, 2008,25(1):17-22
19   Maman E, Geva LL, Yerushalmi G, *et al*. ICSI increases
     ongoing pregnancy rates in patients with poor response
     cycle: multivariate analysis of 2819 cycles. Reprod Bio-
     med Online, 2012,25(6):635-641
20   Hanoch J, Lavy Y, Holzer H, *et al*. Young low respond-
     ers protected from untoward effects of reduced ovarian
     response. Fertil Steril, 1998,69(6):1001-1004
21   Galey-Fontaine J, Cedrin-Durnerin I, Chaibi R, *et al*. Age
     and ovarian reserve are distinct predictive factors of cycle
     outcome in low responders. Reprod Biomed Online,
     2005,10(1):94-99