Taiwanese Journal of Obstetrics & Gynecology 56 (2017) 175–180

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Age is a major prognosticator in extremely low oocyte retrieval cycles

Yu-Ting Su, Pin-Yao Lin, Fu-Jen Huang, Fu-Tsai Kung, Yu-Ju Lin, Yi-Ru Tsai, Kuo-Chung Lan*

Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taiwan

ARTICLE INFO

Article history:
Accepted 18 April 2016

Keywords:
age
Bologna criteria
embryo quality
poor ovarian response
stratification

ABSTRACT

Objective: Clinical prognosis appears to be varied in females with poor ovarian response (POR), and poor responders defined by the Bologna criteria might not be sufficiently homogeneous. The aim of this study was to determine the major predictor of reproductive outcomes in extremely low oocyte retrieval cycles.
Materials and Methods: A cohort of fresh *in vitro* fertilization/intracytoplasmic sperm injection cycles ($n = 858$) was analyzed from January 2001 to September 2014. Females from whom zero, one, two, or three oocytes were retrieved following ovarian stimulation were examined. Univariate analyses were performed to determine the association of pregnancy rate with potential confounding variables. Multiple logistic regression analysis was subsequently performed to identify factors that affected the occurrence of pregnancy.
Results: The clinical pregnancy rate was higher in women aged < 40 years, long protocol, and high embryo score in univariate analysis. After adjusting for confounding factors in multivariate analysis, the maternal age [odds ratio (OR) = 0.91], primary or secondary infertility (OR = 1.99), number of matured oocytes retrieved (OR = 0.64), and score of embryos transferred (OR = 1.39) were significantly associated with the clinical pregnancy rate per cycle and per transfer. In the age subgroup analysis, POR females aged < 35 years significantly demonstrated the highest number of matured oocytes, embryo scores, and clinical pregnancy rates compared with POR females aged 35–40 years and ≥ 40 years.
Conclusion: This study highlights the predictive value of maternal age and embryo quality on the probability of pregnancy in females with extremely low oocyte retrieval cycles. Young females with few eggs collected can still achieve acceptable pregnancy probability as long as they have good-quality embryos. Future randomized control trials for POR using the Bologna criteria should first stratify patients into different age groups.

Introduction

Controlled ovarian hyperstimulation (COH) employs exogenous gonadotropin administration to recruit a large number of oocytes for *in vitro* fertilization (IVF), such that high-quality embryos can be selected for transfer. However, patient response to ovarian stimulation can be highly variable, and this response is a major determinant of treatment outcome. Females with oocyte counts < 5 have a significantly lower cumulative conception pregnancy rate than those with normal responses (> 5 oocytes retrieved) [1].

Reliable predictors of pregnancy outcome would provide physicians with valuable information for counseling patients on whether to start a cycle. The most common tests for predicting ovarian reserve are anti-Mullerian hormone (AMH) and antral follicle counts. These tests have demonstrated a correlation to ovarian response in IVF even though the prediction of successful pregnancy remains controversial [2–4]. Moreover, there is apparently no solid link between the quantity and quality of oocytes.

Despite the unfavorable prognosis of females with poor ovarian response (POR), some studies have reported a reasonable pregnancy rate for such women [5,6]. Thus, it appears that the prognosis varies among poor responders after COH for IVF/intracytoplasmic sperm injection (ICSI) [7]. It is possible that the characteristics or treatments of specific females may provide them with an acceptable prognosis, although they produce an extremely low number of oocytes in the current cycle.

The management of POR has been a major challenge for clinicians because it is difficult to compare different treatments due to the use of different definitions of POR over the past 2 decades. In an effort to standardize the terminology, the European Society of

* Corresponding author. Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Number 123, DaPi Road, Niaosung District, Kaohsiung, Taiwan.
E-mail address: lankuochung@gmail.com (K.-C. Lan).

http://dx.doi.org/10.1016/j.tjog.2016.04.039

Human Reproduction and Embryology (ESHRE) published the Bologna criteria in 2011 to define POR [8]. According to these criteria, at least two of the following three features must be present: (1) advanced maternal age (≥ 40 years) or any other risk factor for POR; (2) previous experience of POR (≤ 3 oocytes with a conventional stimulation protocol); and (3) abnormal ovarian reserve tests (ORTs) result (i.e., antral follicle count of 5–7 or AMH level of 0.5–1.1 ng/mL). However, recent research studies have suggested that females classified as having POR by these criteria are highly heterogeneous [9], and thus, the adoption of these criteria is still debated [10,11].

The aim of present study was to identify significant predictor variables for pregnancy in females from whom an extremely low number of oocytes were collected. On the basis of the results of our analysis, we aimed to determine whether there is a prognosis difference in POR using the Bologna criteria.

Materials and Methods

Participants

This retrospective cohort study reviewed the medical records of infertile couples that underwent IVF/ICSI from 2001 to 2014 at our institution. All females who obtained zero, one, two, or three oocytes at retrieval were included. Cycles in which ovarian stimulation employed clomiphene, gonadotropin, and gonadotropin-releasing agonist/antagonist were examined. Cycles with the administration of Corifollitropin alfa (Elonva; NV Organon, Oss, the Netherlands), frozen–thawed embryo cycles, and donor egg cycles were excluded. To reflect routine clinical practice in which poor responders may be present, there were no other exclusion criteria.

The characteristics of all patients were evaluated, including age, body mass index, family history, risk factors of POR (chronic smoking, drinking, previous ovarian surgery, previous chemotherapy), primary or secondary infertility, cause of infertility, hormone levels [basal follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2) on cycle Day 3 and AMH]. We do not refuse treatment solely based on ultralow AMH levels or higher basal FSH levels [12,13]. Treatment characteristics, such as protocol, IVF or ICSI, and adjuvant r-LH supplement, were analyzed. Follicle size on human chorionic gonadotropin (hCG) day and number of mature oocytes at retrieval were used as determinants of ovarian response. Gonadotropin dose adjustments may affect the response; thus, total gonadotropin dose used during the cycle was also used as a determinant of ovarian response. For the change in IVF practice during a long period of 14 years, the treatment period was considered (2001–2005, 2006–2010, 2011–2014). The score of the embryo transferred was also used as variables to predict clinical pregnancy.

The Institutional Review Board and the Ethics Committee of Chang Gung Memorial Hospital (Kaohsiung, Taiwan) approved this study (CGMF IRB No.: 104-8485C).

Controlled ovarian stimulation

The ovarian stimulation protocols included gonadotropin-releasing hormone (GnRH) agonist long, GnRH agonist short, and GnRH antagonist protocols, all of which were performed following standard clinical practice. The protocol for each patient was selected according to the ovarian reserve, which assessed the patient's age, baseline serum FSH concentration, previous ovarian response to gonadotropins, and the preference of each clinician. Each patient was administered an initial dose of 150–300 IU human menopausal gonadotropin or FSH (purified or recombinant), and dose adjustments during the cycle were determined individually based on the response to gonadotropin as assessed by serum E_2 concentration and sonographic monitoring of follicular growth. In addition, r-LH (Luveris; Serono) was administered in females with poor responses in a previous cycle or suboptimal follicular progression in a current cycle, starting on Day 1 or Day 6 of FSH stimulation at a daily fixed dose of 75 IU throughout the treatment period. Females undergoing the GnRH antagonist protocol that had at least one leading follicle measuring > 14 mm in diameter received an additional 0.25 mg/day GnRH (Cetrotide, Merk Serono) until the day of hCG injection. When two more follicles had matured (follicle diameter ≥ 16 mm), a 6500 IU dose of hCG (Ovidrel, Merk Serono) was administered, and oocyte retrieval was performed 36–38 hours later by transvaginal aspiration under ultrasound guidance. Standard IVF or ICSI procedures were used for oocyte fertilization, as previously described [14].

The choice between Day 3 embryo transfer and extended culture to blastocyst and transfer on Day 5 was based on embryo quality and number. The luteal phase was supported by intravaginal administration of progesterone (90 mg) vaginal gel once daily (Crinone 8%, Serono Pharmaceuticals Ltd.) or micronized progesterone vaginal capsules (200 mg) four times daily (Ultrogestan, Laboratories Besins International), starting on the day after oocyte retrieval.

Hormone measurements

FSH and LH levels were measured on Day 3 of the menstrual cycle before gonadotropin administration. Serum concentrations of progesterone and E_2 were measured on the day of hCG administration during each IVF cycle, where progesterone is expressed as ng/mL and E2 as pg/mL. Serum concentrations of E_2 and progesterone were determined using standard immunoassay systems (ADVIA Centaur ® XP, Siemens, USA). The intra- and inter-assay coefficients of variation were 5.0% and 4.1% for E_2 and 5.2% and 3.5% for progesterone.

Oocyte grading

According to nuclear maturation grading, the oocytes were classified into categories, metaphase II (mature) or non-metaphase II. The latter category included oocytes at the metaphase I and prophase I stages. For IVF, the retrieved oocyte–corona–cumulus complexes were immediately classified according to their maturity. For ICSI, the oocyte–corona–cumulus complexes were denuded and assessed shortly after retrieval. The denuded oocytes were cultured in an M2 culture medium (Medicult, Denmark) for 3–8 hours and were subsequently examined for the presence of the first polar body. After confirmation of the first polar body, ICSI was performed. The oocytes that did not develop to metaphase II after 8 hours of incubation were discarded. The oocyte preparation has been previously described in detail [14].

Assessment of fertilization, embryo culture, and zygote and embryo grading

ICSI and conventional IVF were performed according to standard procedures. Briefly, oocyte–corona–cumulus complexes were cultured in IVF Medium (Medicult, Denmark) for 4–6 hours, and the oocytes were inseminated with approximately 10^5 motile spermatozoa/mL in 1 mL of IVF medium. The oocytes were transferred from the insemination medium to fresh IVF medium and cultured. Our ICSI procedure has been previously described in detail [14].

Fertilization was evaluated after 16–18 hours. Normal fertilization was defined by the formation of zygotes with two pronuclei.

The zygotes were evaluated using the Z-score system [12]. Embryos were cultured on Days 1–3 in G1.2 TM medium (Scandinavian IVF Science) and on Days 3–5 in G2.2 TM medium (Scandinavian IVF Science). On Day 3, all embryos were graded on a scale of 0–4, which was based on a modification of Veeck's morphological grading system and our previous report [14].

Outcome variables

The major outcome was clinical pregnancy, which was defined as the presence of a gestational sac(s) on transvaginal ultrasound after 6–7 weeks of gestation. Clinical abortion was recorded if pregnancy loss occurred between the clinical detection of a pregnancy and the 22nd week of gestation.

Statistical analysis

All data were analyzed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as the mean and standard deviation and compared using Student's *t* test or one-way analysis of variance with least significant difference *post hoc* test. Categorical variables were expressed as the proportion and percentage, and they were compared using the Chi-square test.

For this analysis, the effects of potential clinical and laboratory factors described above were examined. The statistical significance of each variable was first evaluated using univariate analysis. Specifically, multivariate logistic regression analysis was used to identify the independent prognostic factors. A p value of < 0.05 was considered to be statistically significant in all analyses.

Results

We examined the records of 858 IVF/ICSI cycles in 624 females who were treated at our institution during the 14-year study period. Table 1 shows the characteristics of these patients and the types of assisted reproduction techniques that were used.

Table 2 shows the outcomes of the IVF procedures. Overall, no oocytes were retrieved in 77 cycles (8.97%), at least one oocyte was retrieved for culture in 781 cycles (91.0%), and at least one embryo was retrieved for transfer in 629 cycles (73.3%). The clinical pregnancy rate was 21.9% per transfer and 16.0% per cycle.

Different variables including maternal and treatment characteristics associated with pregnancy were determined using univariate analysis (Table 3). The clinical pregnancy rates per cycle and per transfer were both statistically significantly higher in females who were younger and those who received a long protocol, respectively. As long as patients had available embryos for transfer, the clinical pregnancy rate per transfer was significantly higher in females with a higher embryo score. There were no significant effects of menstrual regularity, body weight index, paternal age, chronic smoking, drinking, previous ovarian surgery, previous chemotherapy, duration of subfertility, previous attempts, fertilization methods, different time frames that the data were separated, r-LH administration, endometrium thickness, gonadotropins dose, duration of stimulation, and number of follicles >1.6 cm on hCG day.

Furthermore, we performed a multivariable logistic regression analysis to determine the independent effects of these factors with adjustment for potential confounders (Table 4). These results indicated that the rates of pregnancy per cycle and per transfer were significantly and independently associated with younger maternal age, secondary infertility, higher number of matured oocytes, and a higher embryo score.

These results clearly indicated the importance of maternal age on successful outcome. Thus, we divided all cycles according to maternal age using the Bologna criteria into those in which the female was < 40 years ($n = 592$) and ≥ 40 years ($n = 266$) (Table 5). These results demonstrated that the younger group had significantly more favorable outcomes than the older group, including the score of the transferred embryo (5.1 ± 3.0 vs. 4.2 ± 2.8, $p = 0.042$), implantation rate (19.2% vs. 9.6%, $p < 0.001$), and clinical pregnancy rates per cycle (20.7% vs. 5.3%, $p < 0.001$) and per transfer (27.7% vs. 7.6%, $p < 0.001$).

To clarify the association between age and clinical pregnancy rate, all cycles were divided into females < 35 years ($n = 257$), between 35 years and 40 years ($n = 335$), and 40 years or older ($n = 266$) (Table 6). A higher clinical pregnancy rate per cycle and per transfer were both significantly associated with the younger group, with females aged < 35 years showing a significantly higher pregnancy rate per transfer than the other two groups (28.5%, 27.1%, and 7.6% for < 35 years, 35–40 years and ≥ 40 years, respectively, $p < 0.001$). A higher score of embryo transferred and higher number of mature oocytes were also significantly associated with the younger group.

We further analyzed the correlation between embryo quality and pregnancy rate in only POR females with advanced age. Females aged ≥ 40 years were divided into two subgroups with an embryo score < 4 and score ≥ 4. No statistically significantly difference was observed in the clinical pregnancy rate of the two subgroups (4.1% and 9.8% for embryo score < 4 and score ≥ 4, respectively, $p = 0.254$).

Table 1
Demographic characteristics of women undergoing *in vitro* fertilization/intracytoplasmic sperm injection with extremely low oocyte retrieval cycles ($n = 858$).

Characteristics	mean ± SD (range) or number (%)
Age of female, y	36.9 ± 4.5 (22–48)
Age of partner, y	38.8 ± 5.3 (26–61)
Body mass index, kg/m^2	22.2 ± 3.3 (17.3–34.0)
Menstrual cycle	
Regular	760 (88.6%)
Irregular	98 (11.4%)
Duration of infertility, y	4.9 ± 3.4
Diagnostic category	
Tubal factor	121 (14.1%)
Ovarian factor	252 (29.4%)
Endometriosis	180 (21%)
Uterine factor	31 (3.6%)
Male factor	73 (8.5%)
Combined factor	99 (11.5%)
Unexplained factor	102 (11.9%)
Infertility	
Primary	554 (64.6%)
Secondary	304 (35.4%)
Previous assisted reproduction attempts	1.2 ± 2.0 (1–13)
Protocol	
Antagonist protocol	233 (27.2%)
Short protocol	242 (28.2%)
Long protocol	353 (41.1%)
Others	30 (3.5%)
FSH Ampoules (75 IU)	38.1 ± 17.8
Day 3 FSH, mIU/mL	9.9 ± 6.7
EM thickness on hCG day, cm	1.2 ± 0.3
E_2 on hCG day, pg/mL	877.4 ± 661.7
P on hCG day, pg/mL	1.1 ± 1.5
No. of follicle size (≥ 1.6 cm) on hCG day	2.4 ± 1.5

EM = endometrium; E_2 = estradiol; FSH = follicle stimulating hormone; hCG = human chorionic gonadotropin; P = progesterone; SD = standard deviation.

Discussion

Previous studies attempting to predict the poor response to COH using female age, ORTs, and multivariable prediction models have

Table 2
Results of oocyte retrieval.

Characteristics	Mean ± SD (range) or number (%)
No. of cycles with at least one oocyte retrieved	781 (91.0)
No. of cycles with zero oocytes retrieved	77 (8.97)
Average of oocytes retrieved	2.0 ± 1.0 (0–3)
Average of mature oocytes retrieved	1.0 ± 0.7 (0–3)
Fertilization method	
IVF	564 (72.2)
ICSI	217 (27.8)
No. of cycles of transfer	629 (73.3)
Fertilization rate	68.0%
Cumulative embryo score per transfer	4.8 ± 3.0 (0–12)
Implantation rate	16.8%
Clinical pregnancy rate	
per transfer	137 (21.8)
per cycle	137 (16.0)
Clinical abortion rate	9.6%

ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization; SD = standard deviation.

Table 3
Univariate analysis of variables related to clinical pregnancy.

Variable	% per cycle	*p*	% per transfer	*p*
Age of female, y		0.005		< 0.001
< 35 (n = 257)	21.4		28.5	
35–40 (n = 335)	20.3		27.1	
≥ 40 (n = 266)	5.3		7.6	
Protocol		< 0.001		0.043
Antagonist (n = 233)	11.6		17.8	
Short (n = 242)	10.7		14.9	
Long (n = 353)	22.7		27.4	
Modified NC (n = 30)	6.7		18.2	
Embryo score				< 0.001
< 4 (n = 190)	-		7.9	
≥ 4 (n = 439)	-		27.3	

Data are presented as means.
NC = natural cycle.

been reported [15–17]. In addition, whether a history of poor response in a previous cycle can predict a poor prognosis is still controversial [5]. However, these reports only examined the effects of a small number of parameters. Moreover, other evidence indicates that ORT has limited accuracy in predicting successful IVF [18]. Our study examined a large number of cycles to identify valuable prognostic factors associated with pregnancy in women who were poor responders to IVF. Although ESHRE have provided a standard definition of POR, we were able to apply a stricter definition to examine our data. We also used multivariable analysis to determine the independent effect of these parameters, including numerous patient characteristics, ovarian response, and data related to the IVF cycle. The major finding of our multivariable analysis, which was adjusted for variables known to be associated with pregnancy outcome, was that young maternal age, high embryo quality, large number of mature oocytes, and a history of pregnancy were the most important factors associated with pregnancy in POR.

Maternal age has a well-established value in predicting the outcome of assisted reproductive technologies, and pregnancy rates decline with age [19]. Indeed, the prevalence of POR is greater than 50% in females aged > 40 years [8]. Our results were consistent with those reported in the literature [20], which demonstrated that consideration of age significantly improved the prediction of clinical pregnancy in females with POR. However, in contrast to Zhen et al [20], we investigated more predictive factors and exclusively examined females who produced extremely low numbers of oocytes (≤ 3). Our results indicated that maternal age is the major factor for successful IVF outcome in low oocytes producers, but the number of mature oocytes and embryo quality are also important.

Primordial follicles decline steadily throughout life, with abrupt changes occurring in the exponential rate when females are approximately 38 years old [21]. However, fecundity declines with advancing age due to decreased oocyte quality. The decline in the quality of oocytes appears to result from an increase in meiotic nondisjunction events, resulting in increased aneuploidy in the early embryo and adverse effects on the development and potential for implantation [22]. Interestingly, we demonstrated that clinical pregnancy rates were not significantly different in two embryo score subgroups in POR females aged ≥ 40 years. An explanation for the findings could be the age-related rate of aneuploidy. Aneuploidy is associated with maternal age and is only subtly related to the morphological appearance of the embryo [23]. Some infertile young females present with a poor response to gonadotropin stimulation caused by previous ovarian surgery, chemotherapy or other unexplained mechanisms. We found that females who had POR but were aged < 35 years had significantly better embryo quality and a higher pregnancy rate than those who were aged > 35 years, which was consistent with findings obtained from a large prospective trial including 144,000 cycles [17]. Our finding indicated that maternal age predominantly modulates oocyte quality, although it is also independent of changing the residual follicle pool. Our results are supported by those obtained in a study, demonstrating that the quality of oocytes and embryos from young females with POR was not inferior to that of normal responders

Table 4
Binary logistic regression analysis of variables associated with clinical pregnancy.

Variable	Clinical pregnancy rate *per cycle*					
	B	SEM	Wald	*p*	Exp(B)	95% CI
Age of female	−0.090	0.027	11.094	0.001	0.914	0.875–0.971
Primary/Secondary infertility	0.632	0.271	5.443	0.020	1.882	1.041–2.963
Number of mature oocytes	−0.471	0.210	5.010	0.025	0.625	0.392–0.896
Score of transferred embryo	0.279	0.048	33.286	< 0.001	1.321	1.299–1.565
Variable	Clinical pregnancy rate *per transfer*					
	B	SEM	Wald	*p*	Exp(B)	95% CI
Age of female	−0.090	0.027	11.094	0.001	0.914	0.867–0.964
Primary/Secondary infertility	0.632	0.271	5.443	0.020	1.882	1.106–3.201
Number of mature oocytes	−0.471	0.210	5.010	0.025	0.625	0.414–0.943
Score of transferred embryo	0.279	0.048	33.286	< 0.000	1.321	1.202–1.452

B = intercept; CI = confidence interval; Exp(B) = odds ratio; SEM = standard error of the mean; Wald = Wald statistic.

Table 5
Outcomes of younger (< 40 years) and older (≥ 40 years) women.

	< 40 years (n = 592)	≥ 40 years (n = 266)	p
Age, y	35.6 ± 3.4 (21–39)	42 ± 1.9 (40–48)	
Infertility			0.007
Primary	400 (67.6)	154 (57.9)	
Secondary	192 (32.4)	112 (42.1)	
No. of FSH Ampoules (75 IU)	37.6 ± 16.2	41.0 ± 19.5	0.015
No. of oocytes retrieved	2.0 ± 1.0 (0–3)	1.9 ± 1.0 (0–3)	0.268
No. of mature oocytes retrieved	1.0 ± 0.8 (0–3)	0.9 ± 0.7 (0–2)	0.864
Normal fertilization rate, %	76.3	72.1	0.102
Score of transferred embryo	5.1 ± 3.0	4.2 ± 2.8	0.042
Number of embryos per transfer	1.9 ± 0.7	1.7 ± 0.7	0.134
Implantation rate, %	19.2	9.6	< 0.001
Clinical pregnancy rate, %			
Per cycle	123/592 (20.7)	14/266 (5.3)	< 0.001
Per transfer	123/444 (27.7)	14/185 (7.6)	< 0.001

Values indicate mean ± SD (range) or number (%).

[24]. Weghofer et al [25] also reported that women with extremely low-serum AMH level still demonstrate reasonable pregnancy with assisted reproduction, and the potential is age-dependent.

Thus, even when a young woman has POR, she may still have the potential to produce mature oocytes and high-quality embryos. Moreover, the transfer of one selected high-quality embryo is the current policy used to avoid multiple gestations [26] and is also a promising method for young women with fewer oocytes collected. Thus, it is important to prevent cycle cancellation in young women with POR. Although the young women in our study had a better pregnancy rate, the diminution of the follicle count to 0 remains a significant problem.

The association between the number of oocytes retrieved and pregnancy in fresh cycles has been demonstrated [27]. With more oocytes retrieved for culture, a higher number of embryos to transfer and a higher probability of pregnancy was observed. Multiple regression analysis illustrated the independent effect of the number of matured oocytes collected on pregnancy in poor responders. We emphasize that the degree of poor response to gonadotropins stimulation can be a prognostic factor for POR.

Thus, in females with POR who are undergoing IVF, the fundamental goal is to increase the number of matured oocytes and to improve embryo quality. The present study also analyzed the effect of specific treatment characteristics that have been investigated. Although numerous strategies had been proposed for the management of poor responders, no effective treatments are currently available [28].

High doses of gonadotropins are commonly administered to females who are expected to be poor responders, but Berkkanoglu and Ozgur [29] have shown no benefit of using this approach. Similarly, our results suggested no benefit from using a higher dose of FSH. When examining the effect of the fertilization method, our data were consistent with those obtained in previous studies [30], such that the pregnancy rate was similar in females with POR who were administered ICSI or IVF. One meta-analysis suggested that there were insufficient data to support a beneficial effect of administering r-LH during pregnancy [31], but a more recent meta-analysis demonstrated an increased rate of clinical pregnancy when r-LH and FSH were administered to females who had POR [32]. Our logistic regression analysis indicated no significant effect of r-LH administration.

A systematic review indicated that 41 different definitions have been used for POR [10], and this incongruence has significantly limited the validity of the meta-analysis studies of this phenomenon [33,34]. To overcome this challenge, ESHRE published a consensus statement in Bologna in 2011 to provide a uniform definition of POR that can be used for subsequent studies in the field of assisted conception [8].

We support the effort to standardize the definition of POR because this will help to reduce sample heterogeneity and improve the external validity and generalizability of future trials. However, the prognostic value of the Bologna criteria in defining POR remains problematic due to the clinical heterogeneity of females classified as having POR and because of the variation in the underlying unclear mechanisms and risk factors [35,36]. For example, advanced age appears to be a distinct risk factor, while other factors, such as ovarian surgery, remain unclear [37]. In our study of females who produced low numbers of oocytes, we observed no significant effect of previous ovarian surgery on the rate of pregnancy. The result of this study suggests that a prognostic difference exists between maternal age and other risk factors.

Some researchers propose that the Bologna criteria might group together diverse subpopulations that have different prognostic classifications [9,38]. Papathanasiou [39] suggested that stratified randomized controlled trials (RCTs) provide an alternative methodological approach to achieving a balanced patient allocation. We agree with the spirit of this approach but believe that the strata should be as small as possible [40]. In addition, on the basis of our data, female age is one of the most important confounders. In particular, young and old women defined as having POR by the Bologna criteria have very different prognoses. Thus, when designing RCTs based on the Bologna criteria, bias may be introduced if female age is not considered during the allocation into different intervention groups. We suggest that age stratification be performed when designing RCTs that test the effects of different interventions based on the Bologna criteria.

A limitation of our study is the retrospective design in a single hospital. However, our study provides statistically significant evidence that female age is the crucial factor by a number of cases. Further RCTs based on the Bologna criteria with age stratification are suggested to confirm these findings. Another limitation of our study could be that AMH levels have been checked only within recent years because it has been gaining popularity recently. We hypothesized that the female with extremely low oocytes yield had a lower serum AMH level. Although the data obtained from 14 years might be obscured, the treatment period as a variable has no significant correlation with pregnancy outcome in the multivariate analysis.

In conclusion, this study highlights the importance of female age and embryo quality on the probability of pregnancy in females who produce extremely few oocytes for COH. When clinicians encounter patients with extremely low numbers of oocytes in current cycles, continuation of the program is still recommended in young patients because they have a greater probability for pregnancy even if

Table 6
Comparison of clinical outcome between different age group of POR women.

	< 35 years (n = 257)	35–40 years (n = 335)	≥ 40 years (n = 266)	p
Age, y	31.5 ± 2.5	37.0 ± 1.4	41.9 ± 3.1	
No. of mature oocytes retrieved	1.0 ± 0.7	1.0 ± 0.7	0.9 ± 0.7	0.015[a/b]
Score of embryo transferred	5.13 ± 3.00	5.10 ± 3.05	4.20 ± 2.87	0.002[a/b]
Clinical pregnancy rate, %				
Per cycle	55/257 (21.4)	68/335 (20.3)	14/266 (5.3)	0.005[a/b]
Per transfer	55/193 (28.5)	68/251 (27.1)	14/185 (7.6)	<0.001[a/b]

Data are presented as mean ± SD or number (%).
p^a value correspond on *post hoc* analysis between < 35 years and ≥ 40 years. p^b value correspond on *post hoc* analysis between 35–40 years and ≥ 40 years.

one embryo develops for transfer. In addition, we suggest that future RCTs that examine the effects of different treatment on POR by the Bologna criteria should first stratify patients into different age groups.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

We thank Embryologist, Chi-Yi Lin who provided helpful and useful assistance. We also acknowledge Registered Nurse, Yun-Fang Chiang for assistance in patient registration and data collection.

References

[1] Sharma V, Allgar V, Rajkhowa M. Factors influencing the cumulative conception rate and discontinuation of in vitro fertilization treatment for infertility. Fertil Steril 2002;78:40–6.
[2] Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Hum Reprod Update 2013;19:26–36.
[3] Fleming R, Broekmans F, Calhaz-Jorge C, Dracea L, Alexander H, Nyboe Andersen A, et al. Can anti-Mullerian hormone concentrations be used to determine gonadotrophin dose and treatment protocol for ovarian stimulation? Reprod Biomed Online 2013;26:431–9.
[4] Iliodromiti S, Kelsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-Mullerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. Hum Reprod Update 2014;20:56070.
[5] Klinkert ER, Broekmans FJ, Looman CW, Te Velde ER. A poor response in the first in vitro fertilization cycle is not necessarily related to a poor prognosis in subsequent cycles. Fertil Steril 2004;81:1247–53.
[6] Hendriks DJ, te Velde ER, Looman CW, Bancsi LF, Broekmans FJ. Expected poor ovarian response in predicting cumulative pregnancy rates: a powerful tool. Reprod Biomed Online 2008;17:727–36.
[7] Oudendijk JF, Yarde F, Eijkemans MJ, Broekmans FJ, Broer SL. The poor responder in IVF: is the prognosis always poor?: a systematic review. Hum Reprod Update 2012;18:1–11.
[8] Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, et al. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod 2011;26:1616–24.
[9] Ferraretti AP, Gianaroli L. The Bologna criteria for the definition of poor ovarian responders: is there a need for revision? Hum Reprod 2014;29:1842–5.
[10] Polyzos NP, Devroey P. A systematic review of randomized trials for the treatment of poor ovarian responders: is there any light at the end of the tunnel? Fertil Steril 2011;96. 1058–61 e7.
[11] Younis JS, Ben-Ami M, Ben-Shlomo I. The Bologna criteria for poor ovarian response: a contemporary critical appraisal. J Ovarian Res 2015;8:76.
[12] Seifer DB, Tal O, Wantman E, Edul P, Baker VL. Prognostic indicators of assisted reproduction technology outcomes of cycles with ultralow serum anti-mullerian hormone: a multivariate analysis of over 5,000 autologous cycles from the society for assisted reproductive technology clinic outcome reporting system database for 2012–2013. Fertil Steril 2016;105. 385–93 e3.
[13] Goswami D, Arif A, Saxena A, Batra S. Idiopathic primary ovarian insufficiency: a study of serial hormonal profiles to assess ovarian follicular activity. Hum Reprod 2011;26:2218–25.
[14] Lan KC, Huang FJ, Lin YC, Kung FT, Hsieh CH, Huang HW, et al. The predictive value of using a combined Z-score and day 3 embryo morphology score in the assessment of embryo survival on day 5. Hum Reprod 2003;18:1299–306.
[15] La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 2010;16:113–30.
[16] Younis JS, Jadaon J, Izhaki I, Haddad S, Radin O, Bar-Ami S, et al. A simple multivariate score could predict ovarian reserve, as well as pregnancy rate, in infertile women. Fertil Steril 2010;94:655–61.
[17] Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from in vitro fertilisation: a prospective study of 144,018 treatment cycles. PLoS Med 2011;8:e1000386.
[18] Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006;12:685–718.
[19] Horcajadas JA, Pellicer A, Simon C. Wide genomic analysis of human endometrial receptivity: new times, new opportunities. Hum Reprod Update 2007;13:77–86.
[20] Zhen XM, Qiao J, Li R, Wang LN, Liu P. The clinical analysis of poor ovarian response in in-vitro-fertilization embryo-transfer among Chinese couples. J Assist Reprod Genet 2008;25:17–22.
[21] Faddy MJ. Follicle dynamics during ovarian ageing. Mol Cell Endocrinol 2000;163:43–8.
[22] Younis JS. Ovarian aging: latest thoughts on assessment and management. Curr Opin Obstet Gynecol 2011;23:427–34.
[23] Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril 2014;101. 656–63 e1.
[24] De Sutter P, Dhont M. Poor response after hormonal stimulation for in vitro fertilization is not related to ovarian aging. Fertil Steril 2003;79:1294–8.
[25] Weghofer A, Dietrich W, Barad DH, Gleicher N. Live birth chances in women with extremely low-serum anti-Mullerian hormone levels. Hum Reprod 2011;26:1905–9.
[26] Land JA, Evers JL. Risks and complications in assisted reproduction techniques: Report of an ESHRE consensus meeting. Hum Reprod 2003;18:455–7.
[27] Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod 2011;26:1768–74.
[28] Kyrou D, Kolibianakis EM, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Fertil Steril 2009;91:749–66.
[29] Berkkanoglu M, Ozgur K. What is the optimum maximal gonadotropin dosage used in microdose flare-up cycles in poor responders? Fertil Steril 2010;94:662–5.
[30] Ou YC, Lan KC, Huang FJ, Kung FT, Lan TH, Chang SY. Comparison of in vitro fertilization versus intracytoplasmic sperm injection in extremely low oocyte retrieval cycles. Fertil Steril 2010;93:96–100.
[31] Bosdou JK, Venetis CA, Kolibianakis EM, Toulis KA, Goulis DG, Zepiridis L, et al. The use of androgens or androgen-modulating agents in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Hum Reprod Update 2012;18:127–45.
[32] Lehert P, Kolibianakis EM, Venetis CA, Schertz J, Saunders H, Arriagada P, et al. Recombinant human follicle-stimulating hormone (r-hFSH) plus recombinant luteinizing hormone versus r-hFSH alone for ovarian stimulation during assisted reproductive technology: systematic review and meta-analysis. Reprod Biol Endocrinol 2014;12:17.
[33] Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update 2003;9:61–76.
[34] Pandian Z, McTavish AR, Aucott L, Hamilton MP, Bhattacharya S. Interventions for 'poor responders' to controlled ovarian hyper stimulation (COH) in in-vitro fertilisation (IVF). Cochrane Database Syst Rev 2010 Jan 20;(1):CD004379.
[35] Frydman R. Poor responders: still a problem. Fertil Steril 2011;96:1057.
[36] Younis JS. The Bologna criteria for poor ovarian response; has the job been accomplished? Hum Reprod 2012;27:1874–5. author reply 5–6.
[37] Roustan A, Perrin J, Debals-Gonthier M, Paulmyer-Lacroix O, Agostini A, Courbiere B. Surgical diminished ovarian reserve after endometrioma cystectomy versus idiopathic DOR: comparison of in vitro fertilization outcome. Hum Reprod 2015;30:840–7.
[38] Venetis CA. The Bologna criteria for poor ovarian response: the good, the bad and the way forward. Hum Reprod 2014;29:1839–41.
[39] Papathanasiou A. Implementing the ESHRE 'poor responder' criteria in research studies: methodological implications. Hum Reprod 2014;29:1835–8.
[40] Pocock SJ. Allocation of patients to treatment in clinical trials. Biometrics 1979;35:183–97.