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ARTICLE

Live birth rates in Bologna poor responders treated with ovarian stimulation for IVF/ICSI

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Abstract This retrospective study determined the efficacy of ovarian stimulation for IVF/intracytoplasmic sperm injection (ICSI) in poor ovarian responders fulfilling the Bologna criteria for poor ovarian response and identified predictors of live birth rates. Overall, 485 patients undergoing 823 ovarian stimulation cycles for IVF/ICSI with maximum gonadotrophin dose (≥300 IU) between January 2009 and December 2011 were included. Patients were considered eligible, irrespective of the treatment protocol, if they were classified as poor responders based on the recently developed definition for poor ovarian response by the European Society of Human Reproduction and Embryology, the Bologna criteria. Live birth rates did not significantly differ between women aged <40 and women aged ≥40 years either per cycle (7.1 versus 5.2%, OR 1.38, 95% CI 0.77–2.46) or per patient (11.6 versus 8.8%, OR 1.36, 95% CI 0.75–2.46). In logistic regression analysis, the number of oocytes retrieved was the only variable significantly associated with live births (OR 1.92, 95% CI 1.03–3.55 for >3 versus 1–3 oocytes). Bologna poor responders demonstrate very low live birth rates, irrespective of age and treatment protocol used. An increase in the number of oocytes retrieved is an independent variable related to live birth rates.

KEYWORDS: Bologna criteria, IVF, live birth, ovarian stimulation, poor ovarian response, poor responders

Introduction

Treatment of poor ovarian responders has been the subject of research for numerous randomized trials over the last 2 decades. However, despite the significant amount of evidence published, the diversity in the definition of poor ovarian response is striking with 47 randomized trials published until 2011, using 41 different definitions (Polyzos and Devroey, 2011). The first systematic effort to define women with poor ovarian response to stimulation has been published in 2011, with the so-called 'Bologna criteria' developed by the European Society of Human Reproduction

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and Embryology (ESHRE) consensus for poor ovarian response (Ferraretti et al., 2011). Nevertheless, despite this effort, the Bologna criteria received extensive criticism due to the wide diversity of patients included (Younis, 2012).

The first studies published including poor responders fulfilling these criteria resulted in very poor outcomes in terms of pregnancy rates, irrespective of the protocol used. Natural-cycle IVF resulted in disappointingly low live birth rates, regardless of patients' age (Polyzos et al., 2012) whereas ovarian stimulation with widely accepted treatment modalities such as the short agonist protocol also demonstrated very low ongoing pregnancy rates (Polyzos et al., 2013a). Furthermore, pregnancy rates did not prove to substantially improve even after the administration of new treatment molecules, such as corifollitropin alpha, followed by recombinant FSH (Polyzos et al., 2013a).

Although the aforementioned evidence highlights the poor prognosis of this population, to date no study has attempted to determine the reproductive potential of poor responders according to the Bologna criteria and provide evidence on what a clinician can anticipate from available ovarian stimulation protocols in these women. The estimation of the reproductive potential of this population would be ideal to be performed through prospective clinical trials; nonetheless, the low incidence of poor ovarian response and the short interval since the development of the new ESHRE definition for poor ovarian response makes it difficult to conduct such a study. Moreover, given the scarce number of outcome events among women with poor ovarian response, large cohorts are needed to provide reliable estimates of outcome event rates and to determine potential determinants of outcome.

The current study, taking advantage of one of the largest clinical databases available worldwide, had three purposes: (i) to determine live birth rate among a large group of women fulfilling the Bologna criteria for poor ovarian response; (ii) to quantify the impact of age on live birth rate based on an age threshold of 40 years as defined by the Bologna criteria; and (iii) to explore potential determinants of live birth while simultaneously adjusting for potential confounders.

Materials and methods

Institutional review board approval was obtained for this study from the Ethical Committee of Universitair Ziekenhuis Brussel (decision no. B.U.N. 143201214980, approved 20 September 2012).

Eligibility criteria

All women who underwent ovarian stimulation with a starting gonadotrophin dose of $\geq$300 IU for IVF/intracytoplasmic sperm injection (ICSI) between January 2009 and December 2011 and were poor responders according to the recently developed criteria for poor ovarian response, the Bologna criteria (Ferraretti et al., 2011) were considered eligible.

Two individual investigators (MN and RC) manually scrutinized patients' files and examined previous cycles' characteristics, age and ovarian reserve tests (anti-Müllerian hormone and antral follicle count). Based on these characteristics, patients were considered eligible if they fulfilled the Bologna criteria. Results were compared and any discrepancies were resolved with the involvement of a third investigator (NPP).

Bologna poor responders should fulfil two out of the three following criteria: (i) advanced maternal age ($\geq$40 years) or any other risk factor for poor ovarian response; (ii) poor ovarian response ($\leq$3 oocytes with a conventional stimulation protocol); and (iii) an abnormal ovarian reserve test (anti-Müllerian hormone $<$1.1 ng/ml or antral follicle count $<$7). In addition, patients who had at least two previous trials with $<$4 oocytes retrieved after administration of $>$300 IU of gonadotrophins were considered poor responders (Ferraretti et al., 2011).

Patients were excluded from the study if they had planned to undergo ovarian stimulation for preimplantation genetic diagnosis or screening.

Cycle characteristics such as treatment protocol (short agonist, long agonist or antagonist), gonadotrophin preparation (FSH, human menopausal gonadotrophin (HMG) or both) and starting dose were recorded for every eligible patient.

Main outcome measures

The primary outcome was live birth rate and the secondary outcomes were the ongoing and clinical pregnancy rates per treatment cycle and per patient. Ongoing pregnancy was defined as the presence of intrauterine sac with an embryonic pole demonstrating cardiac activity at 10 weeks of gestation, whereas clinical pregnancy was defined as the presence of a gestational sac with an embryonic pole and positive heart beat at 7 weeks of gestation.

Additional outcomes were cycle cancellation rate, number of oocytes retrieved and cycles with oocyte retrieval. Outcome data were analysed according to age at the threshold of 40 years ($<$40 and $\geq$40 years old groups), since this threshold is one of the criteria in the new ESHRE definition. Further analyses were performed in more refined age categories ($<$35 years, 35–39 years and $\geq$40 years) in line with previous reports (Sunkara et al., 2011; Ulug et al., 2003).

Statistical analysis

For each group of interest, categorical data are described by number of cases, including numerator and denominator, and percentages. Continuous data are presented as mean ± standard deviation (SD) or median with interquartile range (IQR).

The impact of age (women aged $<$40 years versus women aged $\geq$40 years) on outcome events was quantified by computing odds ratios (ORs) along with corresponding 95% confidence intervals (CIs).

To identify characteristics that may be related with the live birth, logistic regression analysis was performed with live birth as the dependent variable and patient age (two categories: women aged $<$40 years or women aged $\geq$40 years), number of oocytes retrieved (two categories: 1–3 or $>$3 oocytes), type of treatment protocol used (three categories: antagonist, long agonist or short agonist) and type of gonadotrophins used (three categories: FSH, HMG or both) as independent variables. All independent variables were simultaneously entered into the logistic regression model.

Analyses were performed using SPSS version 20 (SPSS, Chicago, IL, USA).

Results

Patient and cycle characteristics

Overall, the analysis included 485 poor responders according to the Bologna criteria undergoing 823 ovarian stimulation cycles for IVF/ICSI. Among them, 201 women were <40 years old and underwent 327 treatment cycles and 284 were ≥40 years and underwent 496 treatment cycles.

The patients' baseline characteristics are presented in Table 1. The most common infertility cause was unexplained infertility for women ≥40 years old and male factor infertility for patients <40 years old. In addition, the short agonist protocol was the most frequently used protocol in young poor responders whereas older women were more frequently treated with an antagonist protocol. Finally, the type of gonadotrophin differed between age groups, with younger women being treated more frequently with HMG compared with older patients.

Ovarian response

Overall, a median (IQR) of 3 (0—5) of oocytes were retrieved, with 24% of treatment cycles cancelled due to poor ovarian response. When analysing the number of oocytes retrieved according to age, women of younger age (<40 years) demonstrated significantly lower number of oocytes compared with women aged ≥40 years (2 (0—4) versus 3 (0—5), $P = 0.003$). Similarly, women of younger age had a lower number of cycles with an oocyte retrieval compared with older women (70.3 versus 76.4%, OR 0.73 (95% CI 0.54—1.00) although results were borderline nonsignificant ($P = 0.052$; Table 2).

Reproductive outcome

Overall, live birth rates were 6.0% per cycle and 9.9% per patient. Analysis according to age showed no significant differences between younger (<40 years) and older (≥40 years) poor responders in live birth rates, either per cycle (7.1 versus 5.2%, OR 1.38, 95% CI 0.77—2.46) or per patient (11.6 versus 8.8%, OR 1.36, 95% CI 0.75—2.46). Similarly, no significant differences among age groups were observed for ongoing and clinical pregnancies (Table 2).

Further analyses according to more refined age limits showed that pregnancy rates did not significantly differ among different age categories. Live birth rates per cycle were comparable between women aged <35, 35—39 and ≥40 years (8.3%, 6.5% and 5.3%, respectively). Similarly, no significant differences in live birth rates were observed per patient (12.2%, 11.1% and 8.8%, respectively).

Variables associated with live birth rates

Multivariable logistic regression analysis demonstrated that age, type of protocol and type of gonadotrophin used were not associated with live birth rates. On the contrary, number of oocytes retrieved appeared to be the only

Table 1 Patients' baseline characteristics.

Characteristic	*All patients*	*<40 years*	*≥40 years*
Patients	485	201	284
Body mass index (kg/m^2)	25.2 ± 5.1	25.3 ± 5.0	25.0 ± 5.2
Infertility cause			
Idiopathic	198 (41)	61 (30)	137 (48)
Male factor	183 (38)	85 (42)	94 (33)
Tubal factor	57 (12)	20 (10)	37 (13)
Endometriosis	32 (7)	25 (12)	7 (2)
Other	15 (3)	10 (5)	5 (2)
No. of previous treatment cycles	2 (0—3)	2 (0—3)	2 (0—3)
Treatment cycles	823	327	496
Protocol			
Antagonist	341 (41)	108 (33)	233 (47)
Short agonist	405 (49)	177 (54)	228 (46)
Long agonist	77 (9)	42 (13)	35 (7)
Type of gonadotrophins			
FSH	345 (42)	119 (36)	226 (46)
HMG	467 (57)	202 (62)	265 (53)
FSH + HMG	11 (1)	6 (2)	5 (1)

Values are *n*, mean ± SD or *n*/total (%).

Table 2 Ovarian response and reproductive outcomes.

	All patients	*<40 years*	*≥40 years*	*OR (95% CI)*
No. of patients	485	201	284	–
No. of treatment cycles	823	327	496	–
Ovarian response				
Oocytes retrieved	3 (0–5)	2 (0–4)	3 (0–5)	NA
Cycles cancelled	196 (23.8)	88 (26.9)	108 (21.8)	1.33 (0.96–1.83)
Cycles with oocyte retrieval	609 (74.0)	230 (70.3)	379 (76.4)	0.73 (0.54–1.00)
Reproductive outcomes				
Clinical pregnancy				
Per patient	68 (14.8)	29 (14.4)	39 (13.7)	1.06 (0.63–1.78)
Per cycle	71 (8.6)	29 (8.9)	42 (8.5)	1.06 (0.64–1.73)
Ongoing pregnancy				
Per patient	61 (12.6)	27 (13.4)	34 (12.0)	1.14 (0.66–1.96)
Per cycle	63 (7.7)	27 (8.3)	36 (7.3)	1.15 (0.68–1.94)
Live birth				
Per patient	48/483 (9.9)[a]	23/199 (11.6)[a]	25 (8.8)	1.36 (0.75–2.46)
Per cycle	49/821 (6.0)[a]	23/325 (7.1)[a]	26 (5.2)	1.38 (0.77–2.46)

Values are median (interquartile range), *n* (%) or *n*/total (%).
NA = not applicable.
[a]Data regarding live births could not be retrieved for two patients/cycles with an ongoing pregnancy.

independent variable and was significantly associated with the live birth rate with OR 1.92 (95% CI 1.03–3.55) for live birth for women having >3 oocytes retrieved compared with women having 1–3 oocytes retrieved, even after adjusting for all other characteristics in the logistic regression model.

Discussion

The current study is, as far as is known, the first to examine live birth rates in poor responders fulfilling the recently developed Bologna criteria after treatment with ovarian stimulation and highlights the extremely poor prognosis of this population, irrespective of age and treatment protocol used.

Previous studies have shown that pregnancy rates in Bologna poor responders are low, irrespective of age, either after treatment with natural-cycle IVF or following ovarian stimulation (Polyzos et al., 2012, 2013a). Furthermore, one of the largest series of poor responders (who did not meet the Bologna criteria) demonstrated that young poor responders have similarly low chances of a live birth as women of advanced age (El-Toukhy et al., 2002). Thus, it appears that age may not be a strong determinant of treatment success in poor responders.

These results are in contrast with several previous studies, which demonstrated that age is a strong determinant of treatment success in poor responders (Biljan et al., 2000; De Sutter and Dhont, 2003; Galey-Fontaine et al., 2005; Hanoch et al., 1998; Ulug et al., 2003; Zhen et al., 2008). However, it is important to highlight that none of them utilized the Bologna criteria. Thus, this contradiction with previous studies does not necessarily imply different results, but indeed reflects the diversity in the definition of poor ovarian response in this study compared with the previous ones. Per definition, the Bologna criteria either refer to women of advanced age, in whom the live birth rate is very low due to poor oocyte quality (Hourvitz et al., 2009; van Rooij et al., 2003), or to younger women with poor ovarian reserve, in whom the likelihood of experiencing response to ovarian stimulation is extremely low. As shown in the current study, women of younger age experienced significantly lower number of oocytes retrieved, and there was a nonsignificant trend for lower number of cycles with oocyte retrieval compared with women ≥40 years. This is in accordance with previous studies demonstrating that young poor responders may experience higher cycle cancellation rates compared with women of advanced age (van Rooij et al., 2003) and should be mainly attributed to the definition used to identify this population in the current study. As described by the ESHRE definition, women < 40 years old are considered poor responders only if they have had an abnormal ovarian reserve test and a previous cycle with ≤3 oocytes retrieved, or at least two previous cycles with ≤3 oocytes retrieved after maximum ovarian stimulation (Ferraretti et al., 2011). Thus, based on the definition of the Bologna criteria, young poor responders have the worst prognosis in terms of ovarian response and this is reflected in the lower number of oocytes retrieved presented here.

An important observation of this work is the significant association of the number of oocytes retrieved with live birth rates. This finding is in accordance with previous large series in a general infertile population (Sunkara et al., 2011) and in poor responders (Oudendijk et al., 2012), which highlight that the number of oocytes retrieved is strictly related

to the chance for pregnancy in current and subsequent cycles. The current data confirm these findings in Bologna poor responders and clearly support that the threshold of 3 oocytes adopted by the ESHRE consensus is adequate to identify patients with the poorer prognosis in terms of live birth rates.

Nevertheless, this study has limitations. First of all, this is a retrospective analysis and in this regard it is subject to biases related to such study design. Due to the retrospective study design, several of the baseline and stimulation characteristics significantly differed between young and older poor responders. Nonetheless, none of these variables was related with live birth rates in the final logistic regression model, suggesting that they were not independently associated with the final outcome. Although the retrospective study design should be considered when interpreting the results, such a study would have been very difficult to conduct in a prospective manner due to the low incidence of poor response in the infertile population and the extremely low live birth rate in this population. Thus, a larger number of patients would have been needed to provide information regarding the actual reproductive potential of these women. Furthermore, the Bologna criteria have been developed only recently, in 2011, and therefore a longer timeframe would be needed until trustworthy results could have been available.

In addition, this work examined live birth rates in poor responders fulfilling the Bologna criteria within a single centre study. Thus, it may not be appropriate to generalize the results, and the Bologna poor responders might either perform slightly better or worse in other IVF units. Therefore, confirmatory studies from other research groups are essential in order to determine the actual reproductive potential of Bologna poor responders after ovarian stimulation. However, given that the figures presented here are very similar to previous studies in poor responders using criteria for poor response similar to the Bologna criteria (El-Toukhy et al., 2002), it is highly likely that this population has very limited prospects in terms of live birth rates.

Finally, given the scarce number of outcome events (live births) among women with poor ovarian response in this study, the sample size of several hundred women included may not be considered adequate to allow comparisons between women of younger and advanced age. Nonetheless, our work represents the largest available study including this population and it is relatively clear that both young and older poor responders fulfilling the Bologna criteria demonstrate very low live birth rates following treatment with ovarian stimulation for IVF/ICSI. In spite of the limitations described above, this is the first attempt to examine the actual reproductive potential of women who are considered poor responders according to the new ESHRE definition and may provide important information for everyday clinical practice.

The clinical implications of the current study are important for several reasons. First of all, it appears that Bologna criteria refer to a very poor prognosis group of patients. Although the specific criteria could be a first step toward the adoption of a uniform definition of poor responders (Polyzos and Devroey, 2011), they actually refer to the worst prognosis IVF population. The anticipated outcome in these women should be very low and certainly, many of the patients fulfilling the criteria should be considered as potential candidates for oocyte donation programmes. Furthermore, pregnancy rates described here should be used for sample-size calculation for future randomized controlled trials, given that this is the first study to examine pregnancy rates after ovarian stimulation in poor responders according to the Bologna criteria.

Secondly, given that the number of oocytes retrieved is an independent variable associated with live birth rates in this population, future studies need to focus on an increase in the oocyte yield in this population. However, this might preferably include women of younger age (<40 years), since reduced oocyte quality due to age appears to be a detrimental factor for pregnancy rates as shown by other cohort studies (van Rooij et al., 2003), while, as shown in the current study, young poor responders appear to experience equivalent pregnancy rates with older patients in spite of the lower number of oocytes retrieved. Data from novel protocols in Bologna poor responders also point towards such a direction, with preliminary results from a pilot study demonstrating that corifollitropin alpha followed by hpHMG may benefit only young poor responders fulfilling the Bologna criteria (Polyzos et al., 2013b). However, validation of these results from a large ongoing multicentre randomized controlled study will provide conclusive evidence on the superiority or not of such a protocol compared with standard ovarian stimulation (Polyzos et al., 2013c).

In conclusion, this study, as far as is known for the first time, provides robust evidence regarding the reproductive potential of women who fulfil the Bologna criteria undergoing ovarian stimulation for IVF/ICSI. Live birth rates among these patients are consistently low, irrespective of patients' age, and an increase in oocyte yield may significantly increase live birth rates.

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References

Biljan, M.M., Buckett, W.M., Dean, N., Phillips, S.J., Tan, S.L., 2000. The outcome of IVF-embryo transfer treatment in patients who develop three follicles or less. Hum. Reprod. 15, 2140–2144.

de Sutter, P., Dhont, M., 2003. Poor response after hormonal stimulation for in vitro fertilization is not related to ovarian aging. Fertil. Steril. 79, 1294–1298.

El-Toukhy, T., Khalaf, Y., Hart, R., Taylor, A., Braude, P., 2002. Young age does not protect against the adverse effects of reduced ovarian reserve – an eight year study. Hum. Reprod. 17, 1519–1524.

Ferraretti, A.P., la Marca, A., Fauser, B.C., Tarlatzis, B., Nargund, G., Gianaroli, L., 2011. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum. Reprod. 26, 1616–1624.

Galey-Fontaine, J., Cedrin-Durnerin, I., Chaibi, R., Massin, N., Hugues, J.N., 2005. Age and ovarian reserve are distinct predictive factors of cycle outcome in low responders. Reprod. Biomed. Online 10, 94–99.

Hanoch, J., Lavy, Y., Holzer, H., Hurwitz, A., Simon, A., Revel, A., Laufer, N., 1998. Young low responders protected from unto-

ward effects of reduced ovarian response. Fertil. Steril. 69, 1001—1004.
Hourvitz, A., Machtinger, R., Maman, E., Baum, M., Dor, J., Levron, J., 2009. Assisted reproduction in women over 40 years of age: how old is too old? Reprod. Biomed. Online 19, 599—603.
Oudendijk, J.F., Yarde, F., Eijkemans, M.J., Broekmans, F.J., Broer, S.L., 2012. The poor responder in IVF: is the prognosis always poor?: a systematic review. Hum. Reprod. Update 18, 1—11.
Polyzos, N.P., Devroey, P., 2011. 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. 96 (1058—61), e7.
Polyzos, N.P., Blockeel, C., Verpoest, W., de Vos, M., Stoop, D., Vloeberghs, V., Camus, M., Devroey, P., Tournaye, H., 2012. Live birth rates following natural cycle IVF in women with poor ovarian response according to the Bologna criteria. Hum. Reprod. 27, 3481—3486.
Polyzos, N.P., Devos, M., Humaidan, P., Stoop, D., Ortega-Hrepich, C., Devroey, P., Tournaye, H., 2013a. Corifollitropin alfa followed by rFSH in a GnRH antagonist protocol for poor ovarian responder patients: an observational pilot study. Fertil. Steril. 99, 422—426.
Polyzos, N.P., de Vos, M., Corona, R., Vloeberghs, V., Ortega-Hrepich, C., Stoop, D., Tournaye, H., 2013b. Addition of highly purified HMG after corifollitropin alfa in antagonist-treated poor ovarian responders: a pilot study. Hum. Reprod. 28, 1254—1260.
Polyzos, N.P., Camus, M., Llacer, J., Pantos, K., Tournaye, H., 2013c. Corifollitropin alpha followed by menotropin for poor ovarian responders' trial (COMPORT): a protocol of a multicentre randomised trial. BMJ Open 3.
Sunkara, S.K., Rittenberg, V., Raine-Fenning, N., Bhattacharya, S., Zamora, J., Coomarasamy, A., 2011. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum. Reprod. 26, 1768—1774.
Ulug, U., Ben-Shlomo, I., Turan, E., Erden, H.F., Akman, M.A., Bahceci, M., 2003. Conception rates following assisted reproduction in poor responder patients: a retrospective study in 300 consecutive cycles. Reprod. Biomed. Online 6, 439—443.
van Rooij, I.A., Bancsi, L.F., Broekmans, F.J., Looman, C.W., Habbema, J.D., te Velde, E.R., 2003. Women older than 40 years of age and those with elevated follicle-stimulating hormone levels differ in poor response rate and embryo quality in in vitro fertilization. Fertil. Steril. 79, 482—488.
Younis, J.S., 2012. The Bologna criteria for poor ovarian response; has the job been accomplished? Hum. Reprod. 27, 1874—1875 (author reply 1875—6).
Zhen, X.M., Qiao, J., Li, R., Wang, L.N., Liu, P., 2008. The clinical analysis of poor ovarian response in in-vitro-fertilization embryo-transfer among Chinese couples. J. Assist. Reprod. Genet. 25, 17—22.

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