Addition of highly purified HMG after corifollitropin alfa in antagonist-treated poor ovarian responders: a pilot study

N.P. Polyzos*, M. De Vos, R. Corona, V. Vloeberghs, C. Ortega-Hrepich, D. Stoop, and H. Tournaye

Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium

*Correspondence address. Tel: +32-2-477-66-60; Fax: +32-2-477-66-49; E-mail: n.polyzos@gmail.com, nikolaos.polyzos@uzbrussel.be

Submitted on November 30, 2012; resubmitted on January 14, 2013; accepted on February 4, 2013

STUDY QUESTION: Will sequential administration of highly purified (hp)-HMG after corifollitropin alfa in a GnRH antagonist protocol benefit women with poor ovarian response according to the Bologna criteria?

SUMMARY ANSWER: Corifollitropin alfa followed by hp-HMG in a GnRH antagonist protocol results in very promising pregnancy rates, albeit only in young (<40 years old) poor ovarian responders fulfilling the Bologna criteria.

WHAT IS KNOWN ALREADY: Poor ovarian responders fulfilling the Bologna criteria have a very poor prognosis in terms of successful IVF outcome. Although a recent study demonstrated low pregnancy rates in this group of patients after treatment with corifollitropin alfa followed by recombinant FSH in a GnRH antagonist protocol, previous studies showed that the addition of LH activity in 36- to 39-year-old women significantly increases implantation rates.

STUDY DESIGN, SIZE, DURATION: In this retrospective pilot study, we included poor ovarian responders fulfilling the Bologna criteria treated with a completely novel protocol, with corifollitropin alfa followed by hp-HMG in a GnRH antagonist setting. Overall, 51 patients were treated within a period of 1 year (August 2011–August 2012).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Patients received 150 μg corifollitropin alfa on second day of the menstrual cycle followed by a fixed daily dose of 0.25 mg of GnRH antagonist on Day 7 of the cycle onwards. On the ninth day of the cycle, a daily fixed dose of 300 IU hp-HMG was administered until the day of ovulation triggering. The primary outcome was ongoing pregnancy rate per patient.

MAIN RESULTS AND THE ROLE OF CHANCE: Among 47 eligible women, 29 patients were <40 years old and 18 patients were ≥40 years old. No differences were observed in endocrine profile, number of cycles with oocyte retrieval (66 versus 67%) and cycles with embryo transfer (62 versus 61%) in women <40 versus ≥40 years old, respectively. However, 8 of the 29 women <40 years old had an ongoing pregnancy (28%) compared with 0 of 18 patients who were ≥40 years of age (P = 0.017).

LIMITATIONS, REASONS FOR CAUTION: Owing to the specific retrospective study design, bias cannot be ruled out and these results should not be extrapolated to other treatment protocols for poor ovarian responders. Therefore, caution should be taken when interpreting the results.

WIDER IMPLICATIONS OF THE FINDINGS: The promising results from this pilot study of corifollitropin alfa followed by hp-HMG stimulation indicate a potential beneficial effect in young poor ovarian responders fulfilling the Bologna criteria. The data provide the rationale for performing a randomized controlled trial to determine if there is sound evidence for a clinical introduction of this protocol.

STUDY FUNDING/COMPETING INTEREST(S): No conflicts of interest to declare. No specific funding was received for this study.

Key words: corifollitropin alfa / poor ovarian response / Bologna criteria / highly purified HMG / IVF

© The Author 2013. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved.

For Permissions, please email: journals.permissions@oup.com
Introduction

Poor response to ovarian stimulation affects a significant proportion of infertile couples seeking fertility advice. Although during the past few years, a debate has arisen regarding the definition of poor ovarian response, the European Society of Human Reproduction and Embryology (ESHRE) working group on Poor Ovarian Response Definition recently developed new criteria to define patients who respond poorly to ovarian stimulation, the so-called ‘Bologna criteria’ (Ferrarietti et al., 2011). These criteria incorporate age, ovarian reserve tests [anti-Mullerian hormone (AMH) level or antral follicle count (AFC)] and ovarian response in previous IVF/ICSI cycles in the definition, and represent the first realistic attempt by a scientific community (ESHRE) to standardize the definition of poor ovarian response in a simple and reproducible manner.

However, despite the development of these criteria, extensive criticism has arisen due to the wide diversity of patients included (Younis, 2012) and it is still unclear whether their adoption may lead to a substantial improvement in reproductive outcome for these women (Polyzos and Devroey, 2011). Poor responders are not a homogeneous group and the prognosis for these patients may vary greatly depending on patient characteristics, such as age and number of oocytes retrieved (Oudendijk et al., 2012). However, chronological age appears to have a very strong detrimental effect on the final reproductive outcome among women demonstrating poor ovarian response since increased age is associated with lower implantation rates, owing to poorer oocyte quality (van Rooij et al., 2003). Ongoing pregnancy rates do not exceed 17% in women older than 41 years even if they have a normal response to stimulation (van Rooij et al., 2003).

The first studies published including women with poor ovarian response according to the Bologna criteria have shown disappointingly low pregnancy rates, irrespective of age. A recent observational study conducted in our centre demonstrated a very poor prognosis for these women, given that live birth rate following treatment with natural cycle IVF was <3% per patient, irrespective of age, and significantly lower compared with women who did not fulfill the Bologna criteria (Polyzos et al., 2012).

Although the use of new treatment molecules may be considered a promising approach for these patients, a pilot study with the use of corifollitropin alfa in an antagonist protocol has shown that ongoing pregnancy rates are low in these women, similar to treatment with a short agonist protocol (Polyzos et al., 2013). However, a key question to be answered is whether slight modification of the current antagonist protocol with corifollitropin alfa may actually result in a substantial benefit in this patient subgroup.

According to the previous trial conducted by our group, the number of cancelled cycles was high and the cumulative transfer and ongoing pregnancy rates were low in poor responders, although corifollitropin alfa induced a satisfactory hormonal response (Polyzos et al., 2013). Nonetheless, in that previous study, recombinant FSH (rFSH) followed administration of corifollitropin in order to sustain follicular development. Given the fact that previous reports showed that the addition of highly purified (hp)-HMG to FSH may improve embryo quality and result in an increase in pregnancy rate (Requena et al., 2010), we considered that a slightly modified stimulation protocol with the administration of hp-HMG following corifollitropin may augment the benefit related to the satisfactory hormonal response achieved through the use of corifollitropin alfa, and therefore increase pregnancy rates.

Based on the above hypothesis, and following the unsatisfactory results of corifollitropin alfa followed by rFSH in Bologna poor responders, we decided to shift our practice and administer hp-HMG 7 days after treatment with corifollitropin alfa in a GnRH antagonist cycle. The current study is a retrospective pilot study of all the patients fulfilling the Bologna criteria who were treated over a period of 1 year in our centre with corifollitropin alfa followed by hp-HMG in a GnRH antagonist cycle.

Materials and Methods

Institutional Review Board approval was obtained for the current study (B.U.N.143201215289). All patients had given written authorization at the time of treatment for the future use of their clinical data.

Eligible patients and treatment schedule

Patients were considered eligible if they were poor ovarian responders according to the Bologna criteria (Ferrarietti et al., 2011). Two out of three of the following criteria were essential in order to classify a patient as poor ovarian responder: (i) advanced maternal age (≥40 years) or any other risk factor for poor ovarian response; (ii) a poor ovarian response (≤3 oocytes with a conventional stimulation protocol); or (iii) an abnormal ovarian reserve test (AFC, <7 follicles or AMH, <1.1 ng/ml).

In addition, patients should have been treated with 150 μg corifollitropin alfa starting on second day of the menstrual cycle followed by administration of a fixed daily dose of 0.25 mg of GnRH antagonist on Day 7 of the cycle. On the ninth day of the cycle, a daily fixed dose of 300 IU of hp-HMG was administered until the day of ovulation triggering. Ovulation triggering was performed with the administration of 10,000 IU of hCG as soon as two–three follicles of 17 mm diameter were observed by transvaginal ultrasound. In the case of monofollicular development, rescue intrauterine insemination was performed, whereas in the case of no response, the cycle was cancelled.

Women who were planned to receive corifollitropin alfa followed by rFSH were excluded from the study, and only the first treatment cycle with corifollitropin alfa followed by hp-HMG was considered in the analysis.

Main outcomes

Due to the fact that the Bologna criteria refer to young as well as older patients with low ovarian response, results were stratified according to age. Thus, cycle cancellation rate, oocyte retrieval rate, embryo transfer rate and pregnancy rate were compared in women <40 versus ≥40 years old.

The primary outcome was the ongoing pregnancy rate per patient. Secondary outcomes were number of oocytes retrieved, number of cancelled cycles, number of cycles with oocyte retrieval and cycles with embryo transfer. In addition, endocrine profile [FSH, LH, estradiol (E2) and progesterone] during the follicular phase was recorded for all eligible patients. The number of oocytes retrieved in the current cycle was compared with those retrieved in the previous treatment cycle (short GnRH agonist, long GnRH agonist or GnRH antagonist).

Embryo quality assessment

Embryo quality was based on the Istanbul consensus workshop on embryo assessment: top-quality embryos on Day 3 had at least seven blastomeres.
with volumes proportionally to the division pattern and no more than 10% fragmentation, as previously described (De Vos et al., 2013).

**Endocrine profiles**

FSH, LH, E2 and progesterone concentrations in (Li-heparin) plasma were measured with Elecsys electrochemiluminescence immunoassays (Roche Diagnostics, Mannheim, Germany).

**Statistical analysis**

Continuous outcomes were compared with the Mann–Whitney U-test and percentages were compared with the Fisher’s exact test, with a level of significance of $P < 0.05$. Comparisons between the number of oocytes retrieved in the current treatment cycle and the previous cycle were performed by using the Wilcoxon rank-sum test. All analyses were performed using the Statistical Package for the Social Sciences 20 software.

As this is the first study of this specific ovarian stimulation protocol and owing to a lack of previous trials in Bologna poor ovarian responders, no sample size calculation could be performed for the current study. Therefore, we arbitrarily considered that 50 patients could provide preliminary data regarding the potential efficacy of the protocol in order to serve as a reference for future prospective randomized studies.

**Results**

**Patient characteristics**

Overall, 51 patients had been treated with corifollitropin alfa followed by hp-HMG in a GnRH antagonist protocol within a period of 1 year (August 2011–August 2012). However, four patients were excluded from the analysis, one due to administration of 225 IU instead of 300 HMG from stimulation Day 7, and three because they did not fulfill the Bologna criteria. Among 47 analysed patients, 29 women were classified as young poor responders (<40 years old) and 18 were ≥40 years old. Overall, 46 out of 47 patients had at least one previous cycle (29 patients <40 years old and 17 patients >40 years old). Patient characteristics are presented in Table I.

**Endocrine profile and stimulation characteristics**

The plasma endocrine profile during the follicular phase was comparable between patients <40 and ≥40 years old (Fig. 1). As shown, the mean serum FSH levels demonstrated an acute rise during cycle Day 7 in both age groups and remained markedly above 20 IU/l during the follicular phase. E2 levels rose steady in all patients, with older patients maintaining equally high E2 levels compared with women <40 years old. There were no differences in FSH, LH, progesterone and E2 levels at Days 2, 7, 9 and the day of ovulation triggering between the young and older poor responders.

Similarly, no difference existed between age groups regarding the total days of stimulation, days of additional stimulation with hp-HMG and total units of hp-HMG used (Table I).

**Cycle cancellation and oocyte retrieval rate**

Overall, 13 patients (28%) had a cycle cancellation due to no follicular recruitment or monofollicular development and 31 cycles (66%)

**Table I Patient and stimulation characteristics in a pilot study of the addition of hp-HMG after corifollitropin alfa in antagonist-treated poor ovarian responders.**

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>&lt;40 years</th>
<th>≥40 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>47</td>
<td>29</td>
<td>18</td>
</tr>
<tr>
<td>Patients characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years, mean, SD)</td>
<td>37.7 (3.9)</td>
<td>35.6 (3.6)</td>
<td>40.1 (0.8)</td>
</tr>
<tr>
<td>BMI (kg/m², mean, SD)</td>
<td>24.6 (5.1)</td>
<td>24.3 (4.8)</td>
<td>25.2 (5.9)</td>
</tr>
<tr>
<td>Infertility cause, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>29 (62)</td>
<td>15 (52)</td>
<td>14 (77)</td>
</tr>
<tr>
<td>Male factor</td>
<td>8 (17)</td>
<td>7 (24)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>4 (9)</td>
<td>3 (10)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Genetic (PGD)</td>
<td>3 (6)</td>
<td>1 (3.5)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Tubal</td>
<td>2 (4)</td>
<td>2 (7)</td>
<td>–</td>
</tr>
<tr>
<td>Previous chemotherapy</td>
<td>1 (2)</td>
<td>1 (3.5)</td>
<td>–</td>
</tr>
<tr>
<td>Duration of infertility (years, mean, SD)</td>
<td>4.4 (2.6)</td>
<td>4.2 (2.4)</td>
<td>4.7 (2.9)</td>
</tr>
<tr>
<td>Number of oocytes retrieved in the previous cycle* (median, IQR)*</td>
<td>1 (0–3)</td>
<td>1 (0–3)</td>
<td>1 (0–3)</td>
</tr>
<tr>
<td>Endometrial thickness on the day of hCG triggering (mean, SD)</td>
<td>9.9 (2.4)</td>
<td>10.1 (2.8)</td>
<td>9.6 (1.7)</td>
</tr>
<tr>
<td>Stimulation characteristics (mean, SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulation days</td>
<td>10.0 (2.1)</td>
<td>9.7 (2.1)</td>
<td>10.3 (2.1)</td>
</tr>
<tr>
<td>Days of additional hp-HMG</td>
<td>3.0 (2.1)</td>
<td>2.7 (2.1)</td>
<td>3.3 (2.1)</td>
</tr>
<tr>
<td>Stimulation units of hp-HMG</td>
<td>897 (629)</td>
<td>817 (631)</td>
<td>1000 (626)</td>
</tr>
</tbody>
</table>

hp-HMG, highly purified HMG. All comparisons between age groups are NS.

*One patient in the >40 years old group did not have a previous cycle of IVF/ICSI.

*Skewed data are presented as medians (IQR, interquartile range).
resulted in the retrieval of at least one oocyte. As shown in Table II, no significant difference existed in cycle cancellation and oocyte retrieval rates between young and older women. In addition, the mean number of oocytes retrieved did not significantly differ between age groups. However, when comparing the mean number of oocytes retrieved in the current treatment cycle with the previous treatment cycle (data not shown in Table II), corifollitropin alfa followed by hp-HMG significantly increases the mean number of oocytes in young poor responders (mean number of oocytes 3.3 versus 1.5, respectively, \( P = 0.01 \)). In contrast, women \( \geq 40 \) years old did not experience any improvement in terms of the number of oocytes retrieved compared with the previous cycle (2.5 versus 1.9, respectively, \( P = 0.70 \)).

Embryo transfer and embryological data
Twenty-nine patients (62%) had an embryo transfer, with no difference between young and older poor responders (Table II). Although younger women appeared to show a higher proportion of cycles with top-quality embryos (45%) compared with older patients (28%), the difference was not statistically significant, \( P = 0.24 \).

Reproductive outcome
Overall, 12 patients had a positive hCG test (26%) and 8 women had an ongoing pregnancy (17%). However, pregnancy rates per patient were significantly different between young and old women (Table II).

Among 18 women who fulfilled the criterion of age (patients \( \geq 40 \) years old), none of them had an ongoing pregnancy. Only one patient, 42 years old, had a clinical pregnancy which ended in a miscarriage at 8 weeks of gestation.

On the contrary, Bologna poor responders \(< 40 \) years old demonstrated substantially better pregnancy rates compared with older women. Eleven out of 29 patients had a positive hCG test (37%). However, three pregnancies did not progress; one patient had a biochemical pregnancy, one had a clinical pregnancy with a gestational sac without an embryonic pole and one a clinical pregnancy with an embryonic pole with a positive heartbeat which ended in a miscarriage at 10 weeks of gestation. Young, poor ovarian responders demonstrated an ongoing pregnancy rate per patient of 28%, and an even higher 44% ongoing pregnancy rate per embryo transfer.

Ongoing pregnancy rates per patient were significantly higher in younger patients (\(< 40 \) years old) compared with women \( \geq 40 \) years old (28 versus 0%, respectively, \( P = 0.017 \)).
rates per oocyte retrieval and per embryo transfer were also significantly higher in women <40 years old (Table II). Two out of eight ongoing pregnancies were twin pregnancies. Finally, none of the patients reported any serious adverse effects during the treatment period.

**Discussion**

The current study is the first to examine the administration of corifollitropin alfa followed by hp-HMG in women with poor ovarian response according to the Bologna criteria. According to our results, it appears that the sequential administration of hp-HMG after corifollitropin alfa results in very promising ongoing pregnancy rates in women <40 years of age fulfilling the criteria and, therefore, such a protocol should be tested in future prospective randomized trials. On the contrary, poor responders aged ≥40 years did not appear to experience any benefits from this novel protocol.

The results of the current study appear to be substantially better than those obtained previously in patients treated with corifollitropin alfa followed by rFSH (Polyzos et al., 2012, 2013). In addition, it appears that the current protocol performs better compared with a short GnRH agonist protocol and natural cycle IVF, since results from our centre demonstrated a pregnancy rate of ~7% in patients treated with these protocols (Polyzos et al., 2012, 2013). Therefore, we have reasons to believe that corifollitropin followed by HMG may be of benefit in this group of patients. However, we have to acknowledge that comparisons between protocols cannot be made, given that this was not a controlled trial. Thus, caution should be taken when interpreting the current study.

Although in previous trials by our group, Bologna poor responders performed equally poorly either with natural cycle IVF or with corifollitropin alfa followed by rFSH (Polyzos et al., 2012, 2013), irrespective of age, corifollitropin alfa followed by hp-HMG appears to result in very promising pregnancy rates but only in young women and this warrants further scrutiny. Despite the fact that we failed to identify any difference in oocyte retrieval and embryo transfer rates between young and older women, none of the patients exceeding 40 years old had an ongoing pregnancy. The poorer prognosis of older women is also highlighted by our finding that only women aged <40 years appeared to have an increased response after treatment with corifollitropin alfa followed by hp-HMG in terms of the number of oocytes retrieved compared with their previous IVF/ICSI cycle.

The differences regarding the reproductive outcome between young and older poor ovarian responders highlighted in our study are further supported by the fact that the percentage of cycles with top-quality embryos tended to be higher in younger compared with older patients (45 versus 28%, respectively), although results did not reach statistical significance. This finding is in accordance with previous
trials showing that women of advanced age experience lower implantation owing to poor embryo quality (van Rooij et al., 2003), and recent data from comparative genomic hybridization array analysis demonstrating that in women aged ≥40 years, the percentage of aneuploid Day 3 embryos exceeds 70% and explains why they fail to implant (Ata et al., 2012). Consequently, this observation raises concerns regarding the anticipated outcome in advanced age (≥40 years old) Bologna poor responders and should be carefully considered when tailoring future trials, which should preferably focus only on women <40 years old, if any benefit is to be anticipated.

A potential explanation for the promising pregnancy rates obtained following a combination of treatment of corifollitropin alfa with hp-HMG could be the beneficial effects linked to both molecules, the potential of corifollitropin alfa to augment the number of oocytes retrieved and the addition of LH activity through hp-HMG which may increase implantation rates.

First of all, corifollitropin alfa reaches maximum serum concentrations (C_{max}) between 25 and 45 h after injection (Devroey et al., 2004; Fauser et al., 2009), a time interval which is significantly shorter when compared with treatment with fFSH; the rapid increase in serum FSH concentration may result in a significantly higher exposure of the small antral follicles to constant high levels of FSH during the early follicular phase, securing not only recruitment of the follicles but also the continued growth. Due to its different pharmacokinetic profile, corifollitropin alfa results in a significantly higher number of oocytes retrieved compared with rFSH in normal responders (Devroey et al., 2009) and, in this regard, it may increase the oocyte yield in women with poor ovarian response.

On the other hand, administration of hp-HMG in women with poor ovarian response may increase pregnancy rates because the addition of LH activity has been hypothesized to benefit women of advanced age and women with poor ovarian response. In a large randomized trial, addition of rLH was shown to benefit patients aged 35–39 years in GnRH antagonist cycles, yielding higher implantation rates (Bosch et al., 2011). Thus, it may be likely that this subgroup of women may experience more profound LH suppression in GnRH antagonist cycles and, in this regard, benefit from exogenous LH activity through the administration of hp-HMG from Day 7 of the stimulation and onwards. Our results support such a hypothesis; nonetheless, this is applicable only in women <40 years old, who demonstrated a significantly higher number of oocytes retrieved after treatment with this novel protocol compared with their previous cycle, and their pregnancy rates were very promising compared with previous trials in similar populations (Polyzos et al., 2013).

In spite of this plausible hypothesis and the promising results of this novel protocol, we have to highlight that our study is subject to biases related to its retrospective design. Nonetheless, we considered it appropriate not to perform such a study in a controlled setting, given the fact that this protocol has never been tested, while on the other hand the first report of corifollitropin alfa followed by fFSH for poor ovarian responders resulted in very low pregnancy rates (Polyzos et al., 2013). The retrospective design of the current study definitely prevents us from drawing firm conclusions; however, it clearly supports the feasibility of a future prospective controlled study and may serve as a tool to calculate the number of patients needed for such a study design.

Another point of interest, which may guide further research, is the fact that we evaluated the addition of hp-HMG to corifollitropin alfa in the antagonist setting, given that the manufacturer suggests its use in this specific setting (European Medicines Agency, 2011). However, considering that a previous pilot study has shown that corifollitropin alfa may result in high number of oocytes retrieved in a GnRH agonist setting in normal responders, it may be imperative to examine whether such a protocol could be of value for poor ovarian responders (Fatemi et al., 2010). Nonetheless, this should be performed in a prospective pilot study to examine the feasibility of this protocol owing to the lack of data regarding its safety and efficacy.

In conclusion, our pilot study suggests that the addition of hp-HMG after corifollitropin alfa in the antagonist setting results in a very promising pregnancy rate only in women <40 years old with a poor ovarian response according to the Bologna criteria. The limitations related to the methodology of the current study clearly prevent us from supporting the use of the specific protocol in routine clinical practice at present. However, the data do provide the rationale for performing a randomized controlled trial (RCT) to determine if there is sound evidence for a clinical introduction of this protocol. Based on our results, a future RCT should include ~120 young poor responders (<40 years old) fulfilling the Bologna criteria in order to detect a difference from 7% with current treatment protocols to 28% with corifollitropin alfa followed by hp-HMG, with a statistical power of 80% and a level of significance at 0.05.

Acknowledgements
The authors would like to thank Walter Meul for his invaluable help during data collection and abstraction. The authors would like to thank Dr Peter Humaidan for his substantial intellectual input during the study.

Authors’ roles
N.P.P. conceived and designed the study and performed the statistical analysis. N.P.P. and R.C. collected the data. All the authors participated in the writing of the manuscript and the interpretation of the results.

Funding
No specific funding was received for this study.

Conflict of interest
The Center for Reproductive Medicine receives research grants unrelated to this study from Ferring Pharmaceuticals, Merck Serono, and Merck Sharp and Dohme Corporation.

References


Younis JS. The Bologna criteria for poor ovarian response; has the job been accomplished? *Hum Reprod* 2012;27:1874–1875; author reply 5–6.