Corifollitropin alfa compared with follitropin beta in poor responders undergoing ICSI: a randomized controlled trial

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STUDY QUESTION: Does substituting 150 µg corifollitropin alfa for 450 IU follitropin beta during the first 7 days of ovarian stimulation in proven poor responders, result in retrieval of a non-inferior number (< 1.5 fewer) of cumulus oocyte complexes (COCs)?

SUMMARY ANSWER: A single s.c. dose of 150 µg corifollitropin alfa on the first day of ovarian stimulation, followed if necessary, from Day 8 onwards, with 450 IU of follitropin beta/day, is not inferior to daily doses of 450 IU follitropin beta. The 95% CI of the difference between medians in the number of oocytes retrieved was −1 to +1 within the safety margin of 1.5.

WHAT IS KNOWN ALREADY: Recent data from retrospective studies suggest that the use of corifollitropin alfa in poor responders is promising since it could simplify ovarian stimulation without compromising its outcome.

STUDY DESIGN, SIZE, DURATION: Seventy-nine women with previous poor response to ovarian stimulation undergoing ICSI treatment were enrolled in this open label, non-inferiority, randomized clinical trial (RCT).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Inclusion criteria were: previous poor response to ovarian stimulation (≤4 COCs) after maximal stimulation, age < 45 years, regular spontaneous menstrual cycle, body mass index: 18 – 32 kg/m² and basal follicle stimulating hormone ≤ 20 IU/l. On Day 2 of the menstrual cycle, patients were administered either a single s.c. dose of 150 µg corifollitropin alfa (n = 40) or a fixed daily dose of 450 IU of follitropin beta (n = 39). In the corifollitropin alfa group, 450 IU of follitropin beta were administered from Day 8 of stimulation until the day of human chorionic gonadotrophin (hCG) administration, if necessary. To inhibit premature luteinizing hormone surge, the gonadotrophin releasing hormone antagonist ganirelix was used. Triggering of final oocyte maturation was performed using 250 µg of recombinant hCG, when at least two follicles reached 17 mm in mean diameter.

MAIN RESULTS AND THE ROLE OF CHANCE: The number of COCs retrieved was not statistically different between the corifollitropin alfa and the follitropin beta groups [Median 3 versus 2, 95% CI 2 – 4, 2 – 3, respectively, P = 0.26]. The 95% CI of the difference between medians in the number of oocytes retrieved was −1 to +1. A multivariable analysis adjusting for all the potential baseline differences confirmed this finding. No significant difference was observed regarding the probability of live birth between the corifollitropin alfa and the follitropin beta group (live birth per patient reaching oocyte retrieval: 7.9 versus 2.6%, respectively, difference +5.3%, 95% CI: −6.8 to +18.3).

LIMITATIONS, REASONS FOR CAUTION: The present study was not powered to test a smaller difference (e.g. 1 COC) in terms of COCs retrieved as well as to show potential differences in the probability of pregnancy. Moreover, it would be interesting to assess whether the continuation of stimulation in the long acting FSH arm, where necessary, with 200 IU instead of 450 IU of follitropin beta would have altered the direction or the magnitude of the effect of the type of FSH, observed on the number of COCs retrieved.
Introduction

One of the major problems in IVF is the failure of exogenous FSH to induce multi-follicular development. This in turn leads to the retrieval of very few cumulus oocyte complexes (COCs), restricting the probability of transferring good quality embryos, and thus reducing the chance of pregnancy (Templeton and Morris, 1998).

Despite the fact that numerous strategies have been proposed for the management of this condition, known as poor ovarian response, the prognosis of these patients remains poor (Kyrrou et al., 2009; Venetis et al., 2010; Kolibianakis et al., 2011; Bosdou et al., 2012; Lehert et al., 2014).

Currently, evidence exists that co-administration of growth hormone (GH) and FSH (Kolibianakis et al., 2009) as well as treatment with transdermal testosterone prior to initiation of exogenous FSH (Bosdou et al., 2012) is associated with an increased probability of live birth. The most likely mechanism, explaining the higher chance of live birth associated with the above interventions, is an increase in the number of COCs retrieved (Bosdou et al., 2012) and/or in the proportion of patients reaching embryo transfer (Kolibianakis et al., 2009). Apparently, a small increase in the number of COCs retrieved in women with poor ovarian response, might lead to an increase in the proportion of patients reaching embryo transfer and thus in the proportion of patients exposed to the risk of pregnancy.

Corifollitropin alfa, which was recently introduced in clinical practice, has been associated with an increase in the number of COCs retrieved when compared with daily FSH in women ≤36 years of age (+1.7 COCs) (Devroey et al., 2009). In theory, corifollitropin alfa could serve poor responders better than daily FSH, since it has been shown to reach peak circulating levels within 2 days (Fauser et al., 2010) whereas daily FSH does so only after 3–5 days (Mannaerts et al., 1996).

It should be noted, however, that the chosen dose of corifollitropin alfa for use in ovarian stimulation for IVF was the result of simulation based on clinical data, which were not obtained from patients with poor ovarian response (Corifollitropin Alfa Dose-finding Study Group, 2008; de Greef et al., 2010). Nevertheless, recent data from retrospective studies (Polyzos et al., 2013a,b) suggest that the use of corifollitropin alfa is promising in poor responders and warrants further evaluation in properly conducted trials. In the retrospective study by Polyzos et al. (2013a) a historical group of low responders treated with a short agonist protocol was compared with a group of low responders treated by corifollitropin alfa. In that study, evidence was provided regarding the feasibility of simplifying ovarian stimulation by replacing daily injections of FSH for 7 days with a single injection of corifollitropin alpha, maintaining similar (although still low) pregnancy rates. Moreover, in the retrospective pilot study by Polyzos et al. (2013b) corifollitropin alfa followed by hp-HMG in a GnRH antagonist protocol resulted in very promising pregnancy rates in poor ovarian responders <40 years of age.

The purpose of this randomized controlled trial was to evaluate whether the substitution of 450 IU of follitropin beta for the first 7 days of ovarian stimulation with a single dose of 150 μg corifollitropin is associated with a non-inferior number of COCs (non-inferiority margin: −1.5 COCs) retrieved in proven poor responders undergoing intracytoplasmic sperm injection (ICSI).

Materials and Methods

Study population

Seventy-nine women with previous poor ovarian response undergoing ICSI treatment were enrolled in this prospective, randomized, open label, non-inferiority clinical trial. The study was conducted at the Unit for Human Reproduction at Aristotle University of Thessaloniki, from January 2011 to March 2014. Eligible patients asked for their consent and those who agreed to participate were randomized to receive either corifollitropin alfa or follitropin beta, according to a computer-generated randomization list, for which a research nurse, not involved with the recruitment of patients, was responsible. Randomization was performed after the completion of the couples’ evaluation if the inclusion criteria were met. Patients could participate in the study only once.

Inclusion criteria were: previous poor response to ovarian stimulation, defined as retrieval of ≤4 COCs in a previous IVF cycle in which a starting dose of at least 450 IU per day was used, age < 45 years, regular spontaneous menstrual cycle (24–35 days), body mass index (BMI) of 18–32 kg/m² and basal FSH ≤ 20 IU/l. Only fresh ejaculated sperm was used, while no preimplantation genetic screening was allowed.

The threshold used in the current study regarding the number of oocytes retrieved was decided prior to the publication of the Bologna criteria and was based on two previous studies (Aletebi, 2007; Weitzman et al., 2009), included in those used to reach a consensus for the threshold of oocytes retrieved in the Bologna manuscript (Ferraretti et al., 2011).

The FSH threshold used in the inclusion criteria in the current study did not serve as an ovarian reserve test. It was a prerequisite for patients entering the study to have shown a proven poor ovarian response. The rationale for choosing an upper threshold of FSH as an inclusion criterion was based on the fact that if endogenous FSH level is below a certain value, e.g. > 20 IU/l, then it is not possible to create serum FSH levels that are likely to induce multi-follicular development by administering high doses of exogenous FSH. In these patients, the high levels of endogenous FSH already stimulate to a maximum degree the ovaries in order to keep women in a reproductive status and thus the addition of even high doses of exogenous FSH convey no benefit.

If such patients, who will most likely produce a single follicle, despite being stimulated, were allowed to be recruited, any potential differences between...
confollitropin alfa and daily FSH, regarding the primary outcome measure, number of COCs retrieved, would probably have been obscured.

The study was approved by the Ethics Committee Review Board of our hospital and informed consent was obtained from all patients (NCT02046655).

**Ovarian stimulation**

On Day 2 of the menstrual cycle (stimulation day 1), patients were administered either a single s.c. dose of 150 μg confollitropin alfa (Bionva; NL Organon, Oss, the Netherlands) or began a course of seven fixed daily doses of 450 IU of follitropin beta (Puregon; NL Organon, Oss, the Netherlands). In the confollitropin alfa group, 450 IU of follitropin beta were administered from Day 8 of stimulation until the day of human chorionic gonadotrophin (hCG) administration, if necessary. To inhibit premature luteinizing hormone (LH) surge, a daily s.c. dose of 0.25 mg of gonadotrophin releasing hormone (GnRH) antagonist ganirelix (Orgalutran; NL Organon, Oss, the Netherlands) was administered when the leading follicle reached 14 mm in average diameter and continued up to the day of hCG administration, in both groups. Triggering of final oocyte maturation was performed using 250 μg of recombinant hCG (Ovitrelle, Merck Serono Europe Ltd, London, UK), when at least two follicles reached 17 mm in mean diameter. In case of monofollicular development, patients were allowed to proceed to oocyte retrieval.

Oocyte retrieval was performed by ultrasound-guided transvaginal follicular aspiration 36 h later after hCG administration. Intracytoplasmic sperm injection was used in all patients. Up to three embryos, in women ≤40 years of age and up to four embryos, in women >40 years of age were transferred on Day 2 or Day 3 of in vitro culture, if available, according to the Greek law of reproduction. Luteal phase was supported by vaginal administration of 600 mg/day of micronized progesterone (Utrogestan; Basins Iscovecso. Paris, France, vaginal tablets, 200 mg t.i.d) starting on the day of oocyte retrieval.

**Hormonal measurements and ultrasound assessment of follicular development**

Cycles were monitored by hormonal and ultrasound assessments performed at initiation of stimulation (Day 2 of the cycle), on Days 5, 8 of ovarian stimulation and on the day of hCG administration. Serum, LH, estradiol (E2) and progesterone levels were measured by means of the automated Elecsys immunoanalyzer (Roche Diagnostics, Mannheim, Germany). Intra-assay and inter-assay coefficients of variation were <3% and <4% for LH, <5% and <10% for E2 and <3% and <5% for progesterone, respectively.

**Outcome measures**

The primary outcome measure was the number of cumulus oocyte complexes retrieved. Secondary outcome measures included duration of stimulation, the number of metaphase II (MII) oocytes, the number of 2-pronuclei (2pn) zygotes, maturation rate, fertilization rate, the quality of embryos on Day 2 of in vitro culture, the proportion of patients with top quality embryos, the number of embryos transferred, the proportion of patients with embryo transfer, positive hCG per randomized patient and per embryo transfer, clinical pregnancy per randomized patient and per embryo transfer, miscarriage rate, serum hormonal levels of LH, E2 and progesterone during ovarian stimulation, the number of follicles ≥11 mm and ≥17 mm during ovarian stimulation and on the day of triggering final oocyte maturation. Analysis of all secondary outcome measures served as an exploratory analysis only that could lead to hypotheses which could be tested in future trials. For this reason, there was no need for multiplicity correction in the analysis of secondary outcome measures.

Clinical pregnancy was defined as the presence of intrauterine sac with fetal heart activity at 6–8 weeks of gestation. Miscarriage rate was defined as pregnancy loss before 20 weeks of gestation. Maturation rate was defined as the number of MII oocytes divided by the number of COCs retrieved. Fertilization rate was defined as the number of 2pn zygotes divided by the number of MII oocytes or by the number of COCs retrieved. Embryo quality was assessed according to morphological criteria based on the assessment of the blastomeres and the degree of blastomeric fragmentation (Ziebe et al., 1997).

**Sample size**

Sample size estimation, using PASS 11 (Hintze 2011), showed that 39 patients were required in each arm, in order to detect non-inferiority with a margin of −1.5 COCs, using a one-sided, Mann–Whitney test with 80% power, SD of 2.4 and a significance level of 0.025.

The difference in the number of COCs retrieved, on which the power analysis was performed, was chosen on the basis that 1.5 oocyte difference would be likely to result in approximately one embryo difference between groups, assuming a fertilization rate of 65%. A difference on one embryo is likely to be significant in these patients and may distinguish those who will from those who will not undergo embryo transfer.

**Statistical analysis**

Analysis per protocol and per intention-to-treat (ITT) were performed, analyzing patients according to whether they reached oocyte retrieval and thus could be evaluated for the primary outcome measure as well as according to their initial randomization and treatment intent, respectively. In the ITT analysis all patients who started treatment were included in the analysis regardless of whether they proceeded to oocyte retrieval or not. If oocyte retrieval was cancelled patients were considered as having retrieved zero oocytes. Parametric (independent sample Student T-test) and non-parametric (Mann–Whitney U-test) tests were used for comparison of continuous variables and Fisher’s exact test for comparison of binary variables. Values are expressed as median (interquartile range) unless stated otherwise. Generalized estimating equation was used to compare serum hormonal levels of LH, E2 and progesterone, measured multiple times during ovarian stimulation, between the two groups.

An additional per protocol analysis was performed restricting the population to be analysed to those patients conforming to Bologna definition of poor ovarian response (Ferraretti et al., 2011).

**Results**

Seventy-nine women with proven poor ovarian response were included in the current study (confollitropin alfa group: n = 40 versus follitropin beta group: n = 39). Baseline characteristics of patients randomized in the two groups are shown in Table I.

**Primary outcome measure**

Using per protocol analysis, excluding the three patients who did not have oocyte retrieval (as shown in the flow diagram), the median (interquartile range) of COCs retrieved was not statistically different between the confollitropin alfa and the follitropin beta groups [3.0 (4) versus 2.0 (3) COCs, P = 0.26, respectively; 95% CI for the median 2–4, 2–3, respectively]. The 95% CI of the difference between medians in the number of oocytes retrieved was −1 to +1. Analysis per intention to treat did not change the direction of the results obtained. The median (interquartile range) of COCs retrieved was not statistically different between the confollitropin alfa and the follitropin beta groups [2.5 (4) versus 2.0 (3) COCs, P = 0.32, respectively; 95% CI for the median 2–4, 2–3, respectively]. The 95% CI of the difference between medians in the number of oocytes retrieved was −1 to +1.
Although no imbalances were seen after randomization (Tables I and II), a multivariable analysis was performed with dependent variable the number of COCs retrieved and independent variables: age, BMI, infertility duration, number of previous IVF trials, indication for infertility, number of antral follicles, mean ovarian volume and treatment, with either follitropin alfa or follitropin beta. This analysis showed that the only variable that was important for the number of COCs retrieved was the number of antral follicles ($P$, 0.001). The treatment mode, corifollitropin alfa or follitropin beta had no effect on the number of COCs retrieved, after adjusting for all the above variables ($P = 0.98$, coefficient 0.012, 95% CI: $-1.018$ to $0.992$). The adjusted means of COCs (95% CI) in the corifollitropin alfa and the follitropin beta were 2.96 (2.26–3.65) and 2.94 (2.24–3.65), respectively.

**Secondary outcome measures**

Duration of stimulation was comparable between the two groups [10.0 (3) versus 10.0 (3) days, $P = 0.49$, 95% CI of the difference $-1$ to $+1$]. The distribution of patients who met the criteria for hCG administration on a certain day was similar between the corifollitropin alfa and the follitropin beta groups (Fig. 1).

On the day of hCG administration, no significant differences were observed between the corifollitropin alfa and the follitropin beta group regarding the number [median (interquartile range)] of follicles measuring either $\geq 11$ mm [5 (6) versus 5 (6), $P = 0.81$, respectively] or $\geq 17$ mm [2 (2) versus 3 (2), $P = 0.19$, respectively]. Endometrial thickness was not statistically different between the corifollitropin alfa and the follitropin beta groups [10.1 (4) versus 9.6 (3) mm, $P = 0.31$, respectively].

As shown in Table II, there was no significant difference between the corifollitropin alfa and the follitropin beta group in the number of MII oocytes or the number of 2PN zygotes. Similarly, no differences were observed between the two groups regarding maturation rate, fertilization rate and the number of embryos transferred.

The number of embryos classified as top, good or low quality (Table II) and the proportion of patients with top quality embryos (Table III) were

**Table I Baseline characteristics of patients randomised in the corifollitropin alfa group and the follitropin beta group.**

<table>
<thead>
<tr>
<th>Demographics and fertility characteristics</th>
<th>Corifollitropin alfa (n = 40)</th>
<th>Follitropin beta (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (interquartile range, 95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.5 (4.8, 39–42)</td>
<td>41.0 (4.0, 39–42)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>65 (25, 63–72.9)</td>
<td>65 (16, 62.9–70)</td>
</tr>
<tr>
<td>Body mass index (BMI) (kg/m²)</td>
<td>24.8 (7.6, 23.3–27.6)</td>
<td>24.1 (6.3, 23.2–25.4)</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>5 (6, 3–7)</td>
<td>5 (5.3, 9–6)</td>
</tr>
<tr>
<td>Age of first menstruation (years)</td>
<td>13 (2, 12–13)</td>
<td>13 (2, 12–13)</td>
</tr>
<tr>
<td>Duration of menstruation (days)</td>
<td>4 (2, 4–4)</td>
<td>4 (2, 3–4)</td>
</tr>
<tr>
<td>Previous IVF/ICSI trials</td>
<td>2 (2, 2–3)</td>
<td>3 (2, 2–4)</td>
</tr>
<tr>
<td>% (n, 95% CI) Cause of infertility besides poor ovarian response:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal factor</td>
<td>22.5% (9, 12.3–37.5)</td>
<td>20.5% (8, 10.8–35.5)</td>
</tr>
<tr>
<td>Male factor</td>
<td>47.5% (19, 32.9–62.5)</td>
<td>41.0% (16, 27.1–56.6)</td>
</tr>
<tr>
<td>Tubal &amp; male factor</td>
<td>5.0% (2, 1.4–16.5)</td>
<td>10.3% (4, 4.1–23.6)</td>
</tr>
<tr>
<td>Fibroid uterus</td>
<td>7.5% (3, 2.6–19.9)</td>
<td>2.6% (1, 0.5–13.2)</td>
</tr>
<tr>
<td>Other/unexplained</td>
<td>17.5% (7, 8.7–32.0)</td>
<td>25.6% (10, 14.6–41.1)</td>
</tr>
<tr>
<td>Mean ovarian volume (ml)</td>
<td>5.2 (2.3, 4.8–6.2)</td>
<td>5.1 (2.4, 4.9–6.2)</td>
</tr>
<tr>
<td>AFC</td>
<td>7 (4, 5–8)</td>
<td>6 (5, 5–8)</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>10.0 (6.5, 8.5–12)</td>
<td>10.2 (6.1, 7.6–12)</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>5.0 (2.7, 4.0–6)</td>
<td>4.8 (3.4, 4–5.9)</td>
</tr>
<tr>
<td>E₂ (pg/ml)</td>
<td>38 (29, 32.3–49.9)</td>
<td>44 (27, 36.5–53)</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>0.4 (0.3, 0.3–0.4)</td>
<td>0.3 (0.3, 0.2–0.4)</td>
</tr>
</tbody>
</table>

IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; AFC, antral follicle count; FSH, follicle stimulating hormone; LH, luteinizing hormone; E₂, estradiol.
similar in both groups on day two of in vitro culture. Considering the patients who reached oocyte retrieval (per protocol analysis) the proportions \( n \) of patients with no embryo transfer [23.7% (9) versus 26.3% (10)], with a single embryo transferred [23.7% (9) versus 36.8% (14)], with two embryos transferred [15.8% (6) versus 13.2% (5)], with three embryos transferred [36.8% (14) versus 15.8% (6)] and four embryos transferred [0% (0) versus 7.9% (3)] did not differ significantly between the corifollitropin alfa and the follitropin beta group, respectively \( P = 0.11 \).

No significant difference was observed regarding the probability of clinical pregnancy, live birth or miscarriage rate between the corifollitropin alfa and the follitropin beta group (Table III). No multiple pregnancies were achieved.

Cryopreservation was feasible in a single patient in the corifollitropin alfa group, who did not become pregnant in the fresh and had a miscarriage in the subsequent frozen cycle \( n = 2 \).

The mean number and size distribution of follicles on Day 5, Day 8 and on the day of hCG administration were comparable between the two groups (Fig. 2). Serum concentrations of LH, estradiol and progesterone on Day 5, 8 of ovarian stimulation and on day of hCG administration between the corifollitropin alfa and the follitropin beta group are presented in Table IV. In the corifollitropin alfa group, progesterone levels increased with the same rate as in the follitropin beta group after Day 7, when stimulation was continued with a daily dose of follitropin beta, whereas, prior to that date, the rate of increase for progesterone was significantly higher in the follitropin beta group when compared with the corifollitropin alfa group. No differences were observed for LH and E2 during ovarian stimulation between the two groups compared (Table IV).

No adverse events were recorded in either arm of the study.

Per protocol analysis in patients who fully conformed to the Bologna criteria

Per protocol analysis in patients who fully conformed to the Bologna criteria (Ferraretti et al., 2011) is shown in Supplementary Table SI. No differences were seen between the number of COCs retrieved between the corifollitropin alfa and the follitropin beta groups. The 95% CI of the difference in medians was \(-1 \) to \(+1\) COCs.

**Discussion**

The current RCT has shown that corifollitropin alfa for the first 7 days of ovarian stimulation, followed if necessary with 450 IU of follitropin beta/day, is not inferior to 450 IU of daily follitropin beta, when judged by the number of COCs retrieved, using a safety margin of 1.5 COCs. The difference in the number of COCs retrieved was in favour of corifollitropin alfa \((+1)\) COCs, while the 95% CI of the difference between medians was \(-1 \) to \(+1\) COCs and thus the hypothesis of non-inferiority cannot be rejected.

<table>
<thead>
<tr>
<th>Clinical outcome per cycle</th>
<th>Corifollitropin alfa ( n = 40 )</th>
<th>Follitropin beta ( n = 39 )</th>
<th>95% CI of the difference between medians</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>COCs Per protocol analysis</td>
<td>3.0 (4.2–4)</td>
<td>2.0 (3.2–3)</td>
<td>(-1 ) to (+1)</td>
<td>0.26</td>
</tr>
<tr>
<td>COCs ITT analysis</td>
<td>2.5 (4.2–4)</td>
<td>2.0 (3.2–3)</td>
<td>(-1 ) to (+1)</td>
<td>0.32</td>
</tr>
<tr>
<td>Metaphase II oocytes (MII)</td>
<td>2.0 (4.1–3)</td>
<td>2.0 (3.1–3)</td>
<td>(-1 ) to (+1)</td>
<td>0.78</td>
</tr>
<tr>
<td>Maturation rate (MII/COCs) % per patient</td>
<td>84.5 (50, 66.7–100)</td>
<td>100 (33, 79.5–100)</td>
<td>(-13.3 ) to (+0)</td>
<td>0.59</td>
</tr>
<tr>
<td>2-pronuclei oocytes (2PNs)</td>
<td>1.5 (3, 1–2)</td>
<td>1.0 (3, 1–2)</td>
<td>(-1 ) to (+1)</td>
<td>0.59</td>
</tr>
<tr>
<td>Fertilization rate (2PN/MII) % per patient</td>
<td>80.0 (50, 63.3–100)</td>
<td>66.7 (50, 50–77.5)</td>
<td>(-0 ) to (+28.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Fertilization rate (2PN/COCs) % per patient</td>
<td>58.3 (36, 50–80)</td>
<td>50.0 (35, 50–75)</td>
<td>(-12.5 ) to (+16.7)</td>
<td>0.66</td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>1.5 (3, 1–2.7)</td>
<td>1.0 (2, 1–2)</td>
<td>(-0 ) to (+1)</td>
<td>0.48</td>
</tr>
<tr>
<td>Embryo quality on day two of in vitro culture:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top quality embryos</td>
<td>0.0 (0, 0–0)</td>
<td>0.0 (0, 0–0)</td>
<td>(-0 ) to (+0)</td>
<td>0.90</td>
</tr>
<tr>
<td>Medium quality embryos</td>
<td>0.0 (2, 0.3–2)</td>
<td>0.0 (2, 0–1)</td>
<td>(-0 ) to (+1)</td>
<td>0.57</td>
</tr>
<tr>
<td>Low quality embryos</td>
<td>0.0 (1, 0–0)</td>
<td>0.0 (0, 0–0)</td>
<td>(-0 ) to (+0)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

ITT, intention to treat analysis.

*With the exception of COCs all additional analyses are performed in an exploratory fashion.

Table II Clinical outcome in the corifollitropin alfa and in the follitropin beta groups.*
This is the first RCT comparing long acting versus daily recombinant FSH in poor responders. Patients included in the current study must have exhibited poor ovarian response in a previous cycle, in order to be eligible for inclusion. Poor responders were not recruited on the basis of ovarian reserve markers and a potential suboptimal response to ovarian stimulation, since this will inevitably identify as poor responders some patients who will subsequently show an adequate response to ovarian stimulation. For reasons of normal variability, the same reserve was capable of. Nevertheless, using proven ovarian response criteria some patients who will subsequently show an adequate response to ovarian stimulation, since this will inevitably identify as poor responders are too valuable, an additional analysis restricted in 63.3% of the patients included, those conforming to the Bologna criteria (Ferraretti et al., 2011) was performed. The results from this additional analysis, although in a smaller sample size, support the conclusion that corifollitropin alfa for the first 7 days of ovarian stimulation, followed if necessary with 450 IU of follitropin beta/day, is not inferior to 450 IU of rFSH in poor responders, where the number of oocytes retrieved is small, the non-inferiority margin was set lower, at 1.5 oocytes. Only through a new RCT it can be properly inferred if corifollitropin alfa reserve using AMH assessment, the need for these criteria to be updated has been recognized by the same authors of the original manuscript (Ferraretti and Gianaroli, 2014).

Nevertheless, since the need for a more uniform definition of POR is evident and the fact that properly collected data from RCTs in poor responders are too valuable, an additional analysis restricted in 63.3% of the patients included, those conforming to the Bologna criteria (Ferraretti et al., 2011) was performed. The results from this additional analysis, although in a smaller sample size, support the conclusion that corifollitropin alfa for the first 7 days of ovarian stimulation, followed if necessary with 450 IU of follitropin beta/day, is not inferior to 450 IU of daily follitropin beta. These data provide additional insight to the reader about the comparison of corifollitropin alfa with standard rFSH in this population and they might also be useful in a future meta-analysis.

Typically in non-inferiority studies performed in normal responders the non-inferiority margin is usually set at three oocytes (Huirne et al., 2006; Moon et al., 2007). Because the current study was performed in proven poor responders, where the number of oocytes retrieved is small, the non-inferiority margin was set lower, at 1.5 oocytes. Only through a new RCT it can be properly inferred if corifollitropin alfa would still be considered non-inferior to follitropin beta when using a lower non-inferiority margin (e.g. 1 COC). Nevertheless, given the fact

<table>
<thead>
<tr>
<th>Table III</th>
<th>ICSI outcome between the corifollitropin alfa and the follitropin beta groups. *</th>
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<tbody>
<tr>
<td></td>
<td>Corifollitropin alfa (n = 38)**</td>
</tr>
<tr>
<td>% (n)</td>
<td>% (n)</td>
</tr>
<tr>
<td>Proportion of patients with top quality embryos</td>
<td>15.8 (6)</td>
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<tr>
<td>Patients with embryo transfer</td>
<td>76.3 (29)</td>
</tr>
<tr>
<td>Positive hCG per patient reaching oocyte retrieval</td>
<td>15.8 (6)</td>
</tr>
<tr>
<td>Positive hCG per ET</td>
<td>20.7 (6)</td>
</tr>
<tr>
<td>Clinical pregnancy per patient reaching oocyte retrieval</td>
<td>7.9 (3)</td>
</tr>
<tr>
<td>Clinical pregnancy per ET</td>
<td>10.4 (3)</td>
</tr>
<tr>
<td>Live birth per patient reaching oocyte retrieval</td>
<td>7.9 (3)</td>
</tr>
<tr>
<td>Live birth per ET</td>
<td>10.4 (3)</td>
</tr>
<tr>
<td>Miscarriage rate per positive hCG</td>
<td>50.0 (3)</td>
</tr>
</tbody>
</table>

*All analyses are performed in an exploratory fashion. **Per protocol analysis, ET: embryo transfer.
that the comparison performed in the current study showed one oocyte more in the corifollitropin alfa group (per protocol analysis) and the 95% CI for the difference in the median number of COCs retrieved between the two gonadotrophins was $-1$ to $+1$ COCs, it is unlikely that even such lower inferiority margin, e.g. one oocyte, would have made a difference in the conclusions drawn in the current study.
The current study suggests that it is feasible to replace daily FSH injections with a long acting FSH injection without anticipating that the number of COCs retrieved might be less than that achieved with daily FSH injections, considering a safety margin of 1.5 COCs. Consequently and as confirmed in the current study, the proportion of patients reaching embryo transfer is expected to be the same (Table III). Although the present study was not powered to show potential differences in the probability of pregnancy, the fact that a non-significant difference in favour of corifollitropin alfa (+5.3%) was observed in live birth rate is at least reassuring. Nevertheless, this requires further testing in additional trials.

Although each new intervention in assisted reproduction should be evaluated on the basis of its effect on the probability of live birth, the current study intended to test the feasibility of this concept in terms of efficacy, i.e. the outcome of ovarian stimulation per se. Assessment of whether there is a benefit of using corifollitropin alfa in poor responders, in terms of pregnancy rates, will require sample sizes that can be achieved only by multicentre studies.

The underlying problem characterizing poor ovarian response is the severely reduced ovarian reserve. As suggested by the present data an improvement in the number of oocytes retrieved by using corifollitropin alfa instead of follitropin beta is unlikely. This is not surprising given the small number of antral follicles, amenable to FSH stimulation, present in these patients.

In the current study, a high daily follitropin beta dose (450 IU) for the first 7 days of stimulation was replaced by corifollitropin alfa. The dose of corifollitropin alfa, used for this purpose, had been shown to be of equal efficacy to a daily dose of 200 of recombinant daily FSH in normal responders (Devroey et al., 2009). This questions the benefit of using high-dose daily FSH stimulation in poor responders with the aim of retrieving more oocytes (Lefebvre et al., 2014). Moreover, it signifies the limited flexibility of manipulating ovarian response in these patients from whom it is not possible to retrieve COCs that do not exist.

It would be interesting to assess whether the continuation of stimulation in the long acting FSH arm, where necessary, with 200 IU instead of 450 IU of follitropin beta would have altered the direction or the magnitude of the effect of the type of FSH, observed on the number of COCs retrieved. In that case, if a lower daily FSH dose was used in the corifollitropin alfa group then the comparison performed would be between two different ovarian stimulation protocols and would not be focusing on the replacement of daily FSH for the first 7 days of ovarian stimulation by corifollitropin alfa, as in the current study.

Corifollitropin alfa when compared with 450 IU of daily follitropin beta does not compromise the outcome of ovarian stimulation; however, it undoubtedly simplifies IVF treatment as it is employed in a GnRH antagonist environment and is replacing seven daily FSH injections with a single one of a long acting FSH. Thus, its use in poor responders is an interesting concept to further evaluate, since it would greatly reduce the burden of treatment to which these patients are exposed. Moreover, its cost, although variable between countries, is likely to be less than the cost of seven daily injections of 450 IU of recombinant FSH.

In the current study, in the context of an exploratory analysis, significantly higher progesterone levels were observed in patients treated with daily when compared with long acting FSH (Table IV). Given that the number of COCs was not significantly different between the two groups compared, this might be associated with a differential effect of FSH type on intra-follicular progesterone production. This seems to be supported by the fact that in the corifollitropin alfa group, progesterone seems to be increased with the same rate as in the daily follitropin beta group, when ovarian stimulation is intensified by the daily use of 450 IU of follitropin beta (after Day 7) (Table IV). Previous data have suggested that the levels of progesterone seem to be associated with the intensity of stimulation in terms of the total dose of FSH (Venetis et al., 2007, 2013; Kolibianakis et al., 2012). In the Engage trial (Devroey et al., 2009), higher, although not significantly so, progesterone levels were observed in the daily FSH (200 IU) group when compared with the long acting FSH on Day 8 of stimulation and on the day of hCG administration (Fauser et al., 2010). Although the peak level of progesterone on the day of hCG was not particularly elevated, it has been recently reported that a detrimental effect of progesterone on pregnancy rates is present with thresholds as low as 0.8 ng/ml. Thus, even small differences in progesterone levels might be clinically relevant for pregnancy achievement in poor responders, where embryo quality is also compromised (Venetis et al., 2013).

In conclusion, the present study has shown that corifollitropin alfa for the first 7 days of ovarian stimulation, followed if necessary with 450 IU of follitropin beta/day, is not inferior to 450 IU of daily follitropin beta, considering the number of COCs retrieved, using a safety margin of 1.5 COCs (95% CI of the difference between medians in the number of COCs retrieved −1 to +1).

Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors’ roles
E.M.K.: conceived the idea of the study, contributed towards the analyses and interpretation of the data and drafted the manuscript. C.A.V.: contributed in the analyses and interpretation of the data, and revised the manuscript for important intellectual content. J.K.B.: contributed towards the interpretation of the data and drafted the manuscript. C.A.V.: contributed towards the interpretation of the data and revised the manuscript for important intellectual content. L.Z., K.C., A.Ma., S.T., S.M., A.Mi. contributed in the interpretation of the data and revised the manuscript for important intellectual content. None declared.

Conflict of interest
None declared.

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