human reproduction

ORIGINAL ARTICLE *Reproductive endocrinology*

High ovarian response does not jeopardize ongoing pregnancy rates and increases cumulative pregnancy rates in a GnRH-antagonist protocol

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STUDY QUESTION: Is the ovarian response to controlled ovarian stimulation (COS) related to the ongoing pregnancy rate when taking into account the main covariates affecting the probabilities of pregnancy following fresh embryo transfer?

SUMMARY ANSWER: In patients treated with corifollitropin alfa or daily recombinant FSH (rFSH) in a GnRH-antagonist protocol, a high ovarian response did not compromise ongoing pregnancy rates and increased cumulative pregnancy rates following fresh and frozen-thawed embryo transfer.

WHAT IS KNOWN AND WHAT THIS PAPER ADDS: A strong association between the number of oocytes and pregnancy rates has been described but this is the first comprehensive analysis assessing important confounders that might affect pregnancy rates.

STUDY DESIGN: In a large, prospective, double-blind, randomized trial (Engage; $n = 1506$), patients were treated with either a single dose of 150 μg corifollitropin alfa or daily 200 IU rFSH for the first 7 days of COS in a GnRH-antagonist (ganirelix) protocol. In this retrospective analysis, patients were categorized into five groups according to the number of oocytes retrieved (0–5, 6–9, 10–13, 14–18 and >18 oocytes). The number of good-quality embryos obtained and transferred, as well as the ongoing pregnancy rates, live birth rates and cumulative ongoing pregnancy rates per started cycle by group were evaluated. Univariate analysis was performed to identify factors that predict the chance of ongoing pregnancy. Logistic regression analysis on the dependent variables ongoing pregnancy and cumulative ongoing pregnancy, respectively, including oocyte category as an independent factor in the model, was performed by treatment group (corifollitropin alfa and rFSH) and overall. The likelihood of ongoing pregnancy and cumulative ongoing pregnancy was then evaluated taking into account ovarian response as well as other identified significant predictors of success.

PARTICIPANTS AND SETTING: In total, 1506 patients had been randomized in a ratio of 1:1 to either of the treatment groups. Patients were aged ≤36 years and had a body weight >60 kg.

MAIN RESULTS AND THE ROLE OF CHANCE: The ongoing pregnancy rates per started cycle increased in the corifollitropin alfa and rFSH groups from 31.9 and 31.3%, respectively, in the lowest response group (0–5 oocytes) to 41.9 and 43.4% in the highest response group (>18 oocytes) with a significant linear trend ($P = 0.04$). The cumulative pregnancy rates taking frozen–thawed embryo transfers into account increased from 33.0 and 31.3% to 60.8 and 55.9% in the corifollitropin alfa and rFSH groups, respectively. Univariate logistic regression analyses of ongoing pregnancy showed significant effects for the following factors: embryo transfer (double or single, $P < 0.01$), region of treatment (North America or Europe, $P < 0.01$), progesterone level on the day of hCG (>1.5 or ≤1.5 ng/ml, $P < 0.01$), start day of the stimulation (cycle day 2 or 3, $P = 0.02$) and age ($P = 0.04$). Logistic regression analysis of ongoing pregnancy using 10–13 oocytes as the reference category, per treatment group and overall revealed estimated odds ratios (OR) close to 1.0 versus the reference, without statistically significant differences with and without adjustment for significant predictive factors affecting pregnancy rates. Unadjusted OR for cumulative pregnancy reflected significantly lower odds of pregnancy for the lowest response group and significantly higher odds of pregnancy for the highest response group in comparison with the reference. When adjusted for the predictive factors, the cumulative ongoing pregnancy

OR (95% confidence interval) of the highest response group versus the reference group was 1.87 (1.34–2.59) when the data of both treatment groups were pooled.

BIAS, CONFOUNDING AND OTHER REASONS FOR CAUTION: The number of covariates included in the final model was limited to five major factors and not all other potentially significant predictive factors were available for evaluation.

GENERALIZABILITY TO OTHER POPULATIONS: This analysis is limited to IVF patients with a regular menstrual cycle up to 36 years of age and a body weight >60 and ≤90 kg treated with a GnRH-antagonist protocol and cannot be extrapolated to other patient populations or treatment regimens.

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Introduction

To date, several studies suggest an optimal range of oocytes obtained in response to ovarian stimulation for IVF, below and above which outcomes are compromised (van der Gaast et al., 2006; Verberg et al., 2009b; Sunkara et al., 2011). This hypothesis is generally accepted, as retrieval of fewer than 6–8 oocytes per started cycle is known to be associated with compromised chance of success (Heijnen et al., 2007; Fauser et al., 2010a), whereas more than 18–20 oocytes is associated with an increased risk of ovarian hyperstimulation syndrome (OHSS) (Papanikolaou et al., 2006). However, it is still uncertain whether an optimal number of oocytes exists within the range of 8–18 oocytes in terms of endometrial receptivity and whether this optimum could differ between GnRH-antagonist and long GnRH-agonist protocols. The scientific debate is further fuelled by the comparison of mild stimulation, aiming to limit the number of oocytes obtained to <8, with conventional stimulation, with the target to generate 8–15 oocytes (Fauser et al., 2010a). Mild stimulation certainly may benefit in decreasing the risk of OHSS, which increases with the degree of stimulation (Papanikolaou et al., 2006; Tarlatzis et al., 2012). Additional advantages have been reported to include improved oocyte and embryo quality (Baart et al., 2007) and endometrial receptivity (Devroey et al., 2004; Kyrou et al., 2009).

Alternatively, one could directly compare the implantation rates of patients with mild or conventional stimulation according to the number of oocytes retrieved either following a GnRH-antagonist protocol or a long GnRH-agonist protocol. A retrospective analysis of 1849 patients treated with a long GnRH-agonist protocol did not reveal an association between a high number of oocytes and decreased pregnancy outcome (Kok et al., 2006). In a meta-analysis including three RCTs, Verberg (Verberg et al., 2009a) showed that following mild stimulation the retrieval of a low number of oocytes (median value of five) was associated with the highest chance of ongoing pregnancy rate per embryo transferred when using a GnRH-antagonist protocol, whereas the highest ongoing pregnancy rates were associated with a median of 10 oocytes in a conventional long protocol of GnRH agonist (Verberg et al., 2009b). These preliminary data suggested that the association between the number of oocytes and chance of pregnancy may be different in mild stimulation/GnRH-antagonist and conventional stimulation/long GnRH-agonist protocols.

A large study by Sunkara et al. (2011), including 400 135 treatment cycles without distinguishing the treatment regimen applied, demonstrated that there is a strong association between the number of oocytes and the live birth rates, which increased with the number of oocytes up to ∼15 oocytes, plateaued between 15 and 20 oocytes and steadily declined beyond 20 oocytes. The latter effect may be related to the endometrial receptivity, as recent studies demonstrated a significant association between pre-ovulatory progesterone levels and the number of follicles, whereas a progesterone rise >1.5 ng/ml was associated with a decreased probability of pregnancy in patients treated either with a GnRH agonist or GnRH antagonist (Bosch et al., 2010; Kyrou et al., 2011; Al-Azemi et al., 2012; Kolibianakis et al., 2012).

The current retrospective analysis was undertaken to further examine the possible association between the degree of ovarian response and the chance of pregnancy in a fixed GnRH-antagonist protocol. In an analysis of a large, randomized, double-blind trial (Devroey et al., 2009; Boostanfar et al., 2012), the relationship between the number of oocytes and ongoing pregnancy and live birth rates following treatment with 150 μg corifollitropin alfa or daily 200 IU rFSH was evaluated. In contrast to previous studies (van der Gaast et al., 2006; Sunkara et al., 2011) confounding factors impacting the chance of ongoing pregnancy were considered in this evaluation.

Materials and Methods

Engage was a randomized, double-blind, double-dummy, non-inferiority clinical trial carried out in 34 IVF centers (20 in Europe and 14 in North America). Patients aged 18–36 years with a body weight of >60 kg

were treated with either a single dose of corifollitropin alfa (Elonva, N.V. Organon, The Netherlands) ($n = 756$) or daily recombinant FSH (rFSH; follitropin beta, Puregon Pen, N.V. Organon) ($n = 750$) for the first 7 days of controlled ovarian stimulation (COS) in a GnRH-antagonist (ganirelix, Orgalutran, N.V. Organon) protocol. The treatment regimen has been described in detail previously (Devroey *et al.*, 2009). Briefly, patients started stimulation on either menstrual cycle day 2 or 3 with a single injection of 150 μg corifollitropin alfa or daily injections of 200 IU rFSH continuing through the first 7 days of stimulation. In both treatment arms, from stimulation day 8 onwards, treatment was continued with daily 200 IU rFSH up to and including the day of hCG administration. The dose was allowed to be reduced from day 6 onwards only when required in the opinion of the investigator. The protocol allowed for withholding rFSH administration for a maximum of 3 days up to and including the day of hCG administration, at the discretion of the investigator. Ganirelix (0.25 mg) was administered daily from stimulation day 5 up to and including the day of hCG.

Investigators could decide to cancel a treatment cycle in case of too low or too high an ovarian response at their own discretion. In addition, if more than 30 follicles ≥ 11 mm were observed on ultrasound, hCG was to be withheld and the cycle was to be cancelled.

Urinary hCG 10 000 IU (or 5000 IU in the case of too high an ovarian response in the investigator's view) was administered to induce final oocyte maturation as soon as at least three follicles ≥ 17 mm were observed by ultrasound scan, or the next day.

After ∼34–36 h, oocyte retrieval followed by standard IVF or ICSI was performed. Embryo quality was evaluated for all available embryos on Day 3 of culture. Embryos graded as Grade 1 (6–10 cells, no fragmentation and equal blastomere size) or Grade 2 (allowing up to 20% fragmentation) were qualified as good-quality embryos. A maximum of two embryos were replaced 3 or 5 days after oocyte retrieval.

To support implantation and early pregnancy, luteal phase support with progesterone (at least 600 mg/day vaginally or at least 50 mg/d i.m.) was started on the day of oocyte retrieval and continued for at least 6 weeks. Ongoing pregnancy was defined as the presence of at least one fetus with heart activity at 10 weeks after embryo transfer.

Statistical analyses

In the current retrospective analysis of the Engage trial, patients were categorized into five groups according to the number of oocytes retrieved: 0–5, 6–9, 10–13, 14–18 and >18 oocytes. Patients who cancelled the IVF cycle owing to too low an ovarian response or too high a response were included in the 0–5 and >18 oocyte categories, respectively. Patients who cancelled the IVF cycle for other reasons were excluded from the analysis. The number of oocytes retrieved was not analyzed as a quantitative variable because then patients who cancelled the IVF cycle because of too high an ovarian response would have to be excluded from the analyses.

Summary statistics of patient baseline characteristics, ovarian stimulation characteristics and efficacy outcomes by oocyte category are presented. The efficacy outcomes are presented per started cycle: patients who cancelled the IVF cycle prior to embryo transfer were included in the summary statistics with zero embryos (obtained, transferred and cryopreserved) and zero implantation rate, and were considered non-pregnant.

The effect of treatment group (corifollitropin alfa, rFSH) and the effect of each of the patient baseline characteristics on the distribution of patients in the categorized oocyte groups (0–5, 6–9, 10–13, 14–18 and >18 oocytes) was tested using a multicategory logit model with oocyte category as the nominal dependent variable and the covariate of interest (treatment group or one of the baseline characteristics) as an independent factor. The *P*-value for the difference between corifollitropin

alfa and rFSH of the incidence of elevated progesterone in the highest oocyte category was calculated using the Fisher's exact test.

The effect of treatment group on the percentage of subjects with elevated progesterone level on the day of hCG (>1.5 ng/ml) across the five oocyte category groups was determined in a logistic regression model including oocyte category group and treatment group as independent factors in the model.

The effect of number of oocytes retrieved on ongoing pregnancy and cumulative ongoing pregnancy rates was estimated using logistic regression analysis. A first logistic model for predicting probabilities of ongoing pregnancy per started cycle after fresh embryo transfer including oocyte category (0–5, 6–9, 10–13, 14–18 and >18) as an independent factor was applied to derive estimated odds ratios (OR) of the different oocyte categories, while a second model including oocyte category, age, start day of the stimulation (cycle day 3 versus 2), region (North America versus Europe), and progesterone level on the day of hCG (>1.5 versus ≤1.5 ng/ml) was applied to obtain OR of the oocyte categories adjusted for predictive factors of pregnancy. Additionally, the same logistic model was applied for the prediction of probabilities of cumulative ongoing pregnancy following fresh and frozen embryo transfer. The 10–13 oocyte category was used as the reference oocyte category in the logistic regression analyses. A linear trend test within the logistic models was applied to the pooled data of the two treatment groups to test for a linear trend in ongoing pregnancy rate across the five oocyte categories. The trend test was first performed without adjustment for the predictive factors of pregnancy (Cochran-Armitage trend test) and subsequently with adjustment for the predictive factors of pregnancy.

Potential predictive factors of ongoing pregnancy were *a priori* identified based on the trial design and a previous analysis for such factors (Doody *et al.*, 2011). These factors were identified by univariate logistic regression per candidate predictive factor. Candidate predictive factors that were evaluated were age, start day of the stimulation (cycle day 3 versus 2), region (North America versus Europe), embryo transfer (double versus single), progesterone level on the day of hCG (>1.5 versus ≤1.5 ng/ml) and delay of hCG administration (no delay versus 1-day delay). The significance level below which candidate predictive factors were considered predictive factors of ongoing pregnancy was set to 0.15 in the univariate logistic regression analyses, i.e. $P < 0.15$.

All calculations were performed in SAS®, version 9.1 (SAS Institute Inc., Carey, NC, USA).

Results

Frequency distribution of oocytes and embryos

The distribution of patients in categorized oocyte retrieval groups 0–5, 6–9, 10–13, 14–18 and >18 oocytes was 12.6, 18.2, 22.6, 21.8 and 24.9% in the corifollitropin alfa arm, and 12.9, 22.9, 25.2, 20.9 and 18.2% in the rFSH arm. The effect of treatment (corifollitropin alfa, rFSH) on the distribution of patients in the different oocyte groups was significant ($P = 0.01$). The distribution of the number of oocytes retrieved following treatment with corifollitropin alfa and daily rFSH is presented in Fig. 1A. The median and the fifth and ninety-fifth percentiles (P5 and P95) of the number of oocytes was 13 (4 and 27) in the corifollitropin alfa group and 12 (4 and 25) in the rFSH group. The distribution of the number of embryos obtained in both treatment groups is shown in Fig. 1B. The median number of embryos obtained was seven in both treatment groups, with P5 and P95 of 0 and 18 in the corifollitropin alfa group and 0 and 16 in the rFSH group.
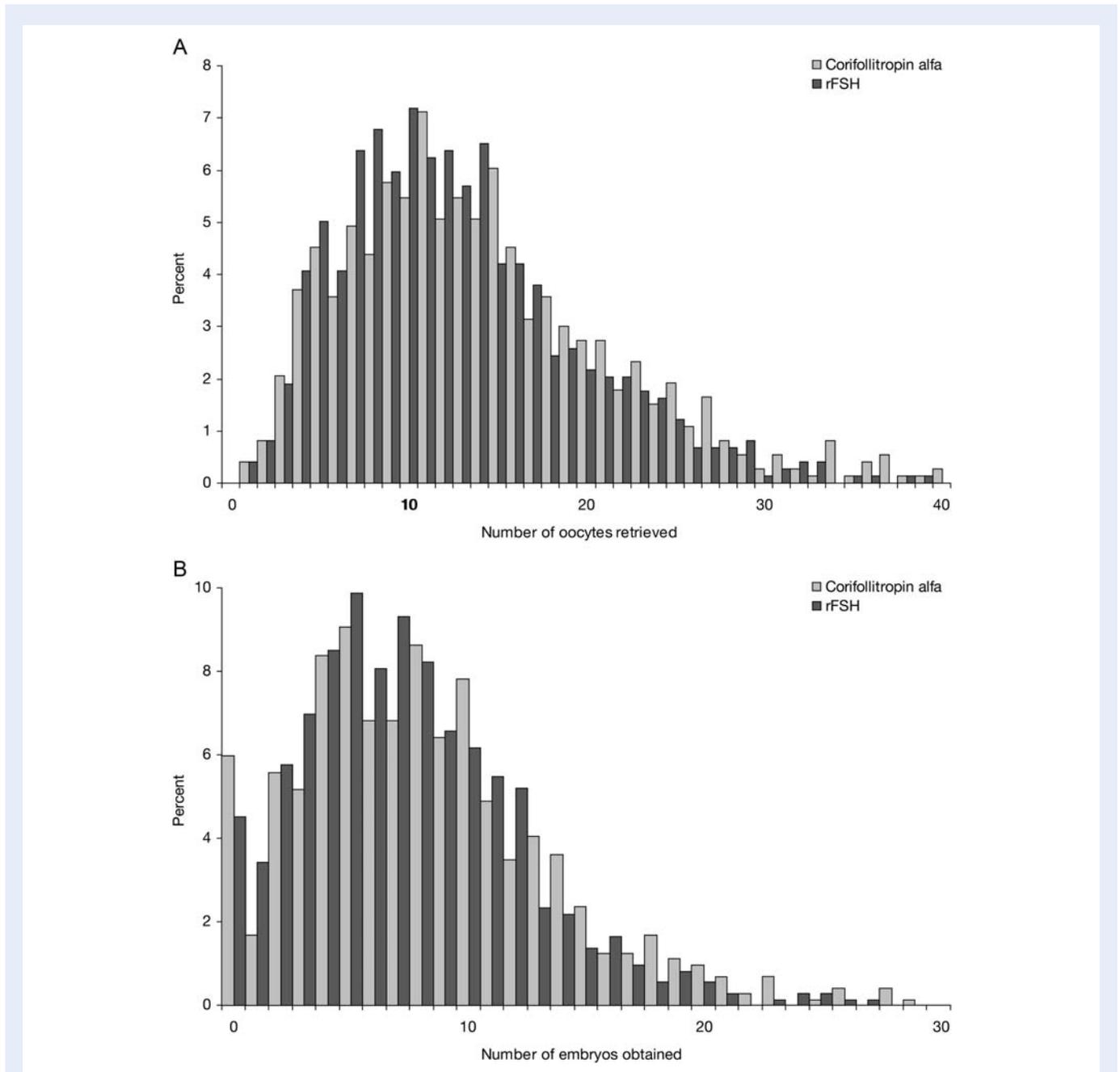
Figure 1 Frequency distribution of the number of oocytes retrieved (**A**) and the number of embryos obtained (**B**) after treatment with corifollitropin alfa or recombinant FSH (rFSH).

Patients' baseline characteristics

Patients' age ($P < 0.01$), antral follicle count (AFC) ($P < 0.01$) and serum FSH level on stimulation day 1 ($P < 0.01$) had a significant effect on the distribution of patients in the five oocyte categories. Across the range from low to high responders, differences in patient demographic and fertility characteristics were similar in the corifollitropin alfa and rFSH arms: high responders tended to be younger than low responders. Accordingly, the mean AFC at stimulation day 1 in the corifollitropin alfa group increased from 9.8 in the lowest response group (0–5 oocytes) to 14.8 in the highest response group (>18 oocytes), and serum FSH declined from 7.5 to 5.6 IU/L, respectively (Table I).

The distribution of patients in the categorized oocyte retrieval groups in Europe was different from North America ($P < 0.01$) which was mainly related to a higher incidence of high responders (>18 oocytes) in North America (Table I).

Follicles and hormone levels on the day of hCG

To reach the same criteria for triggering final oocyte maturation, in both treatment groups the median duration of stimulation was comparable between the different oocyte category groups (Table II). Subjects with 14–18 oocytes or >18 oocytes did not receive on average

Table I Demographics of patients in a study of ovarian response and pregnancy rates in a GnRH-antagonist protocol.

Corifollitropin alfa (n = 748)		Number of oocytes retrieved				
		0–5 (n = 94)	6–9 (n = 136)	10–13 (n = 169)	14–18 (n = 163)	>18 (n = 186)
Age (years)	Mean (SD)	32.0 (3.0)	32.5 (2.8)	31.4 (3.5)	31.7 (3.1)	30.6 (3.6)
BMI (kg/m^2)	Mean (SD)	24.9 (2.8)	24.7 (2.8)	24.9 (2.9)	24.8 (2.7)	24.8 (2.7)
Body weight (kg)	Mean (SD)	69.0 (7.6)	68.6 (7.4)	69.2 (7.9)	68.9 (7.6)	68.4 (7.6)
Serum FSH on Day 1 (IU/L)	Median (P5, P95)	7.5 (4.8, 17.1)	7.2 (4.8, 11.0)	6.4 (4.5, 9.6)	6.2 (4.0, 8.7)	5.6 (3.9, 8.1)
Antral follicle count (size: <11 mm)	Mean (SD)	9.8 (3.8)	10.4 (3.7)	11.8 (4.8)	13.1 (4.1)	14.8 (4.2)
Region, n (%)	Europe	49 (52.1)	73 (53.7)	78 (46.2)	77 (47.2)	74 (39.8)
	North America	45 (47.9)	63 (46.3)	91 (53.8)	86 (52.8)	112 (60.2)
rFSH (n = 747)		Number of oocytes retrieved				
		0–5 (n = 96)	6–9 (n = 171)	10–13 (n = 188)	14–18 (n = 156)	>18 (n = 136)
Age (years)	Mean (SD)	32.8 (2.8)	32.3 (2.9)	31.7 (3.2)	31.0 (3.2)	30.2 (3.4)
Body mass index (kg/m^2)	Mean (SD)	25.1 (2.9)	24.6 (2.6)	24.9 (2.8)	24.6 (2.6)	25.1 (2.8)
Body weight (kg)	Mean (SD)	67.6 (7.8)	68.1 (7.0)	68.6 (7.2)	68.4 (7.2)	68.9 (7.8)
Serum FSH on Day 1 (IU/L)	Median (P5, P95)	7.7 (4.6, 12.5)	7.0 (4.6, 10.6)	6.5 (4.3, 9.0)	5.8 (4.2, 8.1)	5.6 (3.7, 7.5)
Antral follicle count (size: <11 mm)	Mean (SD)	9.5 (4.3)	11.3 (4.0)	12.0 (4.1)	13.5 (4.1)	15.0 (4.1)
Region, n (%)	Europe	44 (45.8)	83 (48.5)	97 (51.6)	82 (52.6)	40 (29.4)
	North America	52 (54.2)	88 (51.5)	91 (48.4)	74 (47.4)	96 (70.6)

rFSH, recombinant FSH; P5, fifth percentile; P95, ninety-fifth percentile.

more FSH from stimulation day 8 onwards than subjects in the other categories.

The number of follicles ≥11 mm per oocyte category group is presented in Fig. 2. The two treatment groups showed a parallel rise in the number of follicles and the number of oocytes recovered. In Table II serum estradiol (E$_2$) levels and the number of subjects with elevated progesterone (>1.5 ng/ml) on the day of hCG are presented per oocyte category group. Serum E$_2$ levels progressively increased with the number of oocytes and elevated progesterone (>1.5 ng/ml) was more frequently observed in the high-responder groups in both treatment groups. The effect of treatment on the distribution of patients with elevated progesterone across the oocyte category groups almost reached significance ($P = 0.05$). The incidence of elevated progesterone in the highest oocyte category was numerically lower in the corifollitropin alfa group than in the rFSH group ($P = 0.05$).

Clinical outcome

The total number of embryos and the number of good-quality embryos obtained is presented per oocyte category in Table III. The mean (SD) number of good-quality embryos obtained increased with the ovarian response from 1.2 (1.2) in the lowest response group to 8.0 (5.8) in the highest response group in the corifollitropin alfa arm, and from 1.1 (1.2) in the lowest response group to 8.0 (5.5) in the highest response group in the rFSH arm. The number of

embryos cryopreserved increased with the ovarian response from 0.2 to 4.9 embryos in the corifollitropin alfa group and from 0.2 to 4.2 in the rFSH group (Table III). Whereas the number of embryos transferred was not very different among the five groups (mean ranged from 1.2 to 1.7), in both treatment groups the implantation rate was 10% higher in the highest response group compared with the lowest response group (Table III).

The ongoing pregnancy rates following fresh transfer in patients per started cycle increased from 31.9% in the lowest response group to 41.9% in the highest response group in patients treated with corifollitropin alfa and from 31.3 to 43.4% in patients treated with rFSH (Fig. 3A). Statistical testing by means of the Cochran-Armitage trend test revealed a linear trend in ongoing pregnancy rate from the lowest to the highest response group, $P = 0.04$. The ongoing pregnancy rates per embryo transfer differed to a lesser extent (than per started cycle) between the oocyte categories, as these incidences increased in particular in the lowest response group in both treatment arms (Table III). The applied Cochran-Armitage trend test showed no linear trend, $P = 0.24$.

If pregnancies from cryopreserved embryos were included, the cumulative ongoing pregnancy rate per started cycle ranged between 33.0 and 60.8% in the corifollitropin alfa group and between 31.3 and 55.9% in the rFSH group (Fig. 3B).

Live birth rates per oocyte category reflected the ongoing pregnancy rates for both the corifollitropin alfa and rFSH treatment groups (Table III).

Table II Stimulation characteristics, and serum estradiol and rises of serum progesterone on the day of hCG administration.

Corifollitropin alfa		Number of oocytes retrieved				
		0–5 (n = 94)	6–9 (n = 136)	10–13 (n = 169)	14–18 (n = 163)	>18 (n = 186)
Total dose of rFSH from Day 8 onwards (IU)	Median (P5, P95)	400.0 (0.0, 1000.0)	400.0 (0.0, 1000.0)	400.0 (0.0, 1000.0)	350.0 (0.0, 900.0)	300.0 (0.0, 800.0)
Total duration of stimulation (days)	Median (P5, P95)	10.0 (7.0, 12.0)	10.0 (8.0, 12.0)	9.0 (8.0, 13.0)	9.0 (8.0, 12.0)	9.0 (8.0, 12.0)
Estradiol (pmol/l) on the day of hCG	Median (P5, P95)	2452 (998, 7303)	3435 (1523, 7927)	4074 (1762, 10 313)	6092 (2092, 13 396)	7487 (2683, 14 717)
Progesterone on the day of hCG, n (%)	>1.5 ng/ml	4 (4.3)	2 (1.5)	12 (7.1)	15 (9.2)	24 (12.9)
rFSH		**Number of oocytes retrieved**				
		0–5 (n = 96)	6–9 (n = 171)	10–13 (n = 188)	14–18 (n = 156)	>18 (n = 136)
Total dose of rFSH from Day 8 onwards (IU)	Median (P5, P95)	400.0 (0.0, 1000.0)	400.0 (0.0, 800.0)	400.0 (0.0, 800.0)	300.0 (0.0, 800.0)	212.5 (0.0, 800.0)
Total duration of stimulation (days)	Median (P5, P95)	9.0 (7.0, 12.0)	9.0 (7.0, 11.0)	9.0 (7.0, 11.0)	9.0 (7.0, 11.0)	9.0 (8.0, 11.0)
Estradiol (pmol/l) on the day of hCG	Median (P5, P95)	2613 (1277, 6790)	3187 (1365, 7450)	4477 (2081, 9726)	5982 (2330, 9946)	7267 (2727, 15 010)
Progesterone on the day of hCG, n (%)	>1.5 ng/ml	4 (4.2)	6 (3.5)	10 (5.3)	23 (14.7)	29 (21.3)

Identification of predictive factors of ongoing pregnancy

Univariate regression analyses showed significant effects on ongoing pregnancy rate for the factors embryo transfer (double or single, $P < 0.01$), region (North America or Europe, $P < 0.01$), progesterone level on the day of hCG (>1.5 or ≤1.5 ng/ml, $P < 0.01$), start day of the stimulation (cycle day 2 or 3, $P = 0.02$) and age ($P = 0.04$). The effect of delay of hCG administration (no delay or 1-day delay) was not significant: $P = 0.60$. The significant factors were included as covariates in the logistic regression analyses for ongoing pregnancy and cumulative ongoing pregnancy described below.

Logistic regression analyses for ongoing pregnancy and live birth

Table IV presents the estimated ORs of ongoing pregnancy and cumulative ongoing pregnancy per started cycle between the oocyte categories using the 10–13 oocyte category as a reference, by treatment group and overall. In the corifollitropin alfa group the unadjusted OR [95% confidence interval (CI)] of ongoing pregnancy increased from 0.68 (0.40–1.16) in the lowest response group (i.e. OR of 0–5 versus 10–13 oocytes) to 1.05 (0.69–1.60) in the highest response group (i.e. OR of >18 versus 10–13 oocytes) and similar results were obtained for the rFSH group (Model 1, Table IV). All estimated 95% CIs from Model 1 included 1.0 in both treatment groups. The estimated OR was 0.72 (0.50–1.05) for the lowest response group and 1.16 (0.85–1.58) in the highest response group when the data of both treatment groups were pooled.

For the cumulative pregnancy per started cycle (see Table IV) the unadjusted OR (Model 1) reflected a significantly lower odds of pregnancy for the lowest response group and a significantly higher odds of pregnancy for the highest response group in each treatment group and when the treatment groups were pooled. The estimated OR (95% CI) was 0.58 (0.40–0.84) for the lowest response group and 1.75 (1.29–2.37) for the highest response group in comparison with the reference group when the data of both treatment groups were pooled.

When adjusted for age, region, cycle day and elevated progesterone on the day of hCG (Model 2), the adjusted ORs for ongoing pregnancy were all close to 1.0 versus the reference category (10–13 oocytes) in all comparisons and the estimated OR was 0.87 (0.59–1.30) for the lowest response group and 1.17 (0.84–1.63) for the highest response group when the data of both treatment groups were pooled (Table IV). Again, all 95% CIs of the adjusted ORs included 1.0 in both treatment groups (Model 2, Table IV). The odds of pregnancy for low and high ovarian responders according to oocyte category appear to be similar to the odds of pregnancy for normal responders with 10–13 oocytes. The applied linear trend test showed no linear trend in the ongoing pregnancy rate across the five oocyte categories when adjusted for the above predictive factors of pregnancy, $P = 0.25$. Factors age, region and elevated progesterone (>1.5 ng/ml) on the day of hCG, however, did have a significant effect on ongoing pregnancy rate in one or both treatment groups. For example, the odds of pregnancy decreased by a constant 0.94 (0.90–0.99) for every year increase of age in the rFSH group (Table IV).

When adjusted for age, region, cycle day and elevated progesterone on the day of hCG (Model 2), the adjusted ORs for cumulative ongoing pregnancy were 0.71 (0.48–1.05) for the lowest response
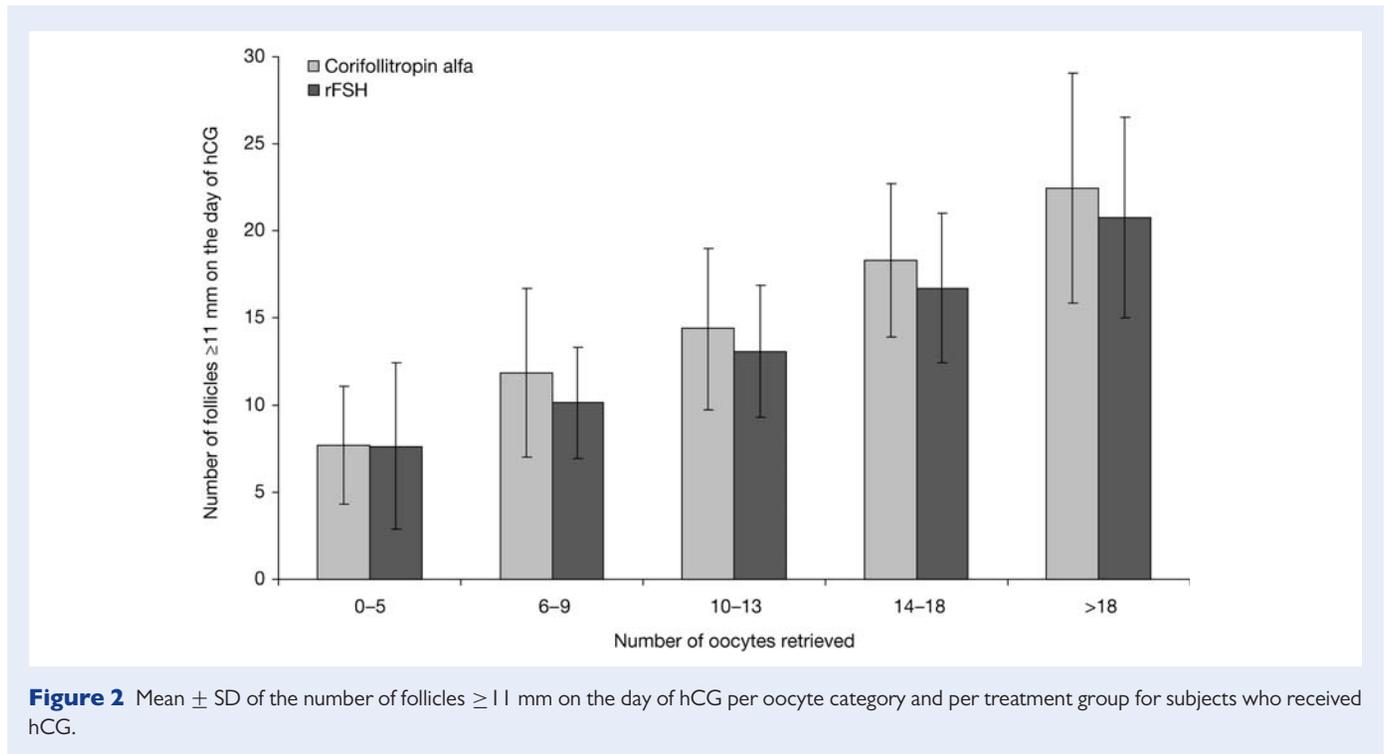
Figure 2 Mean ± SD of the number of follicles ≥11 mm on the day of hCG per oocyte category and per treatment group for subjects who received hCG.

group and 1.87 (1.34–2.59) for the highest response group when the data of both treatment groups were pooled (Table IV). Thus, the odds of cumulative ongoing pregnancy for the highest response group appeared to be significantly higher than the odds of cumulative ongoing pregnancy for normal responders (10–13 oocytes). The adjusted OR per treatment group indicated that this difference is larger in the corifollitropin alfa group than in the rFSH group. The factors region and elevated progesterone (>1.5 ng/ml) on the day of hCG had a significant effect on the cumulative ongoing pregnancy rate in the pooled analysis of both treatment groups.

Discussion

The current study shows that in patients treated with a single dose of corifollitropin alfa or daily rFSH for the first 7 days of COS in a GnRH-antagonist regimen, the ongoing pregnancy rates per started cycle increased with the ovarian response following fresh embryo transfer, although the trend analysis was significant owing to the lower pregnancy rate in the lowest ovarian response group. However, after correction for the effect of main confounders, the ongoing pregnancy rates were less sensitive to ovarian response. Clearly, a high ovarian response did not compromise the chance of ongoing pregnancy following fresh embryo transfer, and even increased the chance of cumulative ongoing pregnancy including both fresh and frozen embryo transfers.

Our results on pregnancy following fresh transfer are in agreement with a previous retrospective study including nearly 1900 women showing that a high number of oocytes are not associated with the decreased pregnancy outcome (Kok *et al.*, 2006). However, it does not confirm the analysis of Sunkara *et al.* (2011) that showed a positive association between the number of oocytes retrieved and the live birth rates, which declined beyond 20 oocytes. In our analysis, 186

subjects had 18 oocytes or more but the success rates in these high responders were at least as high as in patients with fewer than 18 oocytes. This difference may be related to the fact that our retrospective analysis included a prospective randomized trial in which patients were only treated with GnRH antagonist and rFSH. Also, these were women with a normal menstrual cycle and body weight range and those with extremes in AFC and with polycystic ovary syndrome were excluded.

The number of embryos transferred during the treatment cycle was comparable among the oocyte categories with the exception of the lowest response group in which fewer patients reached embryo transfer. However, the number of cryopreserved embryos increased mainly with the number of retrieved oocytes, resulting in an impressive increase in the cumulative pregnancy rate, which nearly doubled when comparing the highest with the lowest response group. When adjusted for predictive factors of pregnancy, the odds of cumulative ongoing pregnancy for the highest ovarian response group was nearly twice as high as the odds for the normal responder group.

Our analysis confirmed that patients with a high ovarian response are generally younger, with a higher AFC and lower serum FSH levels, as previously described in the literature (Broekmans *et al.*, 2006; Broer *et al.*, 2011; Nyboe Andersen *et al.*, 2011). Whereas the number of oocytes retrieved was positively related to the number of follicles and increases of serum E_2 following stimulation, the risk of a pre-ovulatory progesterone rise (>1.5 ng/ml) also increased with the ovarian response. Interestingly, while corifollitropin alfa induced more follicles than daily rFSH (Fauser *et al.*, 2010b), the current study demonstrated at a *P*-value of 0.05 that the incidence of pre-ovulatory progesterone rises was lower in high responders treated with corifollitropin alfa than with rFSH. This may be explained by the step-down of corifollitropin alfa exposure from stimulation

Table III Number and quality of embryos obtained and clinical outcomes.

| Corifollitropin alfa		Number of oocytes retrieved				
		0–5 (n = 94)	6–9 (n = 136)	10–13 (n = 169)	14–18 (n = 163)	>18 (n = 186)
Embryos obtained on Day 3 after oocyte retrieval	Mean (SD)	2.0 (1.6)	4.6 (2.2)	6.6 (3.0)	9.4 (3.3)	13.5 (7.0)
GQEs obtained on Day 3 after oocyte retrieval	Mean (SD)	1.2 (1.2)	2.4 (1.9)	3.5 (2.6)	5.2 (3.2)	8.0 (5.8)
Embryos transferred	Mean (SD)	1.2 (0.9)	1.6 (0.6)	1.6 (0.6)	1.7 (0.5)	1.5 (0.7)
Embryos cryopreserved	Mean (SD)	0.2 (0.5)	0.8 (1.3)	1.4 (1.9)	2.6 (2.7)	4.9 (4.8)
Implantation rate	Mean (SD)	25.4 (38.5)	33.5 (40.4)	32.5 (40.2)	31.6 (41.2)	36.3 (42.7)
Ongoing pregnancy rate/embryo transfer	%	44.1	40.9	43.7	41.1	48.4
Live birth rate/started cycle	%	27.7	36.0	36.7	36.8	38.7
rFSH		**Number of oocytes retrieved**				
		0–5 (n = 96)	6–9 (n = 171)	10–13 (n = 188)	14–18 (n = 156)	>18 (n = 136)
Embryos obtained on Day 3 after oocyte retrieval	Mean (SD)	2.1 (1.5)	4.7 (2.0)	6.9 (2.9)	9.0 (3.5)	13.0 (6.0)
GQEs obtained on Day 3 after oocyte retrieval	Mean (SD)	1.1 (1.2)	2.9 (2.0)	3.9 (2.5)	5.4 (3.5)	8.0 (5.5)
Embryos transferred	Mean (SD)	1.3 (0.8)	1.7 (0.6)	1.7 (0.6)	1.6 (0.5)	1.7 (0.5)
Embryos cryopreserved	Mean (SD)	0.2 (0.6)	1.2 (1.6)	1.8 (2.1)	2.7 (2.6)	4.2 (4.2)
Implantation rate	Mean (SD)	24.5 (38.4)	29.8 (38.6)	29.5 (38.9)	31.7 (41.4)	34.6 (40.6)
Ongoing pregnancy rate/embryo transfer	%	38.0	41.1	38.9	39.7	45.0
Live birth rate/started cycle	%	25.0	37.4	32.4	34.6	40.4

GQEs, good-quality embryos.

Day 3 onwards resulting in less pronounced rises of serum E_2 and progesterone between stimulation days 6 and 8 following a single injection of corifollitropin alfa (Fauser et al., 2010b).

To date, there are numerous studies suggesting that there is a negative association between pre-ovulatory progesterone concentrations and the chance of pregnancy (Kyrou et al., 2011; Kolibianakis et al., 2012) but no association also has been reported (Yding et al., 2011). In the current large study, neither the unadjusted nor the adjusted regression analysis of ongoing pregnancy rate provided an OR that was <1.0 or different from 1.0 for patients with >18 oocytes compared with patients with 10–13 oocytes. This indicates that in high responders the negative effect of elevated progesterone is outweighed by other factors with a positive effect. High responders may have better and faster developing embryos that catch up with the endometrial changes owing to the premature rise in progesterone.

Logistic regression analysis showed that factors age, region, starting day of the treatment and a progesterone elevation on the day of hCG had an effect on the chance of pregnancy. Thus, patients who were older, were treated in Europe, who started on cycle day 2 and who had a pre-ovulatory progesterone rise had a lower chance of pregnancy than patients who were younger, or were treated in North

America, started on cycle day 3 or did not have a pre-ovulatory progesterone rise. A recent comparison of live birth rates and cumulative ongoing pregnancy rates between Europe and North America in this trial confirms the impact of region on pregnancy rates (Boostanfar et al., 2012). Our data also suggest that patients starting stimulation on cycle Day 3 would have a higher chance of pregnancy than those starting on Day 2. Literature data, however, do not suggest that a later start of stimulation would be more favorable (Hohmann et al., 2003; Sterrenburg et al., 2011) and compared with Europe, slightly more patients in North America started treatment at cycle day 3.

As elevated progesterone had a significant impact one could reason that the prevention of pre-ovulatory progesterone rises could further increase the ongoing pregnancy and live birth rates in high responder patients. However, since the incidence of progesterone rises was relatively low, the increase would be limited to 1–2%. For example, in the corifollitropin alfa group, the overall ongoing pregnancy rate per embryo transfer was 39.3% (294 out of 748), with 40.5% (280 out of 691) for subjects without elevated progesterone and 24.6% (14 out of 57) for subjects with elevated progesterone. If such a premature progesterone rise could have been prevented, then the estimated pregnancy rate would increase to 42.1% in the patient group
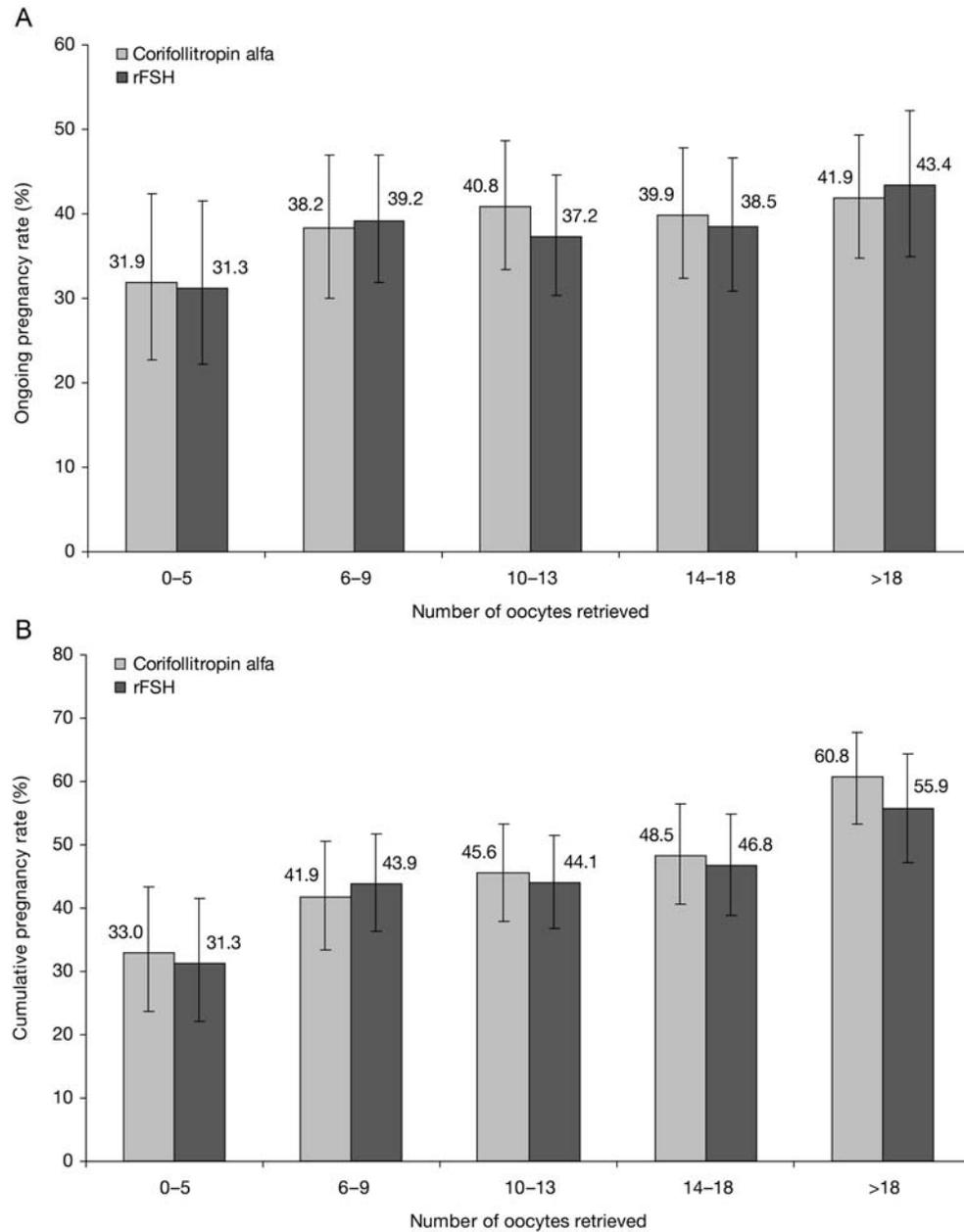
Figure 3 Ongoing pregnancy rate (**A**) and cumulative pregnancy rate (**B**) per started cycle and 95% confidence interval per oocyte category and per treatment group.

with elevated progesterone. This would lead to an overall ongoing pregnancy rate of 40.6% in the corifollitropin alfa group. The negative impact of premature progesterone rise in high responders seems to have a less severe impact on the pregnancy rate as compared with other patients.

A further direct comparison of implantation rates revealed an increase of 10% when comparing the lowest response group with the highest response group, indicating that uterine receptivity is not obviously impaired by a higher ovarian response. This increase is partly related to the increase in the number of good-quality embryos, which caused a doubling of the cumulative pregnancy rate when

comparing the lowest to the highest oocyte response group. The fact that the two highest response groups required less rFSH but the same duration of stimulation to reach the criteria for hCG is explained by patients who were coasted, which was allowed during the study from stimulation day 8 onwards for a maximum of 3 days.

In conclusion, in regularly cycling patients <36 years of age treated with corifollitropin alfa or daily rFSH in a GnRH-antagonist regimen, a high ovarian response did not jeopardize ongoing pregnancy rates and live birth rates following fresh embryo transfer. The cumulative pregnancy rates per started cycle, including pregnancies of cryopreserved embryos, largely increased with the ovarian response.

Table IV Logistic regression analysis of ongoing pregnancy and cumulative ongoing pregnancy per started cycle, by treatment group and overall

Factors included in model	Categories	Corifollitropin alfa		rFSH		Overall	
		Model 1 [OR (95% CI)]	Model 2 [OR (95% CI)]	Model 1 [OR (95% CI)]	Model 2 [OR (95% CI)]	Model 1 [OR (95% CI)]	Model 2 [OR (95% CI)]
Ongoing pregnancy							
Oocytes	0–5 versus 10–13	0.68 (0.40–1.16)	0.84 (0.47–1.50)	0.77 (0.45–1.29)	0.91 (0.52–1.58)	0.72 (0.50–1.05)	0.87 (0.59–1.30)
	6–9 versus 10–13	0.90 (0.57–1.42)	1.00 (0.61–1.64)	1.09 (0.71–1.66)	1.05 (0.67–1.64)	0.99 (0.73–1.36)	1.04 (0.74–1.44)
	14–18 versus 10–13	0.96 (0.62–1.49)	1.06 (0.66–1.69)	1.05 (0.68–1.63)	0.96 (0.60–1.54)	1.01 (0.74–1.38)	1.02 (0.74–1.42)
	>18 versus 10–13	1.05 (0.69–1.60)	1.23 (0.78–1.94)	1.29 (0.82–2.03)	1.05 (0.64–1.73)	1.16 (0.85–1.58)	1.17 (0.84–1.63)
Age	Per year increase		0.97 (0.92–1.02)		0.94 (0.90–0.99)		0.96 (0.92–0.99)
Cycle day	Day 3 versus day 2		1.12 (0.82–1.54)		1.30 (0.95–1.78)		1.21 (0.97–1.51)
Region	NA versus EUR		1.85 (1.34–2.54)		2.09 (1.51–2.90)		1.96 (1.56–2.46)
Progesterone level on the day of hCG	>1.5 versus ≤1.5 ng/ml		0.41 (0.22–0.78)		0.51 (0.29–0.90)		0.46 (0.30–0.70)
Cumulative ongoing pregnancy							
Oocytes	0–5 versus 10–13	0.59 (0.35–0.99)	0.71 (0.40–1.26)	0.58 (0.34–0.97)	0.70 (0.40–1.20)	0.58 (0.40–0.84)	0.71 (0.48–1.05)
	6–9 versus 10–13	0.86 (0.55–1.36)	0.96 (0.59–1.56)	0.99 (0.65–1.50)	1.00 (0.65–1.55)	0.93 (0.68–1.26)	0.99 (0.71–1.36)
	14–18 versus 10–13	1.12 (0.73–1.73)	1.18 (0.75–1.87)	1.11 (0.73–1.70)	1.11 (0.71–1.74)	1.12 (0.83–1.52)	1.15 (0.83–1.58)
	>18 versus 10–13	1.85 (1.21–2.82)	2.16 (1.37–3.41)	1.60 (1.03–2.50)	1.50 (0.92–2.45)	1.75 (1.29–2.37)	1.87 (1.34–2.59)
Age	Per year increase		0.97 (0.93–1.02)		0.97 (0.93–1.02)		0.97 (0.94–1.01)
Cycle day	Day 3 versus Day 2		1.20 (0.88–1.65)		1.20 (0.88–1.63)		1.21 (0.97–1.50)
Region	NA versus EUR		1.58 (1.15–2.17)		1.76 (1.29–2.42)		1.66 (1.33–2.07)
Progesterone level on the day of hCG	>1.5 versus ≤1.5 ng/ml		0.57 (0.32–1.02)		0.60 (0.35–1.02)		0.57 (0.39–0.85)

NA, North America; Eur, Europe. Estimated OR and associated 95% confidence intervals (CIs) of Model 1 include oocyte category as an independent factor and in Model 2 include oocyte category and several other covariates of pregnancy as independent factors.

Authors' roles

H.F., K.D., G.G., H.W. and B.M. took part in the analysis and interpretation of data, writing the manuscript and in the final approval of the version to be published.

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