

Predictive factors of ovarian response and clinical outcome after IVF/ICSI following a rFSH/GnRH antagonist protocol with or without oral contraceptive pre-treatment

A. Nyboe Andersen¹, H. Witjes², K. Gordon³, and B. Mannaerts^{4,*}
on behalf of the Xpect investigators[†]

¹Rigshospitalet, Fertility Clinic Copenhagen University Hospital, Copenhagen 2100 O, Denmark ²Biostatistics and Research Decision Sciences, Women's Health and Endocrine, MSD, Oss, PO Box 20, Oss 5340 BH, The Netherlands ³Global Medical Affairs, Women's Health and Endocrine, Merck and Co., Kenilworth, NJ 07033, USA ⁴Global Clinical Research, Women's Health and Endocrine, MSD, Oss, PO Box 20, Oss 5340 BH, The Netherlands

*Correspondence address. Tel: +31-412663353; Fax: +31-412662555; E-mail: b.mannaerts@merck.com

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BACKGROUND: Prediction of ovarian response prior to the first controlled ovarian stimulation (COS) cycle is useful in determining the optimal starting dose of recombinant FSH (rFSH). However, potentially predictive factors may be subject to inter-cycle variability and many patients are pre-treated with oral contraceptives (OC) for scheduling purposes. Our objective was to determine predictive factors of ovarian response for patients undergoing COS with rFSH in a gonadotrophin-releasing hormone antagonist protocol and to determine the inter-cycle variability of these factors.

METHODS: In this multinational trial, 442 patients were randomized to receive either OC treatment or no treatment prior to their first COS cycle. For candidate predictive factors, patient characteristics were collected at screening, and endocrine and sonographic data were collected during the early follicular phase of the two subsequent cycles. A treatment regimen of 200 IU rFSH and 0.25 mg ganirelix was applied during the second cycle. Predictive factors of ovarian response and of too low (<6 oocytes) or too high (>18 oocytes) ovarian responses were determined using stepwise linear regression and stepwise logistic regression, respectively.

RESULTS: Anti-Müllerian hormone (AMH) and basal FSH were statistically significant predictors of the number of oocytes retrieved and of an excessive ovarian response. For low ovarian response, AMH was the only significant predictive factor. In the non-OC group, the predictive value was higher than in the OC group and higher at the early follicular phase of the stimulation cycle than of the previous cycle. The inter-cycle variation for AMH was low compared with the inter-cycle variation of other hormones. Between the two groups, there were no differences in the number or quality of embryos obtained or transferred, but the implantation rate was significantly lower in the OC group (24.1 versus 30.1%, $P = 0.03$), resulting in an ongoing pregnancy rate of 26.3% compared with 35.7% in the non-OC group ($P = 0.05$).

CONCLUSIONS: The best predictive model of ovarian response was in the non-OC group and included both AMH and basal FSH determined at the early follicular phase of the stimulation cycle. In the proceeding cycle, AMH alone had sufficient predictive value since it was not affected by inter-cycle variability or OC pretreatment.

Clinical trial identifier: **NCT00778999**.

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[†] The list of Xpect investigators is available in the Appendix.

Introduction

Predicting the ovarian response is helpful in determining the optimal starting dose of recombinant FSH (rFSH), especially prior to the first controlled ovarian stimulation (COS) cycle. Selection of the starting dose based on the predicted ovarian response increases the proportion of patients with a normal ovarian response and decreases the need for dose adjustments during stimulation (Popovic-Todorovic et al., 2003b). Previously published data indicate that several factors, including age, BMI, menstrual cycle length, basal FSH and antral follicle count (AFC) (Popovic-Todorovic et al., 2003a; Fauser et al., 2008; Verhagen et al., 2008; Olivennes et al., 2009), are clinically relevant predictors of oocyte yield, which may be used in a multivariate model or as a single test for the assessment of ovarian response. Often, such tests of ovarian response have assessed the prediction of poor ovarian response rather than the prediction of hyper-response (Broekmans et al., 2006). More recently, it has been suggested that anti-Müllerian hormone (AMH) is a better marker in predicting ovarian response to COS than age, FSH, estradiol (E_2) and inhibin B. The performance of AMH as a predictor of poor ovarian response is anticipated to be very similar to AFC (Broer et al., 2009); however, AFC should be assessed during the early follicular phase to minimize the effect of intra-cycle fluctuations (Broekmans et al., 2010), whereas serum AMH levels are generally thought to remain stable throughout the menstrual cycle (La Marca et al., 2007). The dose-response relationship between AMH and ovarian response to FSH explains why AMH levels have been shown to be useful in both the prediction of poor response and hyper-response. As such, patients with high AMH levels could have an increased risk of developing ovarian hyperstimulation syndrome (OHSS).

To date, however, the role of predictive factors of ovarian response has most frequently been studied in patients treated with a long gonadotrophin-releasing hormone (GnRH) agonist protocol. Predictive factors for GnRH antagonist protocols are lacking. In addition, it is unknown whether predictive factors remain stable between menstrual cycles. Therefore, the accuracy of these predictions may be affected by inter-cycle variability. Pre-treatment regimens, such as the use of oral contraceptives (OC) [often used to assist in scheduling assisted reproductive technology (ART) cycles], may also influence some of the factors used as predictors.

The primary objective of this randomized, open-label, multicentre clinical trial was to identify factors capable of predicting ovarian response in patients undergoing their first treatment cycle with a daily dose of 200 IU rFSH in a GnRH antagonist protocol. In the present study, patients were randomized into two groups with or without OC pre-treatment, to investigate the predictive value in both groups separately. Randomization to OC versus non-OC treatment was performed since OC pre-treatment is more often applied by US clinics than by European clinics, which prevents bias due to regional differences. The inter-cycle variability of endocrine and sonographic factors was assessed by measuring those factors twice, during the early follicular phase of two successive menstrual cycles.

Materials and Methods

The Xpect trial was a randomized, open-label, multicentre clinical trial involving eight centres in the USA and six centres in Europe (one in

Denmark and Germany, and two in Spain and Turkey) conducted between October 2006 and July 2008. The study protocol was approved by the independent medical ethics committee or institutional review board for each centre and was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonisation guidelines for Good Clinical Practice and current regulatory requirements. Written informed consent was provided by all patients.

Study population

Women aged 18–39 years with BMI of ≤ 32 kg/m², a menstrual cycle length of 24–35 days, access to ejaculatory sperm, an indication for COS and IVF and/or ICSI and who were scheduled for their first COS cycle and were willing and able to give written informed consent were eligible to enrol in the study. Patients were excluded from the study due to a history of an endocrine abnormality, less than two ovaries or any other ovarian abnormality (including endometrioma > 10 mm visible on ultrasound), presence of unilateral or bilateral hydrosalpinx, any clinically relevant pathology affecting the uterine cavity (upon discretion of the investigator), fibroids ≥ 5 cm, a history of recurrent miscarriage (three or more) and/or FSH or LH levels > 12 IU/l in the early follicular phase.

Study design

The trial was designed to identify endocrine and/or sonographic predictors of ovarian response, including too high (> 18 oocytes) and suboptimal (< 6 oocytes) ovarian response. Subjects were randomized to receive a fixed daily dose of 200 IU rFSH in a GnRH antagonist protocol either with OC pre-treatment (OC group) or without any OC pre-treatment (non-OC group). Randomization was done by central remote allocation using an interactive voice response telephone system, which allocated each subject to a treatment group. Randomization was stratified for centre and age (≤ 32 and > 32 years).

The study design is summarized in Fig. 1. The OC group received OC (30 μ g ethinyl E_2 /150 μ g desogestrel; Marvelon, NV Organon, The Netherlands) for 14–21 days and started daily rFSH [foliotropin beta (Puregon/Follistim AQ Cartridge, NV Organon, The Netherlands)] 5 days after stopping OC treatment (stimulation day 1) provided a withdrawal bleeding had occurred. In the absence of bleeding, the start of COS was delayed up to the first day of full bleeding. If bleeding did not occur within 7 days, the subject was withdrawn from the study. The non-OC group started daily rFSH on Day 2 or 3 of their next menstrual cycle (stimulation day 1). In both groups, a single subcutaneous injection of 200 IU rFSH was initiated on stimulation day 1 and continued daily up to and including the day of triggering of final oocyte maturation by urinary hCG. Based on the observed follicular response, the rFSH dose from stimulation day 6 onwards could be reduced but only in such cases where the investigator considered there was a risk of development of OHSS. The maximum total duration of stimulation was 19 days. Starting on stimulation day 5, all patients received 0.25 mg ganirelix daily. The criteria for giving hCG was the development of three or more follicles ≥ 17 mm. Approximately 34–36 h after induction of oocyte maturation, oocyte pick up followed by IVF or ICSI was performed. At embryo transfer, 3 or 5 days after oocyte pick up, a maximum of two embryos (subjects aged ≤ 36 years) or three embryos (subjects aged > 36 years) could be replaced. All patients received daily progesterone (≥ 600 mg/day vaginally or ≥ 50 mg/day intramuscularly) for luteal phase support for ≥ 6 weeks in case of pregnancy or until menses or up to a negative pregnancy test performed ≥ 14 days after embryo transfer.

Assessments of potential predictive factors

At screening, the following demographic data were collected: age, BMI (kg/m²), cycle length, age at menarche, duration of infertility, smoking

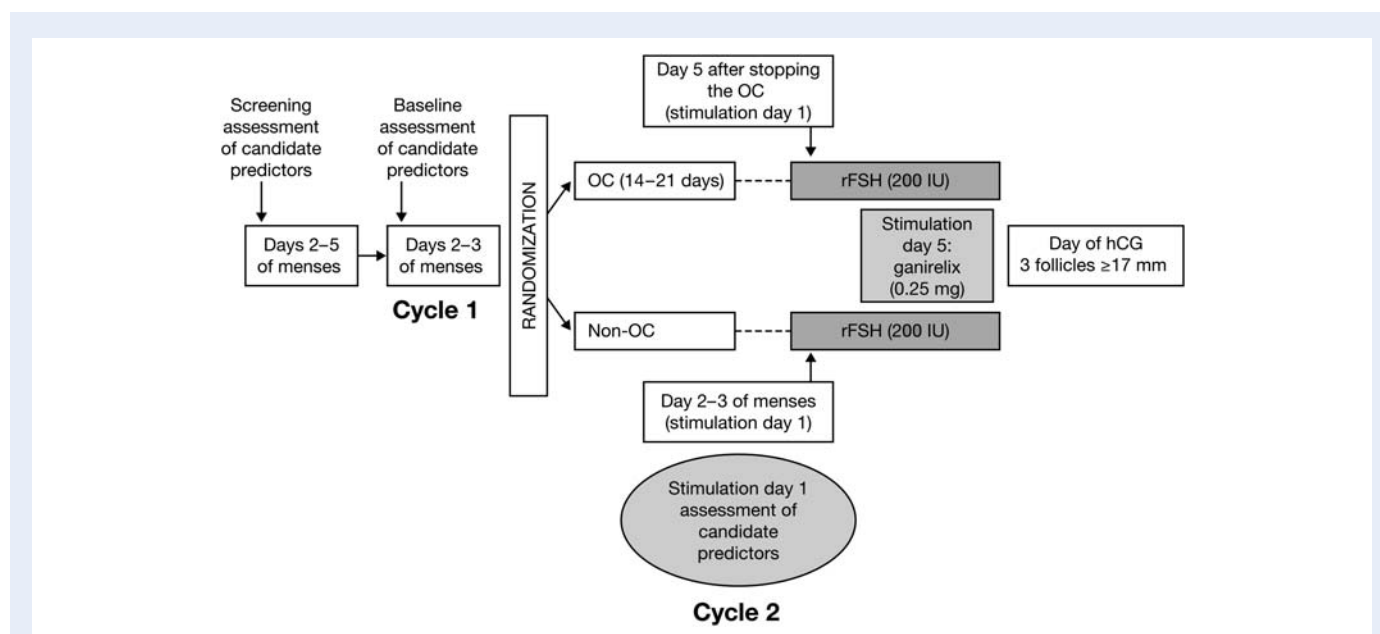


Figure 1 Study design. hCG, human chorionic gonadotrophin; OC, oral contraceptive; rFSH, recombinant follicle-stimulating hormone.

(yes/no) and alcohol use (yes/no). In addition, candidate predictors were assessed in two subsequent cycles: ovarian volume, AFC, basal FSH, LH, testosterone, progesterone, E_2 , inhibin B and AMH. In the non-OC group, these assessments were made at randomization on cycle day 2 or 3 (referred to as 'cycle 1') and at stimulation day 1 on cycle day 2 or 3 (referred to as 'cycle 2'). In the OC group, these assessments were made at randomization on cycle day 2 or 3 and at stimulation day 1 at least 5 days after the last OC pill intake.

Assessments during assisted reproduction

Ultrasonographic investigation was performed during rFSH treatment at stimulation day 5 prior to the start of ganirelix treatment, on stimulation day 8, then daily up to and including the day of hCG. The number and quality of embryos retrieved was assessed 3 days after oocyte pick-up. Two weeks after embryo transfer, a pregnancy test (serum or urinary hCG) was performed. Vaginal and/or abdominal ultrasonographic investigation was performed to confirm pregnancy at 5–6 weeks after embryo transfer, and at 10 weeks after embryo transfer to confirm ongoing pregnancy.

Assays

Validated immunoassays were performed at a central laboratory (Essex Pharma Development GmbH, Germany) to measure serum hormone levels of FSH, LH, inhibin B, E_2 and progesterone. Levels of FSH, LH, E_2 and progesterone were determined by time-resolved fluoroimmunoassay (AutoDelfia® immunofluorometric assay, PerkinElmer Life and Analytical Sciences, Brussels, Belgium) with a coefficient of variation of <10%. Detection limits were 0.25 IU/l, 0.6 IU/l, 49.9 pmol/l and 0.38 ng/ml for FSH, LH, E_2 and progesterone, respectively. Serum inhibin B levels were determined by using a validated immunoassay by Diagnostic Systems Laboratories (DSL; Webster, TX, USA) with a coefficient of variation of <10% and a detection limit of 10.0 pg/ml. All serum samples of a subject were measured with the same inhibin B lot; in total, two lots were applied. Serum AMH levels were determined at a central laboratory

(Analytisch Biochemisch Laboratorium BV, The Netherlands) using a validated enzyme-linked immunosorbent assay (DSL, Webster, TX, USA; values presented in nanogrammes per millilitre) with a detection limit of 0.1 ng/ml. All serum samples of a subject were measured with the same AMH lot. However, in this trial serum AMH was measured by three different lots; control samples indicated considerable variability among lots. Around 30% of the AMH measurements were not reported because either the serum sample was received unfrozen at the laboratory, or the sample was analysed after the guaranteed stability period of AMH in human serum.

Clinical outcome parameters

The primary parameter for ovarian response was the number of oocytes obtained and the secondary efficacy parameters were the number of follicles ≥ 11 mm at Day 8 and the number of follicles ≥ 11 mm at day of hCG. In addition, the following outcome parameters are reported: duration of stimulation, total rFSH dose, number of embryos, number of embryos transferred, implantation rate, clinical pregnancy rate and ongoing pregnancy rate. Implantation rate was calculated per patient as the number of gestational sacs observed compared with the number of embryos transferred.

Statistical analysis

In the present prognostic study, the following 16 baseline characteristics were planned to be evaluated for ovarian response prediction: age, age at menarche, menstrual cycle length, duration of infertility, alcohol use (Y/N), smoking (Y/N), BMI, ovarian volume, AFC (<11 mm) and serum FSH, LH, E_2 , progesterone, inhibin B, AMH and testosterone. For the planned sample size, it was expected that up to five predictive factors were selected in each logistic regression model. Assuming that for each predictive factor at least 10 events are required (Moons et al., 2009), a total of around 50 events are needed. A total sample size of 200 randomized subjects per treatment group was planned, with an additional 20 subjects (10%) to compensate for discontinued subjects.

All analyses were performed in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and were based on the intent-to-treat (ITT) population. The ITT population consisted of all randomized subjects who received at least one dose of rFSH. All analyses were performed 'per started cycle': if a treated subject did not reach a certain stage in IVF treatment, then zero values were imputed. For example, if a particular subject did not have oocyte retrieval, then the number of oocytes was set to zero and the pregnancy outcome was set to 'not pregnant'. Hormone values below the lower limit of quantification (LLOQ) were set to 0.5 times the LLOQ for all calculations. Serum progesterone was omitted from the analyses because 139 subjects (66.5%) in the OC group and 93 subjects (46.7%) in the non-OC group had at least one serum progesterone sample level below the LLOQ in the early follicular phase of cycles 1 or 2.

The Spearman rank correlation coefficient (ρ) was calculated to assess the correlation between cycles 1 and 2 for each of the candidate predictive factors. The inter-cycle variability of the candidate predictors was estimated by fitting an analysis of variance model to the data of cycles 1 and 2, including subject as a covariate for each of the candidate predictors separately. Patients with hormone values below the LLOQ on both assessment days were excluded from the analyses to prevent overestimation of the correlation and inter-cycle variability.

Stepwise linear regression was applied to identify the predictive factors of the number of oocytes retrieved and the predictive factors of the number of follicles ≥ 11 mm on Day 8 and on day of hCG, respectively. Stepwise logistic regression was applied to identify significant factors for prediction of high ovarian response (>18 oocytes) and low ovarian response (<6 oocytes), respectively. The significance level of the candidate predictive factors to enter the model was set to 0.15 and to stay in the model, it was set to 0.10. After selection of the candidate predictive factors using stepwise selection, the final model selects those prognostic factors with statistical significance, i.e. $P \leq 0.05$. The goodness-of-fit of the normal regression models was quantified by the coefficient of determination R^2 . Receiver operating characteristic (ROC) curves were calculated and the area under the curves (AUC) was used to assess the discriminative power of the logistic regression models.

Regression models for cycle 1 include the candidate predictive factors measured at screening and in cycle 1. Regression models for cycle 2 include the candidate predictive factors measured at screening and in cycle 2. Cycle 1 models were fitted for the combined treatment groups (OC, non-OC), and treatment group was included as a candidate predictive factor, while cycle 2 models were fitted per treatment group. Only additive models (i.e. without potential interactions of factors) were considered. The regression models were not adjusted for the AMH inter-lot variability, i.e. AMH lot was not added as a factor to the list of candidate predictive factors to be included in the models. Three subjects were excluded from the regression analyses because they discontinued the trial prior to oocyte retrieval for reasons other than too low ovarian response. However, one subject who discontinued the trial due to risk of OHSS was excluded from the linear regression analyses but included in the logistic regression analyses as a high responder (>18 oocytes). Because AMH appeared to be a strong predictive factor for ovarian response in this study, subjects without AMH measurements (around 30% of the total sample size) were not included in the regression analyses.

To explore the potential predictive factors of ongoing pregnancy, the data of the two treatment groups were combined, and the treatment group was included in the regression models as a candidate predictive factor.

Difference in implantation rate between the two treatment groups was evaluated using a cumulative logit model with proportional odds property for the implantation rate, including the factors: treatment group, age class as stratified (≤ 32 and >32 years) and region (Europe, USA). Difference in ongoing pregnancy rate between the two treatment groups was estimated

using a generalized linear model with identity link for the ongoing pregnancy rate including the factors: treatment group, age class as stratified and region.

Results

Patient demographics and disposition

The present manuscript focuses on the prediction of ovarian response. In addition, inter-cycle variability of the candidate predictors and the impact of OC pre-treatment on the values of the candidate predictors are addressed.

A total of 442 subjects were randomized—223 subjects to the OC group and 219 subjects to the non-OC group (Fig. 2). Altogether, 34 subjects did not receive rFSH, including 14 subjects in the OC group and 20 subjects in the non-OC group. In the OC group, 19 subjects did not receive hCG for final oocyte maturation compared with 27 subjects in the non-OC group. A total of 380 subjects (86.0%) had embryo transfer, 195 subjects (87.4%) in the OC group and 185 subjects (84.5%) in the non-OC group. Of the subjects who started stimulation, 6.7% (14/209) of subjects in the OC group and 7.0% (14/199) in the non-OC group did not have embryo transfer.

The two treatment groups were similar with respect to mean age, BMI, age at menarche, duration of infertility, cycle length, alcohol use and smoking (Table I).

Inter-cycle variability

Candidate endocrine and sonographic predictive factors were assessed in the early follicular phase at randomization (cycle 1) and at stimulation day 1 (cycle 2) (Table II). Table II shows the median and the 5th and 95th percentiles of the endocrine (serum FSH, LH, E₂, testosterone, inhibin B and AMH) and sonographic (AFC, ovarian volume) factors on the day of randomization (cycle 1) and on stimulation day 1 (cycle 2). In addition, it shows the correlation between cycles 1 and 2 and the cycle-to-cycle variation of the candidate predictive factors. In the non-OC group, the correlation between cycles 1 and 2 was the highest for AMH ($\rho = 0.88$) and testosterone ($\rho = 0.84$), which appeared to be less influenced by inter-cycle variability than basal FSH, LH, E₂, inhibin B, AFC and ovarian volume (Table II). The inter-cycle variability is illustrated in Fig. 3 for AFC, FSH and AMH. The figure illustrates the larger inter-cycle variability of serum FSH and basal AFC compared with AMH.

For the OC group, five days after the last OC intake, at stimulation day 1, circulating FSH, LH and E₂ levels were comparable to those at the start of OC pre-treatment, whereas the AFC and ovarian volume were still slightly reduced. As in the non-OC group, the highest correlation between cycles 1 and 2 was found for AMH ($\rho = 0.90$) and testosterone ($\rho = 0.79$). In comparison with the non-OC group, a lower correlation between cycles 1 and 2 was found for FSH, E₂ and inhibin B (Table II).

Clinical outcome

There was no difference between the two treatment groups in terms of duration of stimulation, total dose of rFSH administered, or the number and size of follicles recruited (Table III and Fig. 4).

Clinical outcomes, including the number of oocytes, the total number of embryos, number of embryos transferred and the pregnancy rates are presented in Table III. The mean number of oocytes

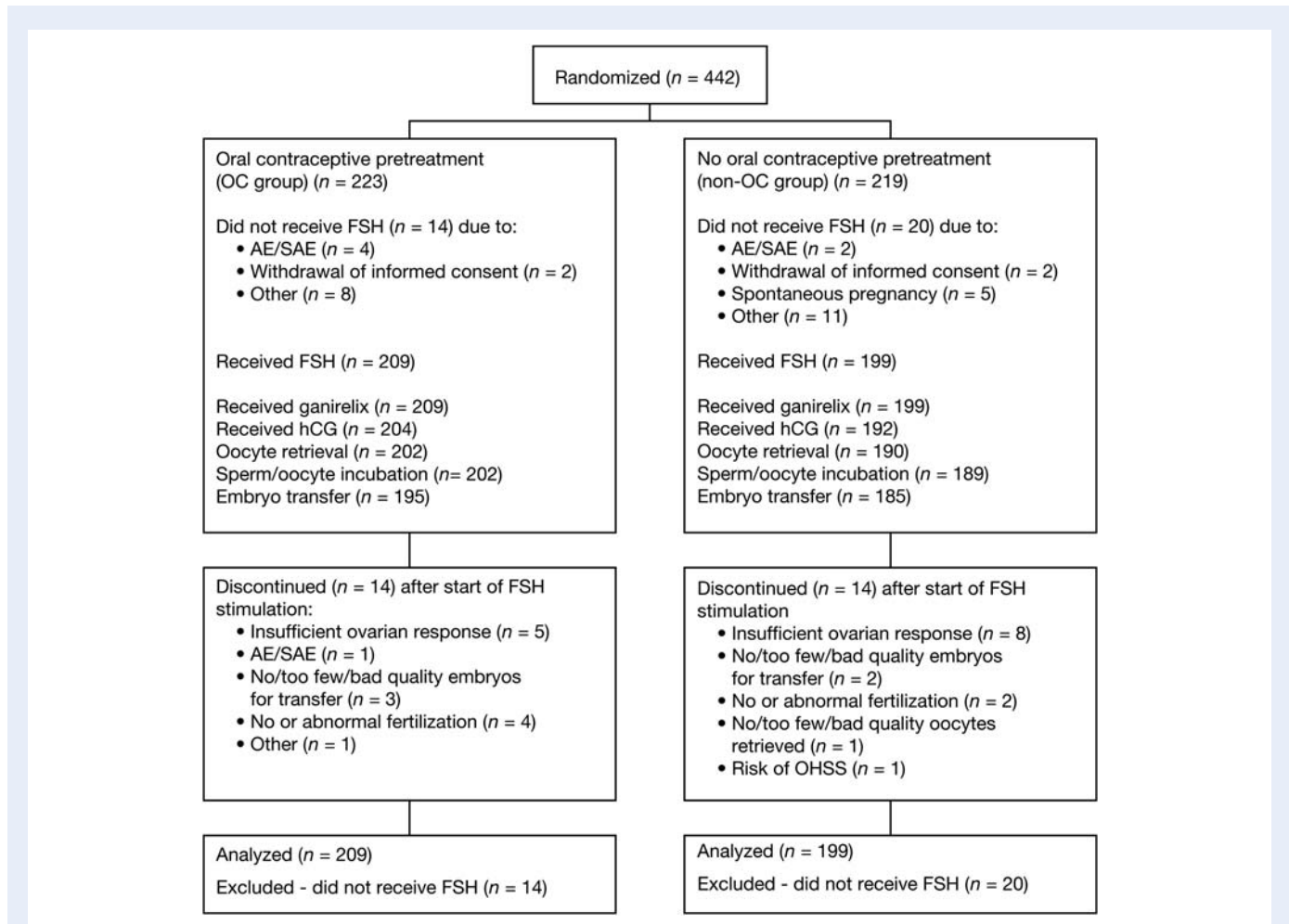


Figure 2 Subject disposition. AE, adverse event; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotrophin; OC, oral contraceptive; OHSS, ovarian hyperstimulation syndrome; SAE, serious AE.

Table 1 Potential predictive factors assessed at screening.

	OC (n = 209)	Non-OC (n = 199)	Overall (n = 408)
Age, years, mean (SD)	31.8 (3.7)	31.6 (4.1)	31.7
BMI, kg/m ² , mean (SD)	24.2 (3.6)	23.6 (3.4)	23.9
Age at menarche, years, mean (SD)	12.6 (1.4)	12.9 (1.5)	12.7
Duration of infertility, years, mean (SD)	3.9 (3.2)	3.7 (3.0)	3.8
Cycle length, days, mean (SD)	28.6 (1.8)	28.5 (1.8)	28.6
Alcohol use, n (%)	86 (41.1)	86 (43.2)	41.7%
Smoking, n (%)	32 (15.3)	34 (17.1)	16.2%

retrieved was 12.4 (SD = 6.7) and 12.1 (SD = 7.7) in the OC and non-OC groups, respectively. Fertilization rates were 66.9% in the OC group and 62.9% in the non-OC group. The mean number of

good quality embryos obtained was 4.4 (\pm 3.6) for the OC group and 4.8 (\pm 4.9) for the non-OC group. An equal mean number of 1.9 embryos was replaced in both treatment groups; implantation rates were 23.4 and 30.4% in the OC and non-OC groups, respectively ($P = 0.03$). In the OC group, three subjects (5.2%) with a vital pregnancy experienced a miscarriage compared with six (7.7%) in the non-OC group. The ongoing pregnancy rate per started stimulation cycle was 26.3 and 35.7% in the OC and non-OC groups, respectively ($P = 0.05$, adjusted for age class and region).

There were in total five cases of OHSS, one moderate and one severe case in the OC group and three moderate cases in the non-OC group.

Predictive factors

Predictive factors for number of oocytes retrieved

Cycle 1 data were less predictive for the number of oocytes retrieved than cycle 2 data. AMH was the only predictive factor that appeared in all models regardless of the cycle or treatment group. In the cycle 1 model, the goodness-of-fit measure R^2 was 0.33, whereas in cycle 2, these values were 0.41 and 0.46 in the OC group and in the non-OC group, respectively. Predictive factors in cycle 1 were AMH

Table II Assessments of hormones and ultrasound scan.

	Cycle 1, median, (P5, P95)	Cycle 2, median, (P5, P95)	Inter-cycle variation, (SD)	Correlation, ρ (95% CI)
Non-OC group				
Serum AMH (ng/ml)	1.84 (0.4, 4.3), $n = 136$	1.91 (0.5, 4.5), $n = 137$	0.45	0.88 (0.83–0.91)
AFC	12.0 (4, 22), $n = 196$	11.0 (3, 22), $n = 198$	3.16	0.67 (0.58–0.74)
Serum FSH (IU/l)	6.16 (3.8, 9.8), $n = 189$	6.67 (4.2, 10.4), $n = 173$	1.33	0.63 (0.53–0.71)
Serum E_2 (pmol/l)	110.5 (59, 212), $n = 189$	100.6 (59, 196), $n = 173$	34.5 ^a	0.54 (0.42–0.64)
Serum inhibin B (pg/ml)	49.8 (13, 108), $n = 189$	47.9 (14, 95), $n = 173$	18.2	0.53 (0.42–0.63)
Serum T (nmol/l)	1.04 (<0.4, 2.1), $n = 189$	1.11 (0.4, 2.6), $n = 173$	0.26	0.84 (0.79–0.88)
Serum LH (IU/l)	4.58 (2.1, 8.5), $n = 189$	4.97 (2.2, 9.1), $n = 173$	1.27	0.56 (0.45–0.66)
Total ovarian volume (ml)	11.98 (5.6, 24.3), $n = 190$	10.92 (5.4, 22.7), $n = 194$	4.27	0.48 (0.36–0.58)
OC group				
Serum AMH (ng/ml)	2.16 (0.5, 6.3), $n = 143$	2.11 (0.6, 4.6), $n = 145$	0.51	0.90 (0.87–0.93)
AFC	11 (5, 23), $n = 208$	10 (5, 21), $n = 207$	2.89	0.67 (0.59–0.74)
Serum FSH (IU/l)	6.16 (4.2, 10.1), $n = 202$	7.28 (3.2, 14.0), $n = 179$	2.42	0.42 (0.29–0.53)
Serum E_2 (pmol/l)	103.9 (63, 208), $n = 202$	101.3 (<50, 255), $n = 179$	51.8 ^b	0.25 (0.10–0.38)
Serum inhibin B (pg/ml)	49.6 (13, 97), $n = 202$	53.1 (<10, 140), $n = 179$	30.4	0.35 (0.21–0.47)
Serum T (nmol/l)	1.15 (0.4, 2.6), $n = 202$	0.91 (<0.4, 2.5), $n = 179$	0.31	0.79 (0.73–0.84)
Serum LH (IU/l)	4.57 (2.4, 7.6), $n = 202$	4.81 (1.7, 9.4), $n = 179$	1.48	0.48 (0.35–0.58)
Total ovarian volume (ml)	10.34 (4.8, 20.2), $n = 200$	8.87 (4.3, 18.4), $n = 201$	4.03	0.52 (0.41–0.61)

AFC, antral follicle count; AMH, anti-Müllerian hormone; CI, confidence interval; E_2 , estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; OC, oral contraceptive; T, testosterone; ρ , Spearman correlation coefficient.

^aFour extreme values ($E_2 > 600$ pmol/l) were omitted for calculation of inter-cycle variation.

^bOne extreme value ($E_2 > 600$ pmol/l) was omitted for calculation of inter-cycle variation.

($P < 0.001$), FSH ($P < 0.001$), AFC ($P = 0.003$) and age at menarche ($P = 0.009$). Predictive factors in cycle 2 were AMH ($P < 0.001$) and FSH ($P < 0.001$) in the non-OC group and AMH ($P < 0.001$), FSH ($P = 0.003$), ovarian volume ($P = 0.02$) and age ($P = 0.04$) in the OC group.

Predictive factors for low (<6 oocytes) and high (>18 oocytes) ovarian response

Table IV presents the final logistic regression models of low and high ovarian response, respectively. The best predictive models were obtained for the non-OC group just prior to the start of stimulation (cycle 2) both for low and high ovarian response (Table IV).

To discriminate low ovarian response (<6 oocytes) from normal or high ovarian response (≥ 6 oocytes), the cycle 2 model of the non-OC group included serum AMH ($P < 0.001$), smoking ($P = 0.04$) and serum FSH ($P = 0.05$) as significant factors (AUC = 0.90; Fig. 5A). In the OC group, the final cycle 2 model for low ovarian response included only AMH as a predictive factor (AUC = 0.84). When, in the non-OC group, only AMH was included in the cycle 2 model, the AUC was 0.88, whereas this value was 0.84 in cycle 1 for the combined groups.

To discriminate high ovarian response (>18 oocytes) from normal or low ovarian response (≤ 18 oocytes), the cycle 2 model of the non-OC group included serum AMH ($P = 0.001$) and serum FSH ($P = 0.002$) as significant factors (AUC = 0.86; Fig. 5B). In the OC group, the cycle 2 model for high ovarian response included

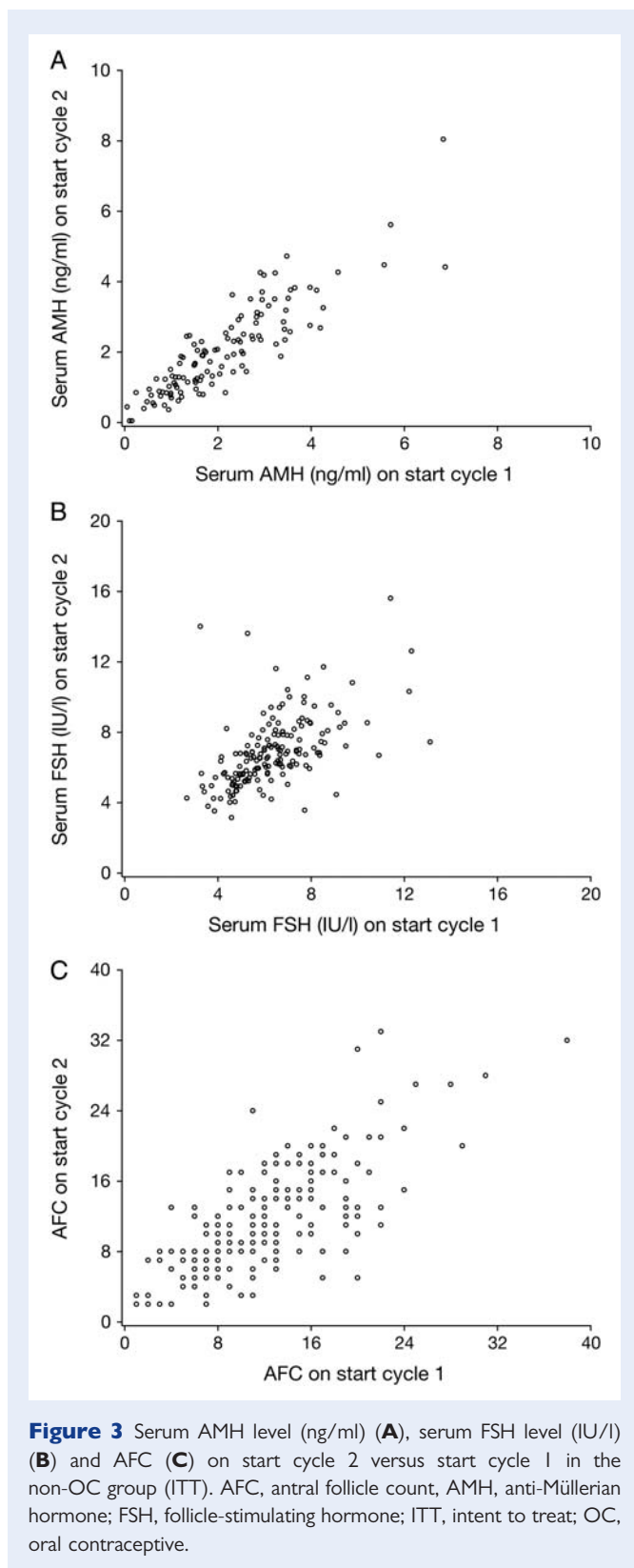
AMH and FSH as predictive factors (AUC = 0.78). When only AMH was included in the cycle 2 model, the AUC was 0.82 in the non-OC group and 0.74 in the OC group, whereas this value was 0.77 in cycle 1 for the combined groups.

AMH levels in subjects with low, normal and high ovarian response

Using cycle 2 data of subjects in the non-OC group, AMH showed a positive association with ovarian response (see Fig. 6). The median AMH levels were 0.88 ng/ml in the low responders ($n = 16$), 1.92 ng/ml in the normal responders ($n = 90$) and 3.32 ng/ml in the high responders ($n = 25$). FSH showed a negative association with ovarian response. Median serum FSH levels were 8.07 ($n = 17$), 6.69 ($n = 116$) and 5.41 ($n = 33$) IU/l in the low, normal and high responder groups, respectively.

Predictive factors for number of follicles (≥ 11 mm)

For the number of follicles at stimulation day 8, the final model included AFC ($P < 0.001$), serum FSH ($P < 0.001$) and serum AMH ($P = 0.001$) as significant predictive factors ($R^2 = 0.54$). For the number of follicles at the day of hCG, the final model included AFC ($P < 0.001$), FSH ($P < 0.001$), serum AMH ($P = 0.001$) and age of menarche ($P = 0.002$) as significant predictive factors ($R^2 = 0.52$). These models were based on the cycle 2 data of the non-OC group. The follicular response increased with lower FSH and with higher AFC, AMH and age of menarche.



Predictive factors for ongoing pregnancy

The overall ongoing pregnancy rate per started cycle is presented in Table III. The final model at randomization (cycle 1) of the combined OC and non-OC groups included FSH ($P = 0.02$), ovarian

Table III Clinical outcome per started cycle.

	OC (n = 209)	Non-OC (n = 199)
Duration of stimulation, days, median (P5, P95) ^a	10.0 (8, 12)	9.0 (7, 12)
Total rFSH dose, IU, median (P5, P95)	1800.0 (1300, 2400)	1600.0 (1200, 2200)
Follicles ≥ 11 mm at Day 8, mean (SD)	9.9 (5.4)	10.6 (5.7)
Follicles ≥ 11 mm at day of hCG, mean (SD) ^a	13.2 (5.9)	12.6 (5.9)
Serum E ₂ at the day of hCG, pmol/l, median (P5, P95) ^a	4624 (1585, 11120)	4954 (1897, 9982)
Oocytes, mean (SD)	12.4 (6.7)	12.1 (7.7)
Mature oocytes, mean (SD)	9.9 (5.7)	9.4 (6.4)
2PN fertilized oocytes, mean (SD) ^b	7.7 (4.9)	7.4 (5.7)
Embryos, mean (SD) ^b	8.5 (5.0)	8.5 (6.3)
Good quality embryos, mean (SD) ^b	4.4 (3.6)	4.8 (4.9)
Embryos transferred, mean (SD) ^c	1.9 (0.4)	1.9 (0.5)
Implantation rate, % ^{c,d}	24.1	30.1
Biochemical pregnancy rate, n (%)	71 (34.0)	101 (50.8)
Clinical pregnancy rate, n (%)	62 (29.7)	86 (43.2)
Ongoing pregnancy rate, n (%) ^e	55 (26.3)	71 (35.7)

ET, embryo transfer; hCG, human chorionic gonadotropin; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; OC, oral contraceptive; 2PN, pro-nucleate; rFSH, recombinant follicle-stimulating hormone.

^aRestricted to subjects with hCG administration for final oocyte maturation.

^bRestricted to subjects with IVF and/or ICSI.

^cRestricted to subjects with ET.

^d $P = 0.03$, adjusted for region (Europe, USA) and age category (≤ 32 and > 32 years).

^e $P = 0.05$, adjusted for region (Europe, USA) and age category (≤ 32 and > 32 years).

volume ($P = 0.03$), inhibin B ($P = 0.04$) and smoking ($P = 0.05$) as predictive factors, while at stimulation day 1 (cycle 2), it only included FSH ($P = 0.01$) as a predictive factor. Note that the treatment group was not a statistically significant factor in these models ($P > 0.05$). The area under the ROC curve was 0.66 and 0.59 at cycles 1 and 2, respectively, which was considered to be low in the sense that (ongoing) pregnancy cannot be reliably predicted for individual subjects.

Discussion

In this prospective trial examining potential predictive factors of ovarian response in a GnRH antagonist protocol, AMH appeared to be an important predictor for the number of oocytes retrieved, whereas AFC was one of the identified predictors for the number

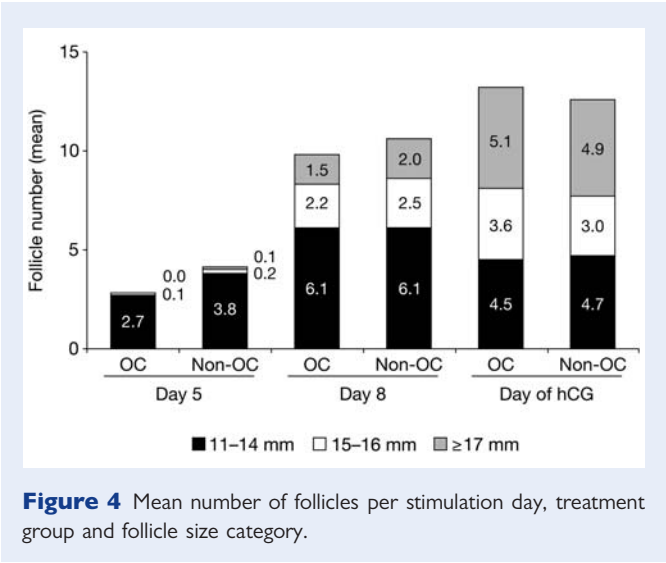


Figure 4 Mean number of follicles per stimulation day, treatment group and follicle size category.

of follicles ≥ 11 mm on the day of hCG. However, it should be noted that the AFC variability in this multicentre trial may have been increased due to the different operators performing the ultrasound measurements. While both factors are direct indicators of ovarian reserve and highly linked to each other, AMH is produced by both antral and pre-antral follicles, while the latter are not included in the AFC (La Marca et al., 2010).

Predictive factors, including demographic, sonographic and endocrine factors, are often obtained prior to the treatment cycle, and are used to determine the best rFSH-starting dose (Olivennes et al., 2009; Popovic-Todorovic et al., 2003a; Popovic-Todorovic et al., 2003b) or the best treatment regimen, i.e. GnRH antagonist versus long GnRH agonist protocol (Nelson et al., 2009). However, the findings of the current study indicated that several of these potential predictive factors, including AFC, FSH and inhibin B, were affected by cycle-to-cycle variability. Accordingly, in the current study, the best predictive models were obtained when using predictors measured at stimulation day 1 of the actual treatment cycle.

Of the candidate predictive factors, serum AMH was found to have the lowest inter-cycle variability, a finding that is consistent with previous studies indicating that serum AMH samples may be taken at any time point prior to the actual IVF treatment cycle (McIlveen et al., 2007). Moreover, this study confirmed that AMH levels remain unmodified due to OC pre-treatment as previously described for pituitary-suppressed patients using GnRH analogues or OC (La Marca et al., 2010). Although our final predictive models often indicated more significant predictive factors than AMH, our data also demonstrate that AMH alone is a reliable predictor of ovarian response. An added value of baseline FSH in the predictive model was observed only in the non-OC group when determined at the early follicular phase of the stimulation cycle.

The predictive factors identified for ovarian response in the current GnRH antagonist protocol were similar to previous reported factors for a long GnRH agonist protocol as reviewed by Broekmans et al. (2006) and Verhagen et al. (2008). However, with the exception of AMH, the cut-off values for high or low ovarian response of those factors may be different under pituitary suppression, and predictive

Table IV Final logistic regression models of low ovarian response (<6 oocytes) and high ovarian response (>18 oocytes), respectively.

		AUC		
Cycle	Treatment group	Model including AMH only	Model including all predictive factors	Identified predictive factors
Low ovarian response (<6 oocytes)				
1	OC + non-OC combined	0.84	0.85	AMH (<i>P</i> < 0.001) Smoking (<i>P</i> = 0.05)
2	OC	0.84	0.84	AMH (<i>P</i> < 0.001)
	Non-OC	0.88	0.90	AMH (<i>P</i> < 0.001) Smoking (<i>P</i> = 0.04) FSH (<i>P</i> = 0.05)
High ovarian response (>18 oocytes)				
1	OC + non-OC combined	0.77	0.80	AMH (<i>P</i> < 0.001) AFC (<i>P</i> = 0.03) FSH (<i>P</i> = 0.03)
2	OC	0.74	0.78	AMH (<i>P</i> < 0.001) FSH (<i>P</i> = 0.03)
	Non-OC	0.82	0.86	AMH (<i>P</i> < 0.001) FSH (<i>P</i> = 0.002)

AUC, area under the curve of the receiver operating characteristics (ROC) curve.

models cannot be extrapolated from antagonist to agonist protocols or vice versa (Pinto et al., 2009).

Previous studies have arbitrarily defined an appropriate ovarian response as retrieval of 5–14 oocytes (Popovic-Todorovic et al., 2003a). Initially, AMH measurements were applied to predict poor ovarian response, and different AMH assays and definitions of poor response were reported ranging between a cut-off value of two and six oocytes. In the largest prospective study by Nelson et al. (2007), poor response was defined as two or less oocytes, whereas excessive response was 21 or more oocytes (Nelson et al., 2007). In the present study, an appropriate response was defined as the retrieval of at least six oocytes and a maximum of 18 oocytes. The rationale for this definition is the observation that pregnancy rates decrease only when <6 oocytes are retrieved (Sharma et al., 2002). In accordance with this, the large National Dutch study of van der Gaast et al. (2006) showed that the increase in pregnancy rates in relation to the number of oocytes increased markedly from one to five oocytes, whereas the increase continued but was more moderate above six

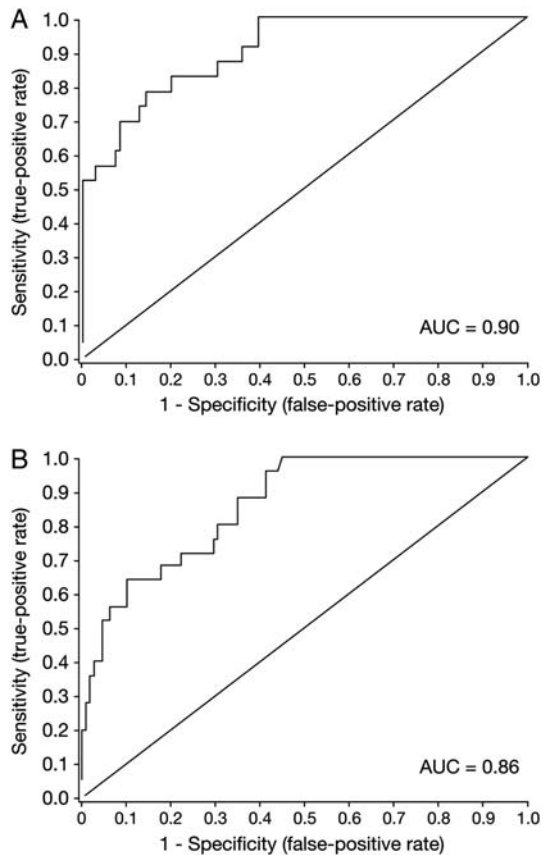


Figure 5 Receiver operating characteristic (ROC) curve of the stimulation cycle prediction model for low ovarian response [<6 oocytes, (A)] and high ovarian response (>18 oocytes, (B)) in the non-OC group. AUC, area under the curve.

oocytes (van der Gaast et al., 2006). Regarding the upper limit of appropriate response, the risk of OHSS increases greatly when more than 18 antral follicles or oocytes are obtained (Papanikolaou et al., 2006).

In a study by Olivennes et al. (2009), a wide range of FSH doses from 75 to 225 IU/day were applied using a dosing algorithm, including four predictive factors, i.e. age, FSH, BMI and AFC. Overall, in this rFSH dosing trial, a median number of nine oocytes were retrieved and the average per FSH dose group varied between 8 and 12 oocytes. This study gives additional evidence that predictive factors may be used to dose in order to obtain appropriate responses.

As reviewed by La Marca et al. (2010), a total of nine earlier studies have compared AMH with AFC in order to predict ovarian response. Five of the studies found that AFC and AMH had a correlation similar to the number of oocytes retrieved, whereas four other studies indicated that AMH was either less good or better. In accordance with our findings, Nelson et al. (2007) showed that AMH was the best predictor compared with age and FSH, but they did not include AFC measurements. More recently in a prospective, non-randomized trial, the same group demonstrated that AMH levels could be successfully applied as single parameter to determine the starting dose of FSH as well as the application of a GnRH antagonist instead of GnRH agonist protocol (Nelson et al., 2009).

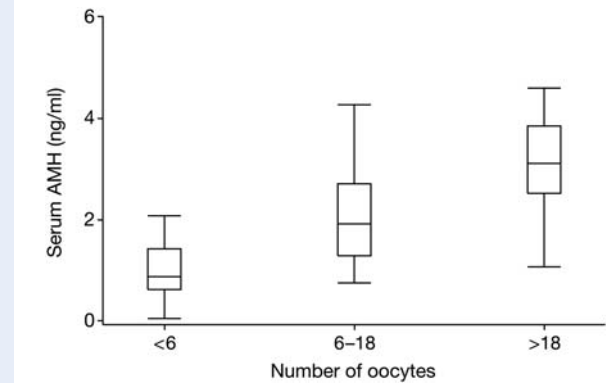


Figure 6 Serum anti-Müllerian hormone (AMH) levels in low, average and high responders. Restricted to cycle 2 data of subjects in the non-OC group who had oocyte pick-up. Figure presents box-plots with median values and the 5th, 25th, 75th and 95th percentiles of serum AMH.

Like the current trial, a recent prospective study showed a significant correlation between basal AMH concentrations and extremes of response in an unselected population of women undergoing their first cycle of ovarian stimulation for IVF (Nardo et al., 2009). Unlike Nelson and colleagues, they included AFC in ROC analyses and demonstrated that AMH is a superior predictor of excessive ovarian response, which enables clinicians to identify women at risk of OHSS better than AFC.

In the current study, some difficulties were encountered during the analyses of serum AMH levels, which may have influenced the results. AMH was measured with three different lots from the same commercial kit, but application of in-house control samples indicated a large inter-lot variability. When tested, the inclusion of the factor AMH lot had a statistically significant impact on the model for the number of oocytes retrieved ($P = 0.01$; non-OC group, cycle 2). As both samples from individual subjects were tested within the same lot, the inter-lot variability did not affect the estimation of the inter-cycle variation in the OC and non-OC groups. However, because of this variability, only the predictive ability of the candidate predictive factors were evaluated and no thorough validation of the models was performed to provide model prediction errors.

In general, the choice of possible AMH cut-off values to predict low, normal or high ovarian response requires consideration when assessment of any inter-lot variability is not part of the assay validation. Methodological problems with the DSL assay have been reported before (Bersinger et al., 2007; La Marca et al., 2010; Streuli et al., 2009) but should be resolved with the launch of the AMH Gen II Elisa (Beckman Coulter Inc., Brea, CA, USA). If so, serum AMH levels of low, normal and high responders may show less overlap and the predictive value of AMH may further increase.

The current study was not designed to detect a clinical relevant difference in ongoing pregnancy rate between OC and non-OC treatment. Randomization to OC versus non-OC treatment was performed since OC pre-treatment is more often applied by US clinics than by European clinics, which prevents bias due to regional differences.

Nevertheless, due to the randomized design of the study, the comparison between the two treatment groups is valid and showed a significantly lower implantation rate and correspondingly lower ongoing pregnancy rate after OC pre-treatment. The possible impact of OC pre-treatment in a GnRH antagonist protocol was first reported in 2006 (Huirne et al., 2006; Kolibianakis et al., 2006; Rombauts et al., 2006) just prior to the start of the current study. Although OC pre-treatment was thought to improve follicular homogeneity and increase the number of oocytes and good quality embryos, a tendency for lower implantation rates was observed in OC recipients. A first meta-analysis (Griesinger et al., 2008) of randomized controlled trials confirmed this trend, and a second meta-analysis after completion of the current trial indicated that the probability of an ongoing pregnancy per randomized subject was significantly lower in those receiving OC ($P = 0.02$) (Griesinger et al., 2010).

Although OC pre-treatment is a convenient way for clinics to schedule oocyte retrievals, it reduces the advantages of a GnRH antagonist protocol by extending the duration of treatment and the amount of FSH required to reach the same criteria of hCG (Rombauts et al., 2006), especially when stimulation is started shortly after OC discontinuation. In the current study, the time interval between the last dose of OC intake and the start of stimulation was 5 days, and all patients had onset of menses before starting COS. Circulating hormone levels measured on stimulation day 1 indicated that 5 days after the last dose of OC, pituitary suppression due to OC pre-treatment was mainly reversed. Despite this, the present study shows that the overall predictive value of sonographic and endocrine tests were less good after OC pre-treatment. The latter facilitated scheduling of oocyte retrieval and did not affect ovarian response, number of oocytes or embryo quality, compared with the results obtained from non-OC subjects. However, it appears that the implantation rate is reduced by OC pre-treatment, whereas no effect was observed on the number or quality of oocytes and embryos. Further research is required to examine whether OC treatment may have any carry-over effect on the endometrial development in the following stimulation cycle.

In conclusion, serum AMH and basal FSH were significant factors for the prediction of the number of oocytes retrieved as well as for the prediction of high responders (>18 oocytes). In low responders (<6 oocytes), only AMH was a significant factor. AFC, basal FSH and AMH were significant factors for the prediction of the number of follicles ≥ 11 mm on the day of hCG. AMH showed less cycle-to-cycle variation than basal FSH and AFC. The use of AMH or AFC, as a single test or in combination, for the prediction of ovarian response will prevent cycle cancellations due to too low or too high ovarian response and reduce the risk of OHSS. The final challenge will be to construct a reliable and simple algorithm that will enable clinicians to choose for each patient the best treatment protocol, which should prospectively reduce cancellation rates and therefore improve the success rates per started cycle.

Authors' roles

A.N.A. was the lead investigator of this multicentre trial and contributed to the trial design and interpretation of data. H.W. was primarily responsible for the data analyses. K.G. contributed to the trial design and interpretation of data and B.M. was responsible for the scientific

content of the trial protocol and report. All four authors contributed equally to the intellectual content of this manuscript.

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Conflict of interest

A.N.A. has been an investigator for trials initiated by Merck-Serono and Ferring. H.W., K.G. and B.M. are employees of Merck, Sharp and Dohme & Co.

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Appendix

Denmark: Nyboe Andersen, Rigshospitalet, København. Germany: Bispink, Praxis für Hormonstörungen und Reproduktionsmedizin, Hamburg. Spain: Musoles, Hospital Clinico Valencia, Valencia; Martínez Navarro, Hospital Virgen de las Nieves, Granada. Turkey: Aksu, Hacett. Univ., Kadın hastalıkları ve Doğum ABD, Üreme Endok. ve Inf. Unit., Ankara, Hacett. Univ., Med. Faculty Hosp. Dept. of Obstetrics and Gynecology, Ankara; Tavmergen, Ege University School of Medicine, Obstetrics and Gynecology, Dep Ege University School of Medicine, Obstetrics and Gynecology Dep, İzmir. United States: Westphal, Stanford University Center for Research on Women's Health and Reproductive Medicine, Dept. of Gynecology & Obstetrics, Stanford University Medical Center, Stanford, California; Buyalos, Fertility & Surgical Associates, Thousand Oaks, California; Jacobs, Fertility & IVF Center of Miami, Miami, Florida; Bopp, Midwest Fertility Specialists, Indianapolis, Indiana; Rosenwaks, New York Presbyterian Hospital/Weill-Cornell Medical College, CRMI, New York, New York; Scheiber, Institute for Reproductive Health, Cincinnati, Ohio; Chantilis, Presbyterian Hospital of Dallas, Texas; Severino, Aurora Fertility Services, Green Bay, Wisconsin.