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Predicting the FSH threshold dose in women with WHO Group II anovulatory infertility failing to ovulate or conceive on clomiphene citrate

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BACKGROUND: The objective of this investigation was to establish independent predictors of follicle-stimulating hormone (FSH) threshold dose in anovulatory women undergoing ovulation induction with FSH preparations. METHODS: One hundred and fifty-one patients with WHO Group II anovulatory infertility failing to ovulate or conceive on clomiphene citrate underwent ovarian stimulation with FSH-only preparations following a low-dose step-up protocol. The individual FSH threshold dose was defined as the FSH dose when meeting the human chorionic gonadotrophin criteria (one follicle ≥17 mm, or 2–3 follicles ≥15 mm). The influence of demographics, physical characteristics, obstetric and infertility and menstrual cycle history, ovarian ultrasonography, endocrine parameters and type of gonadotrophin preparation on the FSH threshold dose was assessed through multiple regression analysis. RESULTS: In the univariate analysis, age, body mass index (BMI), failure to ovulate with clomiphene citrate, menstrual cycle history (amenorrhea, oligomenorrhea or anovulatory cycles of 21–35 days), mean ovarian volume, LH/FSH ratio, testosterone and free androgen index were significant ($P < 0.05$) predictors of FSH threshold dose. In the multivariate analysis, menstrual cycle history, mean ovarian volume and BMI remained significant ($P < 0.001$). CONCLUSIONS: The individual FSH threshold dose for ovulation induction in anovulatory women can be predicted based on three variables easily determined in clinical practice: menstrual cycle history, mean ovarian volume and BMI. A FSH dosage nomogram was constructed based on these parameters.

Keywords: anovulation; follicle-stimulating hormone; threshold dose; predictors; efficiency

Introduction

Approximately 20–30% of the women seeking fertility treatment present with anovulation (Healy *et al*., 1994). Clomiphene citrate has been used as the first line treatment for inducing ovulation in anovulatory women. In most women who failed to ovulate or conceive with clomiphene citrate, ovarian stimulation with gonadotrophins leads to ovulation (Coelingh-Benning *et al*., 1998; Yarali *et al*., 1999; Platteau *et al*., 2006; Balen *et al*., 2007). The use of low-dose step-up protocols has been shown to be successful in producing monofollicular development in more than half of the women undergoing stimulation with gonadotrophins, and thereby minimizing the risk of multiple follicular development and the associated risks of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy (Homburg and Howles, 1999).

A typical low-dose step-up protocol starts with 50–75 IU/day of follicle-stimulating hormone (FSH) or menotrophins for ~7 to 14 days, and the dose is then increased by 37.5–50 IU/day if adequate response is not obtained. For some patients, the dose of gonadotrophin needed to provide adequate response is relatively high and the use of low-dose regimens will in these cases lead to stimulations of long duration. A long ovarian stimulation is associated with more monitoring visits and would be expected to result in increased costs and

inconvenience for the patients. Selection of the most optimal starting dose of gonadotrophin for each individual patient should minimize these disadvantages, while ensuring adequate response.

Imani *et al*. (1998) have identified clinical and laboratory parameters such as body mass index (BMI), menstrual cycle history, mean ovarian volume and free androgen index (FAI) which can predict ovulation in WHO type II anovulatory women undergoing clomiphene citrate treatment. A similar investigation in anovulatory women undergoing ovulation induction with gonadotrophins suggested BMI, clomiphene citrate resistance, serum insulin-like growth factor-1 (IGF-1) and serum FSH as significant predictors of FSH threshold dose (Imani *et al*., 2002). The clinical application of this latter result is complicated by the fact that the IGF-1 assay is not part of most clinics standard battery for laboratory screening of anovulatory patients. Therefore, further data are needed to identify simple parameters which can predict the FSH threshold dose in women who will undergo low-dose protocols with gonadotrophins.

The purpose of the present prospective investigation was to evaluate the influence on the FSH threshold dose of clinical, sonographic and endocrine parameters available at the start of ovarian stimulation as well as type of FSH preparation to be used in a low-dose step-up protocol. The data from this investigation form the basis for constructing a nomogram which provides an indication of the expected FSH threshold dose for the individual patient, facilitating the selection of the most appropriate starting FSH dose for ovarian stimulation.

Materials and Methods

Study population and design

This investigation included 151 women with anovulatory WHO Group II infertility who were included in a randomized, open label, assessor-blind, parallel group, multicentre, multinational, non-inferiority study comparing two different FSH preparations (highly purified (HP)-FSH, BRAVELLE, Ferring Pharmaceuticals A/S, Copenhagen, Denmark and recombinant FSH (rFSH), follitropin alfa, GONAL-F, Serono, Geneva, Switzerland) with respect to ovulation rate. Patients were recruited in 22 fertility centres (12 in Belgium, 7 in Denmark and 3 in the UK) that on average each included seven patients, ranging from 2 to 20. A detailed description of the study population, all inclusion and exclusion criteria, all study procedures, as well as the main outcome of the study, is described in detail in a separate publication (Balen *et al*., 2007).

Patients were to be 18–39 years of age with BMI 19–35 kg/m^2, have been diagnosed with infertility for $\geq$1 year, have chronic anovulation (amenorrhea, oligomenorrhea or anovulatory cycles based on progesterone levels in patients with cycle lengths of 21–35 days) and have failed to ovulate with clomiphene citrate doses of at least 100 mg/day for at least 5 days or failed to conceive after three cycles of ovulation induction with clomiphene citrate. Women with a history of $\geq$12 unsuccessful ovulation induction cycles, persistent ovarian cysts ($\geq$15 mm) for >1 cycle or ovarian endometrioma on ultrasound and any significant systemic disease, endocrine or metabolic abnormalities were excluded. The study was carried out in accordance with the declaration of Helsinki on good clinical practice and ethical committee approval was obtained in all participating centres.

All patients underwent screening assessments prior to start of ovarian stimulation. These included evaluation of demographics (age), physical characteristics (body weight, height, waist and hip circumference), obstetric history (previous pregnancies, previous live births), infertility history (duration of infertility, number of previous ovulation induction cycles with clomiphene citrate and in total, failure to ovulate with clomiphene citrate, failure to conceive with clomiphene citrate) and menstrual cycle history. Just prior to the first dose of gonadotrophin, patients underwent a transvaginal ultrasound and blood sampling for endocrine evaluation. The sonographic recordings comprised dimensions of right and left ovary, number of follicles (antral follicles >2 mm) in right and left ovary and endometrial thickness. The endocrine evaluation included the following parameters: luteinizing hormone (LH), FSH, estradiol (E_2), prolactin, total testosterone, sex hormone binding globulin (SHBG), FAI, glucose and insulin. Chemiluminescent immunometric assays were used for LH, FSH, prolactin, total testosterone and SHBG, and radioimmunoassays were used for E_2 and androstenedione, whereas a hexokinase assay was used for glucose and immulite technology was applied for analysis of insulin. All endocrine analyses were conducted by a central laboratory (Quest Diagnostics—Quest Diagnostics Limited, Heston, UK; Quest Diagnostics Limited, Van Nuys, CA, USA; Nichols Institute, San Juan Capistrano, CA, USA). FAI was calculated as (total testosterone/SHBG) $\times$ 100.

All patients underwent ovarian stimulation following a low-dose step-up protocol. Stimulation was started 2–5 days after a spontaneous or progesterone-induced menstrual bleed. The starting dose of gonadotrophin was 75 IU daily, which was maintained for 7 days. After the first 7 days, the gonadotrophin dose was either maintained or increased by 37.5 IU increments according to individual response. Patients were maintained on any specific dose level for at least 7 days. The maximum allowed daily dose was 225 IU and patients were treated with gonadotrophin for a maximum of 6 weeks. Gonadotrophin stimulation was maintained until at least one of the following criteria for human chorionic gonadotrophin (hCG) administration was met: one follicle with a diameter of 17 mm or greater, or 2–3 follicles with a diameter of 15 mm or greater. Patients were not given hCG in either of the following situations: no follicular response after 6 weeks of gonadotrophin treatment, $\geq$4 follicles with diameters of $\geq$15 mm or serum E_2 levels >2000 pg/ml. Patients who reached the hCG criteria received a single s.c. or i.m. injection of hCG (PROFASI, Serono) at a dose of 5000 IU to trigger ovulation. At least one blood sample was taken during the midluteal phase (6–9 days after hCG administration) and analysed for progesterone by a central laboratory using competitive immunoassay using direct chemiluminometric technology with a sensitivity of 0.48 nmol/l (Quest Diagnostics Limited, UK). Ovulation was defined as a mid-luteal serum progesterone concentration of $\geq$25 nmol/l ($\geq$7.9 ng/ml).

The FSH threshold dose was defined as the dose of FSH that was given when the criteria for hCG was reached. The analysis involved the influence of the type of gonadotrophin preparation (i.e. HP-FSH or rFSH) as well as of clinical, sonographic and basal endocrine parameters on the FSH threshold dose. All potential predictive variables were investigated: age, BMI, waist–hip ratio, previous pregnancy, duration of infertility, failure to ovulate on clomiphene citrate, menstrual cycle history (amenorrhea if the patient had absent menstruation, oligomenorrhea if menstruation intervals were longer than 35 days or cycle length of 21–35 days in which case anovulation had been documented by progesterone levels), total number of follicles, mean ovarian volume (based on length, width and depth of both right and left ovary), LH/FSH ratio, androstenedione, total testosterone, SHBG, FAI, prolactin, estradiol, glucose, insulin and insulin/glucose ratio.

Statistical analysis

The primary end-point of the study was ovulation rate after one cycle of gonadotrophin treatment, and these data have been reported elsewhere (Balen *et al*., 2007). The influence of the selected clinical, sonographic and endocrine parameters as well as type of gonadotrophin on FSH threshold dose were assessed in univariate and multivariate analyses using proportional odds polytomous logistic regression models (an extension of the model and method for binary response). Multivariate analyses were performed including characteristics that had statistically significant influence on the FSH threshold dose in the univariate analysis first by entering all selected characteristics into the polytomous logistic model in a forward selection approach and secondly by entering all selected characteristics into the polytomous logistic model using a backward elimination approach. *P*-values of <0.05 were regarded as statistically significant. Score tests were used to evaluate the proportional odds assumption and Wald tests were used to assess the influence of the baseline characteristics on the FSH threshold dose. For the present analysis, all parameters were analysed as continuous variables, except for type of gonadotrophin, previous pregnancy, failure to ovulate on clomiphene citrate and menstrual cycle history (glucose and insulin were categorical variables as normal/abnormal and insulin/glucose ratio was a continuous variable).

In addition to the above, the model was evaluated on the subset of patients with mono-follicular development (one follicle $\geq$17 mm and no follicles of 15–16 mm). In this evaluation, the same set of variables was used as selected for the main model according to the above procedure. The predictive performance of the model was assessed by using the 'leave-one-out' cross-validation procedure. In addition, the residual standard error after a standard multiple regression analysis was evaluated.

Finally, ROC curves were produced and AUC under the ROC curves were calculated. For the standard situation where ROC curves are used, the classification is binary, whereas the threshold dose had four possible outcomes (87.5, 112.5, 150 and 187.5) and the prediction model therefore also predicts into four classes. A consensus approach on how to handle multiclass ROC has not been reached (Landgrebe and Duin, 2007), but when the outcome classes are ranked, ROC curves can be made for each of the possible levels (Waegeman *et al*., 2006).

Table I. Clinical, sonographic and endocrine parameters just prior to starting ovarian stimulation with FSH (Day 1 of stimulation).

	Total ($n = 151$)
Clinical parameters	
Age (years)	28.9 ± 3.7
Body weight (kg)	68.6 ± 13.2
BMI (kg/m^2)	24.8 ± 4.5
Primary infertility, *n* (%)	97 (64%)
Duration of infertility (years)	2.8 ± 1.7
Previous cycles of ovulation induction (all)	4.9 ± 2.4
Previous cycles of ovulation induction (with clomiphene citrate)	4.1 ± 2.4
Clomiphene citrate non-responders, *n* (%)	
Failure to ovulate on clomiphene citrate[a]	51 (34%)
Failure to conceive on clomiphene citrate[b]	100 (66%)
Menstrual cycle history, *n* (%)	
Amenorrhea	23 (15%)
Oligomenorrhea	84 (56%)
Anovulatory cycles with cycle length 21–35 days	44 (29%)
Sonographic parameters	
Mean ovarian volume (cm^3)	7.1 ± 3.5
Antral follicles >2 mm	14.8 ± 12.5
Endometrial thickness (mm)	4.1 ± 2.2
Endocrine parameters	
LH (IU/l)	7.1 ± 4.6
FSH (IU/l)	5.5 ± 2.4
LH/FSH ratio	1.4 ± 0.8
Prolactin (μg/l)	16 ± 13
Androstenedione (nmol/l)	6.74 ± 3.08
Total testosterone (nmol/L)	1.8 ± 0.6
Sex hormone binding globulin (nmol/l)	61 ± 40
Free androgen index	4.45 ± 3.47
Estradiol (pmol/l)	172 ± 98
Glucose (mmol/l)	
Fasting	5.1 ± 0.6
Non-fasting	5.2 ± 1.5
Insulin (pmol/l)	
Fasting	66.4 ± 43.1
Non-fasting	143.9 ± 192.6

All data are mean ± SD or number of patients (percentage of patients).
[a]On at least 5 days of 100 mg/day.
[b]After three cycles.

Results

The clinical, sonographic and endocrine characteristics of the 151 patients included in the study are shown in Table I. The mean age of the study population was 28.9 years, ranging from 19 to 39 years. The BMI spanned from 18 to 39 kg/m^2, with 54% of the patients having a normal BMI, 32% being overweight and 14% being obese and with the mean BMI being 24.8 kg/m^2. On average, patients had been infertile for ~3 years and had undergone four previous ovulation induction cycles with clomiphene citrate, with 34% of the patients failing to ovulate on clomiphene citrate. Regarding menstrual cycle history, 56% of the patients were oligomenorrheic and 15% amenorrheic. An LH/FSH ratio above 1 was noted for 59% of the patients. Among the 151 patients who started stimulation with FSH (HP-FSH 73, rFSH 78), 132 patients met the hCG criteria (HP-FSH 65, rFSH 67) and contributed with data to the analysis of FSH threshold dose. There were 19 patients who initiated gonadotrophin therapy but did not contribute to this analysis; 10 discontinued from the study during ovarian stimulation (five because of excessive response), 2 had spontaneous ovulation and 7 received hCG despite either not meeting or exceeding the hCG criteria.

Table II provides the outcome of the univariate and multivariate analysis for the predictors of FSH threshold dose. Eight parameters were statistically significant predictors of FSH threshold dose ($P < 0.05$): age, BMI, failure to ovulate with clomiphene citrate, menstrual cycle history (amenorrhea, oligomenorrhea or anovulatory cycles with cycle length 21–35 days), mean ovarian volume, LH/FSH ratio, testosterone and FAI. The type of gonadotrophin preparation, HP-FSH or rFSH, did not predict FSH threshold dose. All eight significant parameters were included in a multivariate analysis which showed that only three of the parameters were independently statistically significant (*P*-values < 0.001) predictors of FSH threshold dose: BMI, mean ovarian volume and menstrual cycle history (Table III). The odds for needing >75 IU FSH were 1.18 [95% confidence interval (CI): 1.07–1.29] for BMI and 1.22 [95% CI: 1.09–1.37] for mean ovarian volume; thus the higher the BMI and ovarian volume prior to start of stimulation, the higher the FSH threshold dose. Regarding the menstrual cycle history, the odds for needing a FSH

Table II. Statistically significant predictors of FSH threshold dose (univariate and multivariate analysis).

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*-value[a]	OR (95% CI)	*P*-value[a]
Age (years)	0.91 (0.83–1.00)	0.040	—	—
BMI (kg/m^2)	1.10 (1.02–1.19)	0.010	1.17 (1.07–1.29)	<0.001
Failure to ovulate on clomiphene citrate: yes versus no	2.27 (1.14–4.52)	0.020	—	—
Menstrual cycle history		<0.001		<0.001
Amenorrhea versus cycle length 21–35 days	8.33 (2.81–24.7)		11.88 (3.35–42.1)	
Oligomenorrhea versus cycle length 21–35 days	3.48 (1.51–8.03)		2.57 (0.97–6.79)	
Mean ovarian volume (cm^3)	1.18 (1.06–1.31)	0.002	1.22 (1.08–1.37)	<0.001
Free androgen index (nmol/l)	1.25 (1.13–1.38)	<0.001	—	—
Total testosterone (nmol/l)	2.14 (1.14–4.03)	0.018	—	—
LH/FSH ratio (IU/l)	1.59 (1.03–2.45)	0.038	—	—

[a]Wald test for effect of factor/covariate.

dose >75 IU were 11.9 [95% CI: 3.35–42.0] and 2.57 [95% CI: 0.97–6.79] for amenorrhea and oligomenorrhea, respectively, compared with anovulatory cycles with a length of 21–35 days. The observed data for these three independent predictors are presented in Table III.

The model predicted the FSH threshold dose to be the same as the dose observed in the study for 59% of the patients. The AUC under the ROC curve was 0.78 for 75 IU (Fig. 1a) and 0.79 for 112.5 IU (Fig. 1b). In a multiple regression analysis with the same three variables as in the logistic model, the FSH threshold dose was estimated on a continuous scale. From this estimation, the residual mean square error was 25 IU and the $R^2 = 25\%$.

A simple nomogram can be constructed based on the two clinical and one sonographic parameters that significantly predict the FSH threshold dose. Figure 2 displays three nomograms—one for each type of menstrual cycle history—where the shaded areas indicate the predicted FSH threshold dose, based on the BMI displayed on the *x*-axis and mean ovarian volume on the *y*-axis. For example, the expected threshold FSH dose for a patient with amenorrhea (Fig. 2a), mean ovarian volume of 10.5 cm^3 and a BMI of 30 kg/m^2 will be 150 IU. In the case that the patient presents with oligomenorrhea (Fig. 2b), but similar mean ovarian volume and BMI, the expected threshold FSH dose would be 112.5 IU. As another example, a threshold FSH dose of 75 IU will be expected for a patient with anovulatory cycles of 21–35 days, mean ovarian volume of 8.5 cm^3 and BMI 27 kg/m^2, whereas it will be 112.5 IU for a patient with oligomenorrhea and similar mean ovarian volume and BMI.

The accuracy of the model was 60% when applied to the subset of patients with mono-follicular development (80 patients), and thus similar to that achieved for the overall study population.

Discussion

The main finding in the present study was identification of the three variables: menstrual cycle history, BMI and mean ovarian volume, as independent predictors of the FSH threshold dose during FSH treatment of anovulatory infertility. For clinical use, such data could be implemented and used as screening predictors to determine the individual FSH threshold dose of any patient before start of gonadotrophin treatment, in order to select the most appropriate starting dose. For this purpose, we have used the three independent predictors to develop the FSH dose nomograms presented in Fig. 2. The proposed model had an accuracy of 59–60%, which is well above the 25% that would be expected by random allocation to one of the four dose levels evaluated in this study.

Our findings are partly in line with those obtained by Imani *et al*. (2002) who studied 90 anovulatory women, also treated with either urinary or rFSH, and showed that BMI, clomiphene resistance, free IGF-1 and basal FSH were independent predictors of the FSH threshold dose. Imani *et al*. (2002) made an equation showing that the predicted dose was: [4 BMI (in kg/m^2)] + [32 clomiphene citrate resistance (yes = 1 or no = 0] + [7 initial free IGF-I (in ng/ml)] + [6 initial serum FSH level (in IU/l)] − 51. Similar to our study, it was found that menstrual bleeding interval as well as clomiphene

Table III. BMI, mean ovarian volume and menstrual cycle history by observed FSH threshold dose.

	BMI (kg/m^2)	Mean ovarian volume (cm^3)	Menstrual cycle history (%) Amenorrhea / oligomenorrhea / cycle length 21–35 days
75 IU ($n = 67$)	23.8 ± 4.2	6.3 ± 2.9	8% / 48% / 45%
112.5 IU ($n = 46$)	25.5 ± 4.8	7.3 ± 3.4	22% / 63% / 15%
150 IU ($n = 15$)	25.8 ± 4.0	9.5 ± 4.8	33% / 47% / 20%
187.5 IU ($n = 4$)	28.1 ± 4.6	10.2 ± 2.3	25% / 75% / 0%

All data are mean ± SD or percentage of patients.

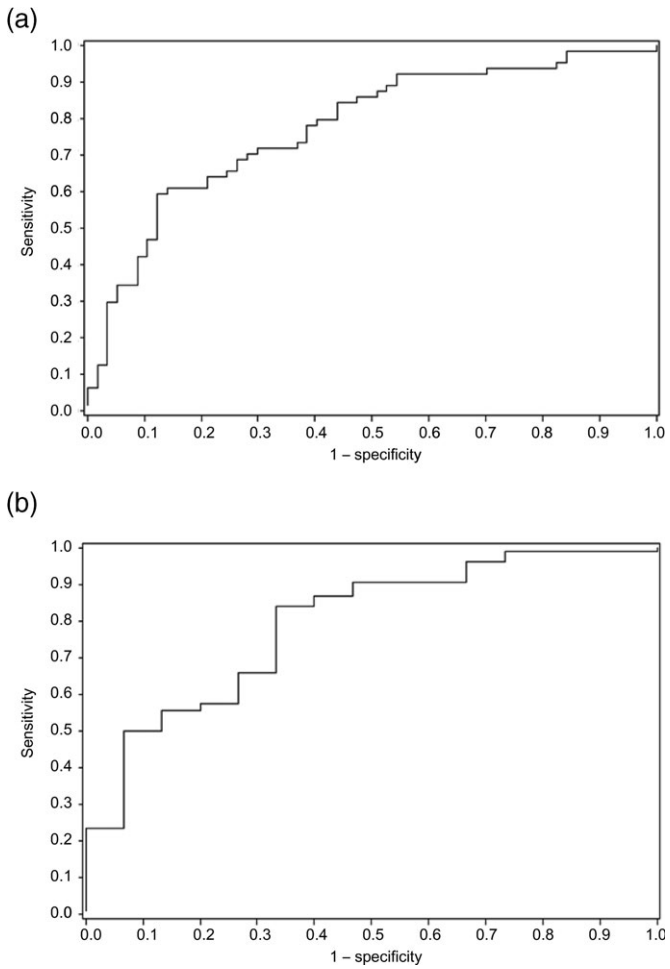

Figure 1: ROC curves for threshold dose (**a**) 75 IU versus <75 (AUC = 0.78) and (**b**) ≤112.5 IU versus >112.5 IU (AUC = 0.79).

resistance were significant predictors in the univariate analysis, but among the possible clinical predictors only clomiphene resistance and BMI remained significant in the multivariable analysis. The population studied by Imani *et al*. (2002) seems very similar to the present study, even though the latter included patients if they failed to conceive after three clomiphene cycles, whereas the study by Imany *et al*. (2002) only included women with six failed ovulatory clomiphene citrate cycles, but it seems unlikely that this would explain the difference in predictive factors. A more plausible explanation could be that the study by Imani *et al*. (2002) included, in the forward step-wise multivariate analysis, some endocrine factors, such as free-IGF-I and its binding proteins, that may alter the predictors that remained in the final model during the analysis.

It is worth briefly discussing a statistical methodological difference between the present model and that suggested by Imani *et al*. (2002). The polytomous logistic regression model used on the present data set predicts FSH threshold doses that correspond to actual dose levels applied in the clinical trial, in contrast to a multiple regression where the predicted dose is on the continuous scale, as the equation published by Imani *et al*. (2002). In our model, the residual mean square error was 25 IU and therefore the individual prediction is at least as good as in the model suggested by Imani *et al*. (2002) where the residual mean square error was 35 IU. The R^2 value of 25% in the present model is explained by the relatively few patients in the two highest dose levels in our trial compared with a more even distribution of patients in the trial conducted by Imani *et al*. (2002) yielding an R^2 of 39% in their model.

Our finding that the menstrual status was important is in line with expectations. Patients with amenorrhea may need more FSH to reach the threshold than a patient with oligomenorrhea, who may also need more FSH compared with patients with anovulatory cycles, indicating that the severity of the ovarian dysfunction may well be reflected in the degree of menstrual cycle disturbance. The relative resistance to FSH in amenorrheic patients is supported by the finding that these patients also have a relative resistance to endogenously released FSH during clomiphene citrate treatment (Imani *et al*., 1998), as patients with clomiphene resistance do release a similar amount of endogenous FSH as the clomiphene sensitive women (Polson *et al*., 1989). This supports the concept that it is indeed the threshold of the ovarian sensitivity that is altered.

A high BMI was associated with a higher FSH threshold dose; an observation that is supported by the findings that the total dose of gonadotrophins needed to induce ovulation is increased in parallel with body weight (Hamilton-Fairly *et al*., 1992; Balen *et al*., 2006). The cause of the association with BMI may be that the endocrine disturbance is related to the degree of body fat and hyperinsulinaemia, but it may also be due to a lower bioavailability of exogenous gonadotrophins in obese women (Chan *et al*., 2003; Steinkampf *et al*., 2003).

The type of gonadotrophin used for stimulation was not a significant predictor of FSH threshold dose, and thus of limited relevance compared with the patient's clinical characteristics.

In theory, the threshold is defined as the lowest dose that induces growth of the single most sensitive follicle. In this clinical trial, a dual criterion for hCG administration was used, covering not only the development of a single dominant follicle, defined as one follicle of 17 mm or greater, but also the presence of 2–3 follicles of 15 mm or greater. Therefore, our threshold is based on the stimulation in those 132 (87%) patients who reached either of these criteria, including those patients with up to 3 follicles ≥15 mm. The threshold dose in the study and subsequently the model could also be termed the 'response dose' and can be viewed as a surrogate for the real threshold. It should be considered that serum FSH may accumulate over time so the theoretically ideal threshold should be defined as the dose that caused growth of a single dominant follicle to around 9–10 mm, where dominance is normally established. The exact timing of dominance was not determined in the present study, but it is important to note that the model for threshold dose predictions also applied in the subset of patients with mono-follicular development defined as only one follicle ≥17 and no follicles of 15–16 mm. The average number of follicles ≥15 mm was 1.5 (Balen *et al*., 2007) indicating that some patients did indeed receive FSH doses slightly above the threshold for single dominant follicle development. However, the analysis restricted to patients with mono-follicular development showed that the accuracy of the model was the same for this subset of patients

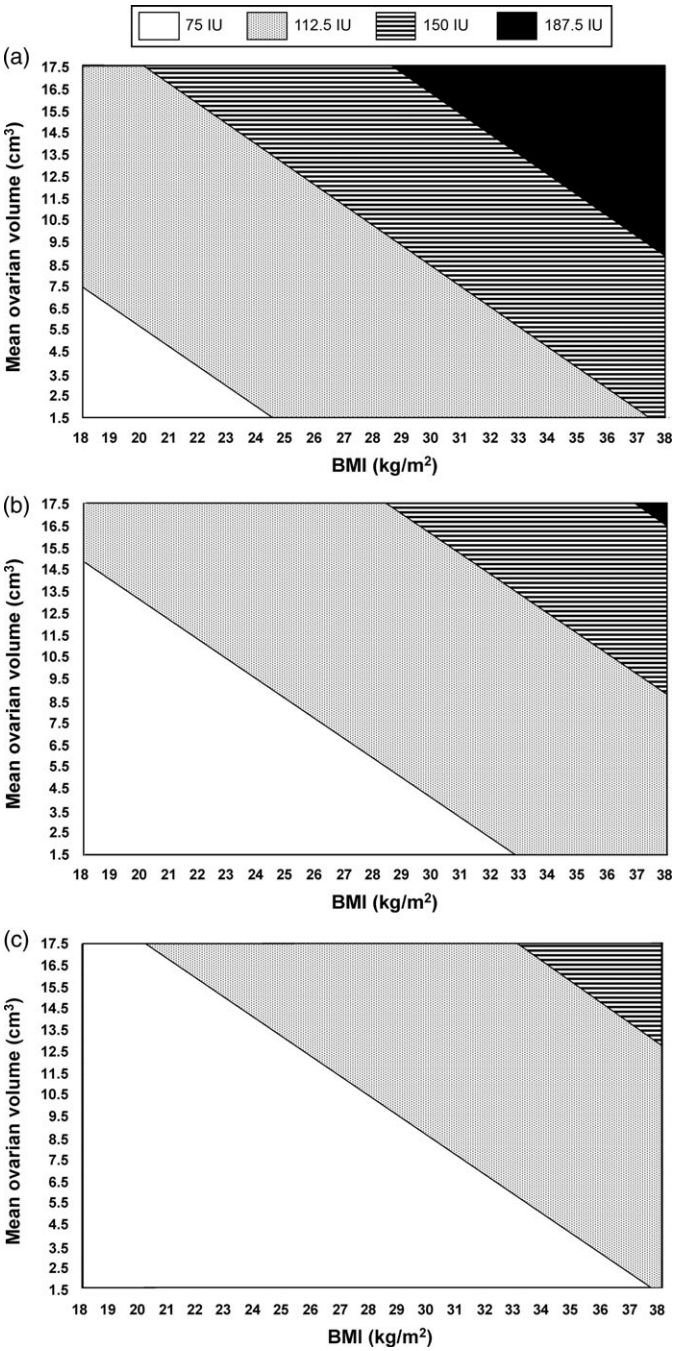

Figure 2: Nomograms for prediction of individual FSH threshold dose in anovulatory patients undergoing ovulation induction with FSH preparations, according to menstrual cycle history, BMI and mean ovarian volume. (a) Amenorrhea, (b) oligomenorrhea and (c) anovulatory cycles with cycle length 21–35 days.

as for the overall study population. On the basis of the intention to treat population in the present study, 92% of the patients received hCG and the ongoing pregnancy rate was 19%, of which 79% accounted for singletons and 21% for multiple pregnancies (Balen *et al*., 2007). These figures are much in line with literature data on 7 day step-up protocols (Homburg and Howles, 1999), but it could be argued that the multiple pregnancy rate is somewhat high, compared with a number of recent studies using starting doses of FSH <75 IU/day (Balasch *et al*., 2000; Calaf Alsina *et al*., 2003). Indeed, multiple pregnancy rates as low as 6% have been reported in a large clinical trial using starting doses of 50 IU/day, even though up to 3 follicles >16 mm before hCG administration was accepted (Calaf Alsina *et al*., 2003). An alternative approach is to restrict hCG administration to those patients who have only one or two mature follicles, but this would increase the cancellation rates. As published (Balen *et al*., 2007), 3.3% (5/151) of the patients had their cycle cancelled before hCG, due to development of multiple follicles. Identification of these patients with a very low threshold would have necessitated a starting dose of <75 IU/l.

Ovarian volume was another significant parameter predicting the FSH threshold dose. The larger the ovaries, the more FSH was needed to obtain adequate ovarian response. This finding is consistent with earlier clinical studies of ovulation induction with gonadotrophins, where it has been shown that patients with larger ovaries need longer duration of stimulation and higher doses of gonadotrophins (Lass *et al*., 2002).

Van den Meer *et al*. (1994) studied three consecutive cycles in 16 patients during gradually increased doses of intravenously infused urinary FSH and elegantly documented how the inter-individual variability in FSH threshold dose was far greater than the variability from cycle to cycle. The key problem is therefore to define the appropriate dose for any individual patient and the present study provides a suggestion for nomograms that may be used clinically. A major concern, if patients were dosed according to the nomograms, would be that 13% would receive a higher starting dose that the observed threshold dose. The implications of an excessive starting dose are likely to be the same as when step-down protocols are used, i.e. risk of multiple follicular development. Indeed, van Santbrink *et al*. (2002) have used the prediction model developed by the same group of investigators (Imani *et al*., 2002), and retrospectively evaluated the doses used in a step-down regimen. In that study, even though the average excessive dose was 28.5 IU/day above the threshold, the step-down approach resulted in an overall multiple pregnancy rate of 17% and not a single case of OHSS (van Santbrink *et al*., 2002).

The expected main advantage of dosing according to the nomogram would be a shortening of the stimulation phase. It is important to note that the suggested dosage nomograms could be easily used clinically because they only include simple characteristics that are routinely recorded in all anovulatory patients. Before using this model in clinical practice, a randomized controlled trial should be conducted to compare the presently used low-dose step-up protocol with a flexible protocol using this model.

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References

Balasch J, Fabregues F, Creus M, Casamitjana R, Puerto B, Vanrell JA. Recombinant human follicle-stimulating hormone for ovulation induction in polycystic ovary syndrome: a prospective, randomised trial of two starting doses in a chronic low-dose step-up protocol. *J Assist Reprod Genet* 2000;**17**:561–565.

Balen A, Platteau P, Nyboe Andersen A, Devroey P, Sørensen P, Helmgaard L, Arce J-C. The influence of body weight on response to ovulation induction with gonadotrophins in 335 women with World Health Organization group II anovulatory infertility. *Br J Obstet Gynaecol* 2006;**113**:1195–1202.

Balen A, Platteau P, Nyboe Andersen A, Devroey P, Helmgaard L, Arce J-C, for the Bravelle Ovulation Induction (BOI) Study Group. Highly purified FSH is as efficacious as recombinant FSH for ovulation induction in women with WHO Group II anovulatory infertility: a randomized controlled non-inferiority trial. *Hum Reprod* 2007;**22**:1816–1823.

Calaf Alsina J, Ruiz Balda JA, Romeu Sarrió A, Caballero Fernandez V, Cano Trigo I, Gómez Parga JL, González Batres C, Rodríguez Escudero FJ. Ovulation induction with a starting dose of 50IU of recombinant follicle stimulating hormone in WHO group anovulatory women: the IO-50 study, a prospective, observational, multicentre, open trial. *Br Journal Obstet Gynaecol* 2003;**110**:1072–1077.

Chan CCW, Ng EHY, Chan MMY, Tang OS, Lau EYL, Yeung WSB, Ho PC. Bioavailability of hCG after intramuscular or subcutaneous injection in obese and non-obese women. *Hum Reprod* 2003;**18**:2294–2297.

Coelingh Bennink HJT, Fauser BCJM, Out HJ. Recombinant follicle-stimulating hormone (FSH; Puregon) is more efficient than urinary FSH (Metrodin) in women with clomiphene citrate-resistant, normogonadotropic, chronic anovulation: a prospective, multicenter, assessor-blind, randomized, clinical trial. *Fertil Steril* 1998;**69**:19–25.

Hamilton-Fairly D, Kiddy D, Watson H, Paterson C, Franks S. Association of moderate obesity with a poor pregnancy outcome in women with polycystic ovary syndrome treated with low dose gonadotrophin. *Br J Obstet Gynaecol* 1992;**99**:128–133.

Healy DL, Trounson AO, Nyboe Andersen A. Female infertility: causes and treatment. *Lancet* 1994;**343**:1539–1544.

Homburg R, Howles CM. Low-dose FSH therapy for anovulatory infertility associated with polycystic ovary syndrome: rationale, results, reflections and refinements. *Hum Reprod Update* 1999;**5**:493–499.

Imani B, Eijkemans MJC, te Velde ER, Habbema JDF, Fauser BCJM. Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotrophic oligomenorrheic infertility. *J Clin Endocrinol Metab* 1998;**83**:2361–2365.

Imani B, Eijkemans MJC, Faessen GH, Bouchard P, Giudice LC, Fauser BCJM. Prediction of the individual follicle-stimulating hormone threshold for gonadotrophin induction of ovulation in normogonadotropic anovulatory infertility: an approach to increase safety and efficiency. *Fertil Steril* 2002;**77**:83–90.

Landgrebe TCW, Duin RPW. Approximating the multiclass ROC by pairwise analysis. *Pattern Recognit Lett* 2007;**28**:1747–1758.

Lass A, Vassiliev A, Decosterd G, Warne D, Loumaye E. Relationship of baseline ovarian volume to ovarian response in World Health Organisation II anovulatory patients who underwent ovulation induction with gonadotropins. *Fertil Steril* 2002;**78**:265–269.

Platteau P, Nyboe Andersen A, Balen A, Devroey P, Sørensen P, Helmgaard L, Arce J-C, for the Menopur Ovulation Induction (MOI) Study Group. Similar ovulation rates, but different follicular development with highly purified menotrophin compared with recombinant FSH in WHO Group II anovulatory infertility: a randomized controlled study. *Hum Reprod* 2006;**21**:1798–1804.

Polson DW, Kiddy DS, Mason HD, Franks S. Induction of ovulation with clomiphene citrate in women with polycystic ovary syndrome: the difference between responders and non-responders. *Fertil Steril* 1989;**51**:30–34.

Steinkampf MP, Hammond KR, Nichols JE, Slayden SH. Effect of obesity on recombinant follicle-stimulating hormone absorption: subcutaneous versus intramuscular administration. *Fertil Steril* 2003;**80**:99–102.

van der Meer M, Hompes PGA, Scheele F, Schoute E, Veersema S, Schoemaker J. Follicle stimulating hormone (FSH) dynamics of low dose step-up ovulation induction with FSH in patients with polycystic ovary syndrome. *Hum Reprod* 1994;**9**:1612–1617.

van Santbrink EJP, Eijkemans MJC, Macklon NS, Fauser BCJM. FSH response-dose can be predicted in ovulation induction for normogonadotropic anovulatory infertility. *Eur J Endocrinol* 2002;**147**:223–226.

Waegeman W, De Baets B, Boullart L. A comparison of different ROC measures for ordinal regression. In: *Proceedings of the ICML Workshop on ROC Analysis in Machine Learning, Pittsburgh, PA, USA, 2006*.

Yarali H, Bukulmez O, Gurgan T. Urinary follicle-stimulating hormone (FSH) versus recombinant FSH in clomiphene citrate-resistant, normogonadotropic, chronic anovulation: a prospective randomized study. *Fertil Steril* 1999;**72**:276–281.

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