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A prospective study of predictive factors of ovarian response in 'standard' IVF/ICSI patients treated with recombinant FSH. A suggestion for a recombinant FSH dosage normogram

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BACKGROUND: The aim was to identify independent predictors of ovarian response to recombinant (r)FSH through a multiple regression analysis. METHODS: Prospective study including 145 'standard' patients treated with 150 IU/day of rFSH during their first IVF/ICSI cycle. Down-regulation was achieved with long agonist protocol. The following were examined as possible predictive factors: age, body mass index, cycle length, smoking status and on day 2–5: total ovarian volume, total number of antral follicles (<10 mm), total Doppler score of the ovarian stromal blood flow, serum FSH, LH, estradiol, inhibin B, and testosterone. RESULTS: Total number of antral follicles, total Doppler score, serum FSH, LH, estradiol, inhibin B, smoking status and cycle length were independent predictors of the number of aspirated follicles. The number of oocytes was predicted by the total number of antral follicles, total Doppler score, serum testosterone and smoking status. In bivariate linear regression analyses ovarian volume was a highly significant predictor of both the number of follicles ($P < 0.001$) and the number of oocytes ($P < 0.001$). CONCLUSIONS: Among 12 investigated possible predictive factors in 'standard' patients, the total number of antral follicles and ovarian stromal blood flow assessed by total Power Doppler score are the two most significant predictors of ovarian response. Suggestion for an rFSH dosage normogram is presented.

Key words: IVF-ICSI patients/rFSH dosage normogram/ovarian response/'standard' patients

Introduction

The optimal starting dose of recombinant (r)FSH during the first treatment cycle in IVF and ICSI remains controversial. The majority of fertility clinics have chosen a 'standard dose' for a 'standard patient'. A number of studies have attempted to define an optimal standard dose (Devroey *et al*., 1998; Out *et al*., 1999; 2000; 2001; The Latin-American Puregon IVF Study Group, 2001). The doses vary between 100 and 250 IU/day, reflecting the range of policies from 'friendly IVF' with a minimal dose, to an approach where a large number of oocytes is considered a criterion of success. Irrespective of the dose used there seems to be a wide range of responses ranging from one oocyte at retrieval to more than 30.

A 'standard patient' is <40 years of age, with two ovaries, a normal serum basal FSH and a regular menstrual cycle. The difficult clinical decision is on the dose to be used for the first week during the first treatment cycle, where the ovarian response to rFSH is basically unknown. From day 8 onwards the response is often evident and adjustments can be made. In subsequent cycles, having assessed the ovarian response in the first cycle, the dosage is far easier.

During recent years data has accumulated, showing that to some extent we are able to predict the ovarian response. The factors which have been investigated as possible predictors include age (Lee *et al*., 1988; Scott *et al*., 1989; Toner *et al*., 1991; Rosenwaks *et al*., 1995) ovarian volume (Lass *et al*., 1997; 1999; Tomas *et al*., 1997; Syrop *et al*., 1999) number of antral follicles (Chang *et al*., 1998; Ng *et al*., 2000; Kupesic *et al*., 2002), evaluation of ovarian stromal blood flow (Zaidi *et al*., 1996; Engmann *et al*., 1999; Kupesic *et al*., 2002), assessment of the hormonal markers such as serum FSH (Bancsi *et al*., 2000;) LH (Noci *et al*., 1998), estradiol (E_2) (Evers *et al*., 1998; Frattarelli *et al*., 2000) and inhibin B (Seifer

et al., 1997) as well as cigarette smoking (Van Voorhis *et al*., 1996; El-Nemr *et al*., 1998).

Although a large number of studies have been conducted in relation to ovarian response a number of methodological problems are encountered including sampling variability and clinical heterogeneity. So far trials have been conducted in relation to single (Scott *et al*., 1989; Lass *et al*., 1997; El-Nemr *et al*., 1998; Frattarelli *et al*., 2000) or combinations of a few predictive factors (Tomas *et al*., 1997; Syrop *et al*., 1999; Tinkanen *et al*., 1999, Ng *et al*., 2000). A wide range of sample sizes was present with many small studies (Engmann *et al*., 1999; Tinkanen *et al*., 1999; Kupesic *et al*., 2002).

There are a number of sources of clinical heterogeneity in the studies. Different starting FSH doses were used for controlled ovarian stimulation among the studies from 150 (Bancsi *et al*., 2002) to 375 IU/day (Engmann *et al*., 1999). Within the studies the starting FSH doses were adjusted for age and basal FSH, i.e. not all the patients received the same starting FSH dose. A starting dose of 150 IU/day was given to women <35 years, and 225 IU/day of FSH for older women (Lass *et al*., 1997). Both first and second stimulation cycles were included, i.e. the starting FSH dose in the second cycle being determined on the basis of the response to stimulation in the previous cycle. The starting doses from 150 to 375 were given depending on age, previous response to ovarian stimulation and basal FSH level (Engmann *et al*., 1999). In other studies women were treated with different stimulation protocols. Additionally, different gonadotrophin preparations were used between and even within the studies.

Among the large number of published studies on ovarian response prediction, only Bancsi *et al*. (2002), used a consistent methodology including only first cycle patients and administering the same starting FSH dose to all the patients.

The main purpose of the present prospective study was, in a well-defined group of 'standard' patients treated in the first treatment cycle, to examine all the possible predictors of ovarian response and to identify independent predictors through multiple regression analysis. The second aim was to use these findings combined with the data from the literature to suggest an rFSH normogram in order to implement the knowledge clinically.

Materials and methods

Between September 2000 and June 2001 we prospectively included 155 first IVF/ICSI treatment cycle patients. The inclusion criteria were: normal basal serum FSH level (with our assays up to 12.5 IU/l), presence of both ovaries, regular spontaneous menstrual cycle (21–35 days), maximum 39 years of age at the onset of treatment and no evidence of endocrine disorders. Exclusion criteria were the presence of ovarian cysts and inaccessible ovaries. The starting dose of rFSH during week 1 of treatment was 150 IU/day for all patients. We defined an appropriate ovarian response as retrieval of 5–14 oocytes, whereas an inadequate response included retrieval of four or less oocytes, and an excessive response was considered as retrieval of 15 or more oocytes.

Upon the onset of menstrual bleeding in a spontaneous cycle preceding GnRH-analogue treatment patients contacted the clinic. They were seen on day 2–5 when they received both oral and written information and clinical history was taken. Ovarian ultrasonography was performed using the Panther 2002 ADI (B-K Medical, Gentofte, Denmark) with a 6.5 mHz transvaginal probe (thermal index 1). Exposure time and acoustic output were kept at the lowest level. The number of antral follicles (<5 mm, and <10 mm) was counted. The maximum longitudinal (D1), antero-posterior (D2) and transverse (D3) diameters of each ovary were measured and ovarian volume calculated (D1 × D2 × D3 × 0.523). Ovarian stromal blood flow was evaluated by Power Doppler and a semi-quantitative score was allocated to each ovary according to the number and area of the Power Doppler signals. Score 1 (poor flow) was given in the presence of only a few and scanty signals, suggesting a poor vascularization. Score 3 (good flow) was given in the presence of several pronounced Power Doppler signals. A score of 2 (moderate flow) was allocated to those ovaries with intermediary findings. Total Doppler score (the sum of scores for each ovary) was analysed as a predictive factor, the values being 2, 3, 4, 5 and 6. Blood samples were drawn for assays of: E_2, FSH, LH, inhibin B and testosterone. Two investigators were present for each ultrasound examination and video recordings were made.

All patients were treated with the long protocol using nafarelin (Synarela®; Pharmacia, Copenhagen, Denmark) 600 µg/day under down-regulation beginning on day 21 of the cycle and with 400 µg/day from day 1 of rFSH stimulation until the day of hCG. The duration of down regulation was at least 14 days.

After pituitary desensitization was confirmed (i.e. no ovarian cysts, endometrial thickness <5mm) the number of antral follicles was counted (<5 mm, and <10 mm), ovarian volume measured and a Power Doppler score was again allocated for each ovary. Blood samples were taken for serum E_2, FSH, LH, and inhibin B. Controlled ovarian hyperstimulation (COH) was commenced with 150 IU/day of rFSH (Puregon®; Organon, Denmark) for the first week of treatment.

On day 8 of stimulation the response was assessed and if considered necessary the dose of rFSH was adjusted accordingly. The dose was increased if the leading follicles were <10–11 mm and in case of asynchrony i.e. >4 mm difference between the leading follicle and the next pool. The number of follicles >11, >13, >15 and >17 mm was noted and the ovarian volume was measured. Blood samples were drawn for E_2, FSH, LH, inhibin B. The same procedures were repeated on the day of/prior to the administration of hCG (10000 IU, Profasi®, Serono, Denmark).

For the purposes of the study we offered aspiration to all patients who had at least one follicle >17mm on day of/prior to hCG. Aspiration was performed 36 h following hCG administration. The number of follicles aspirated and oocytes retrieved was recorded during aspiration. Standard IVF and ICSI procedures were used. The embryos were transferred on day 2. Four cell embryos with <20 % fragmentation were considered as good quality embryos. All patients were treated with vaginal progesterone (Progestan®, Organon) 200 mg three times a day from the day of embryo transfer until hCG measurement 14 days later.

The study was approved by the regional Ethical Committee of Copenhagen Municipality (KF 01-133/00).

Hormone assays

Blood samples were drawn from antecubal vein and centrifuged. Serum was stored at –20 °C until analysis. Serum E_2 was measured by PANTEX $(E_2)^{125}I$ kit using the principles of radioimmunoassay. The sensitivity is 10 pg/ml; the intra-assay variation is 4.3%, and inter-assay variation 5.1%. Serum FSH and LH were measured by time-resolved immunofluorometric assay (DELFIA, Wallac, Inc., Turku, Finland), with detection limits of 0.06 and 0.05 IU/l respectively. Intra- and interassay coefficients of variation were both <8% in the FSH and LH assays. Serum inhibin B was measured by a

double-antibody enzyme immunometric assay using monoclonal antibody raised against the inhibin β_B subunit. The detection limit was 20 pg/ml. Intra- and interassay coefficients of variation were 15 and 18% respectively. Testosterone was determined by radio-immunoassay (Count-a-Count: Diagnostic Products, Los Angeles, CA, USA). The detection limit was 0.23nmol/l, and the intra- and inter-assay coefficients of variation were both <10%.

Statistical analysis

The primary end points were the number of aspirated follicles and oocytes retrieved.

Statistical analysis was performed using multiple regression analysis which was carried out in a backward stepwise manner. All of the predictive variables were entered into the model as independent variables to begin with: age, body mass index (BMI), cycle length, smoking status, day 2–5: total number of antral follicles, total ovarian volume, total power Doppler score, serum E_2, FSH, LH, inhibin B and testosterone, the dependent variable being the number of aspirated follicles and retrieved oocytes respectively. All the variables were continuous except for smoking, which was a binary variable (smoker -1, non-smoker -2). The variables were deleted in a backward procedure in order to determine which variables were independent and were needed for the model. Bivariate linear regression analyses were performed with the number of follicles and the number of oocytes as the dependent variables respectively. Significance level for both the multiple and linear regression analyses was 5% ($P < 0.05$). Statistical analysis of the data was performed with SPSS software (Statistical Package for Social Sciences) for Windows, version 10.0.

Results

In all, 155 patients were enrolled in the study. Following down-regulation 145 patients started COH. The reasons for dropping out of the study were: flare up ($n = 3$), spontaneous pregnancy ($n = 2$), personal ($n = 4$), inadequate down-regulation ($n = 1$). Aspiration was performed in 143 (98.6%) patients, the two cancellations were due to the risk of ovarian hyperstimulation syndrome (OHSS). Embryo transfer was carried out in 133 (91.7%) patients, lack of transfer was caused by: no fertilization ($n = 7$), poor quality embryos ($n = 2$), and risk of OHSS ($n = 1$). Three (2.1%) patients were hospitalized due to OHSS, all three were pregnant and delivered live offspring. Ninety-eight women (67.5%) had appropriate ovarian response i.e. had between 5 and 14 retrieved oocytes.

Table I shows the clinical and treatment cycle data. The patients' age ranged from 25 to 39 years. The age distribution was: 15.2% <30 years, 63.4% between 30 and 35 years of age and 21.4% of the women were >35 years of age. A total of 17.5% had a BMI <20 kg/m^2, 59.5% between 20 and 25, and 23% had a BMI >25 kg/m^2. Forty-seven women were smokers (32.4%) and 98 (67.6%) were non-smokers.

The mean increment in dose was 117 units per day (range 25–150 IU of rFSH). In the group where the dose was decreased on day 8 the mean decrement was 51 IU of rFSH per day (range 10–100). The two cancellations of aspiration due to risk of OHSS were among these latter women. Ongoing pregnancy rates per initiated cycle was 28.3% while the ongoing pregnancy rate per transfer was 30.8%.

Table I. Patient characteristics, treatment and outcome

	Study group $n = 145$
Demographics	
Age (mean ± SD) years	32.6 ± 3.3
BMI (mean ± SD) kg/m^2	23.3 ± 3.8
Cycle length (mean ± SD) days	28.6 ± 2
Causes of infertility[a]	
Tubal factor	43
Male factor	74
Idiopathic	30
Other	5
Treatment	
IVF	81
ICSI	64
Duration of stimulation (days)	
Mean ± SD	10.6 ± 1.4
Range	8–18
Total rFSH administered (IU)	
Mean ± SD	1738.6 ± 449.8
Range	1100–3150
Dose alteration on day 8	
Increase (%)	45 (31)
Decrease (%)	19 (13.1)
Unchanged (%)	81 (55.9)
Peak estradiol level (nmol/l)	
Mean ± SD	7.96 ± 4.62
Range	0.2–22.8
Outcome	
Number of aspirated follicles	
Mean ± SD	15.6 ± 7.2
Range	1–39
Number of retrieved oocytes	
Mean ± SD	11.22 ± 5.31
Range	0–27
No. of embryos transferred (mean, range)	1.9 (0–3)[b]
No. of cryopreserved embryos (mean, range)	2.3 (0–11)
% 4- cell cleavage embryos	61.4
Ongoing pregnancy (%)	41 (28.3)
Biochemical (%)	8 (5.5)
Ectopic (%)	1 (0.7)

[a]some patients had more than one cause of infertility.
[b]1 patient had a triple embryo transfer.

Table II gives the results of endocrine screening and ultrasound assessment of the ovaries on day 2–5 of the menstrual cycle preceding down-regulation.

Twelve predictor variables were entered into a multiple regression model with the dependent variable in the first analysis being the number of aspirated follicles followed by the number of retrieved oocytes in the second analysis.

The multiple regression analyses results showed that the number of follicles was predicted by: total number of antral follicles, total Doppler score, smoking status, cycle length, serum FSH, LH, E_2, testosterone and inhibin B levels. The model given in Table III accounts for 40% of the variability of the number of aspirated follicles ($R = 0.647$, $R^2 = 0.418$, adjusted $R^2 = 0.401$). The total number of retrieved oocytes was predicted by total number of antral follicles, total Doppler score, smoking status and serum testosterone level. The model in Table IV explained 38% of the variability of the number of retrieved oocytes ($R = 0.629$, $R^2 = 0.396$, adjusted $R^2 = 0.379$). The total number of antral follicles on the day of baseline ultrasound was the single most significant predictor of both the number of follicles aspirated ($P < 0.001$) and the number of oocytes retrieved ($P < 0.001$).

Table II. Day 2–5 ultrasound and endocrine parameters

	Mean ± SD range
Total number of antral follicles (<10 mm)[a]	21.5 ± 9.1 5–60
Total ovarian volume (ml)[a]	10.9 ± 3.8 3.0–25
Serum FSH (IU/l)	6.3 ± 1.6 2.2–12.00
Serum LH (IU/l)	4.4 ± 1.6 1.3–10.5
Serum estradiol (nmol/l)	0.2 ± 0.1 0–0.7
Serum Inhibin B (pg/ml)	124.5 ± 50.8 0–278
Serum testosterone (nmol/l)	0.9 ± 0.5 0–2.5
Total Doppler score (2–6)[a]	n (%)
2	3 (2.1)
3	9 (6.2)
4	26 (17.9)
5	50 (34.5)
6	57 (39.3)

[a]sum of both ovaries.

Table III. Significant predictors of number of aspirated follicles in backward stepwise regression analysis

Variable	Regression coefficient	Standard error	*P*-value
Total number of antral follicles	0.327	0.057	< 0.001
Total Doppler score	1.192	0.509	0.021
Smoking status	1.881	0.951	0.050
Cycle length	0.488	0.233	0.039
Serum FSH	–0.579[a]	0.283	0.043
Serum LH	0.665	0.299	0.028
E_2	–8.663[a]	4.199	0.041
Serum testosterone	1.965	0.969	0.045
Inhibin B	0.017	0.01	0.084

[a]–negative for inversely related variables.
Model accounts for 40% variability of the number of aspirated follicles.

Table IV. Significant predictors of number of retrieved oocytes in backward stepwise regression analysis

Variable	Regression coefficient	Standard error	*P*-value
Total number of antral follicles	0.249	0.044	< 0.001
Total Doppler score	1.295	0.396	0.001
Smoking status	1.840	0.748	0.015
Serum testosterone	1.457	0.769	0.060

Model accounts for 38% variability of the number of retrieved oocytes.

Smoking status was a binary variable coded 1 for smokers and 2 for non-smokers. The results of the prediction model suggested that non-smokers had a larger number of follicles aspirated and oocytes retrieved than the smokers.

Bivariate linear regression was performed with the ovarian volume as the independent variable since it was neither in the model with the number of follicles nor the number of oocytes as dependent variables, respectively (Tables V and VI). Ovarian volume in bivariate linear regression was a significant predictor of both the number of follicles ($P < 0.001$) and the number of oocytes ($P < 0.001$). It accounted for the 21% of the variability of the number of follicles ($R = 0.42$, adjusted $R^2 = 0.21$) and for 14% of the variability of the number of oocytes ($R = 0.38$, adjusted $R^2 = 0.14$). Ovarian volume was significantly correlated to both the number of antral follicles ($r = 0.60$, $P < 0.01$) and the total Doppler score ($r = 0.42$, $P < 0.01$). This could in turn explain its absence from the multiple regression models for prediction of both the number of follicles and oocytes, i.e. ovarian volume being the confounding variable.

Although neither the inhibin B ($P = 0.084$) in the follicle model (Table III), nor the testosterone ($P = 0.06$) in the oocyte model (Table IV) reached the significance level of 5%, presence of the variables in the respective models increases the accountancy of the models for variability of the two dependent variables.

Suggestion for a dosage normogram

Based on our study we would like to make a suggestion for a simple 'bed-side' FSH dosage score (Table VII) based on the significant parameters of ovarian sonography (antral follicle count, ovarian volume and Power Doppler score) and also on clinical data (age and smoking habits) but not on endocrine tests. The purpose of a normogram would be to prescribe a dose of FSH that was associated with an appropriate number of retrieved oocytes, arbitrarily defined between 5 and 14. The cut-off values for each variable in the normogram were drawn from our own study population and were defined in relation to our definition of appropriate ovarian response.

The overall scores for the FSH dosages are based on following assumptions. The optimal FSH dose given in order to achieve an appropriate oocyte yield is 150 IU/day in a non-smoking woman, 30–35 years old, with an average number of antral follicles, an average ovarian volume, and average Doppler score. The optimal FSH dose given in order to achieve an appropriate oocyte yield is 100 IU/day in a non-smoking woman, <30 years of age, with large ovaries, many antral follicles and a high Doppler score. The optimal FSH dose given in order to achieve an appropriate oocyte yield is 250 IU/day in a smoking woman, >35 years of age with few antral follicles, small ovaries and a low Doppler score.

The normogram was constructed with the intention of achieving these dosages. The weight of each parameter was based on the most significant and clinically well-established variables (antral follicle count and ovarian volume) given the highest scores, whereas the less significant variables were given the lowest scores. The Doppler score was attributed a score in between. We opted to give it a lower score as this has only been shown in the present study.

It has to be stressed that this FSH dosage normogram is a construction based on scientifically documented variables but additionally on clinical assumptions.

Discussion

Our study confirms and extends earlier studies by showing that the following single factors may predict the number of retrieved oocytes: age, cycle length, smoking status, serum FSH, LH, inhibin B level, total ovarian volume, total number of

Table V. Significant predictors of the number of aspirated follicles in bivariate linear regression

	Model summary		Unstandardized coefficients		Standardized coefficient	*t*	*P*-value
	R	Adjusted R^2	B	SE	Beta		
Age	0.184	0.027	–0.400[a]	0.180	–0.184	–2.221	0.028
Cycle length	0.318	0.095	1.153	0.289	0.318	3.987	< 0.001
Smoking status	0.231	0.047	3.548	1.257	0.231	2.822	0.005
Serum FSH	0.192	0.030	–0.848 [a]	0.364	–0.192	–2.329	0.021
Serum LH	0.256	0.059	1.186	0.378	0.256	3.138	0.002
Testosterone	0.186	0.028	2.941	1.308	0.186	2.248	0.026
Inhibin B	0.191	0.030	0.028	0.012	0.191	2.313	0.022
Ovarian volume	0.462	0.207	0.880	0.142	0.462	6.177	< 0.001
Total number of antral follicles	0.615	0.374	0.491	0.053	0.615	9.265	< 0.001
Total Doppler score	0.469	0.215	3.341	0.529	0.469	6.312	< 0.001

[a] –negative for inversely related variables
B = regression coefficient; SE = standard error; *t*=B/SE

Table VI. Significant predictors of the number of retrieved oocytes in bivariate linear regression

	Model Summary		Unstandardized coefficients		Standardized coefficient	*t*	*P*–value
	R	Adjusted R^2	B	SE	Beta		
Age	0.182	0.026	–0.303[a]	0.138	–0.182	–2.197	0.030
Cycle length	0.244	0.053	0.677	0.226	0.244	2.988	0.003
Smoking status	0.226	0.044	2.652	0.963	2.226	2.753	0.007
Serum FSH	0.188	0.029	–0.635*	0.279	–0.188	–2.277	0.024
Serum LH	0.174	0.023	0.618	0.295	0.174	2.099	0.038
Inhibin B	0.195	0.031	0.021	0.009	0.195	2.358	0.020
Ovarian volume	0.376	0.136	0.549	0.114	0.376	4.825	< 0.001
Total number of antral follicles	0.554	0.302	0.338	0.043	0.554	7.898	< 0.001
Total Doppler score	0.476	0.221	2.594	0.403	0.476	6.429	< 0.001

[a]–negative for inversely related variables.
B = regression coefficient; SE = standard error; *t* = B/SE

Table VII. rFSH dosage normogram

Total number of antral follicles <10 mm day 2–5	FSH score IU/day	rFSH starting dose
<15	90	
15–25	60	
>25	50	
Total ovarian volume day 2–5		Score
<9 ml	90	
9–13 ml	60	
>13 ml	50	
Total Doppler score day 2–5		Score
2–3	30	
4	20	
5	10	
6	0	
Age (years)		Score
>35	20	
>30 – ⩽ 35	10	
⩽ 30	0	
Smoking habits cigarettes/day		Score
>10	20	
⩽10	10	
Non smoker	0	
Total FSH score (sum of scores) same as dose IU/day		

antral follicles and total Doppler score. However, using multiple regression analysis only the total number of antral follicles, total Doppler score, smoking status and serum testosterone level predicted the number of oocytes retrieved. The final model that predicted the number of follicles additionally included cycle length, serum FSH, LH, E_2 and inhibin B. No definite explanation can be given for the difference in the independent predictors of the number of follicles and oocytes. Differences in the oocyte retrieval techniques could account for this phenomenon. The number of follicles is more likely to reflect the true biology of the ovaries but on the other hand the number of oocytes is clinically more important.

Our independent predictors of the number of oocytes as assessed by the multiple regression analysis could account for 38% of the variation of ovarian response in comparison with 25% in the study by Tinkanen *et al*. (1999) and 27% in the work by Ng *et al*. (2000).

The total number of antral follicles on day 2–5 of a spontaneous cycle was an independent predictor of both the number of follicles and oocytes. This finding is in agreement with the results of a number of investigations (Chang *et al*., 1998; Bancsi *et al*., 2002).

With the improvements of the ultrasound equipment colour Doppler sonography (Zaidi *et al*., 1996; Engmann *et al*., 1999) and Power Doppler (Kupesic *et al*., 2002) have been used to assess ovarian circulation in order to predict the ovarian response to COH. While the frequency-based colour Doppler sonography provides directional information of the flow within the vessel, Power Doppler sonography is monochromatic, it

does not provide directional information but its sensitivity to low flow makes it more useful in studying ovarian perfusion. It is also less angle-dependent and not susceptible to aliasing (Meyerowitz *et al*., 1996; Pairleitner *et al*., 1999).

In the present study we assessed ovarian blood flow using a semi-quantitative Power Doppler score (2D). We showed that the total power Doppler score was an independent predictor of both the number of follicles and oocytes. This is a new finding. The technique is easy to perform and it is not time consuming. It is however difficult to make strict criteria for quantification. The 3D Power Doppler indices (Pairleitner *et al*, 1999; Kupesic *et al*., 2002) may help to overcome this problem, although it is fair to say that presently they have not gained wide acceptance in clinical use.

Interesting and new data emerged from our analyses regarding the cycle length. Within the regularly cycling women study population (cycle length 21–35 days), it was an independent predictor of the number of aspirated follicles but not the retrieved oocytes. Women with short cycles had less aspirated follicles compared with women with longer cycles. This is in agreement with the fact that there is a gradual decline in cycle length with the ovarian ageing as shown by Treolar *et al*. (1967) and Münster *et al*. (1992) in a large cohort of regularly cycling women.

Smoking has been associated with an earlier menopause and impaired ovarian function by a number of large epidemiological studies (Jick *et al*., 1977; Adena *et al*., 1982; McKinlay *et al*., 1985). The validity of self-reporting smoking is often questioned but a meta-analysis of published studies comparing self-reported smoking status with biochemical validation suggested generally high levels of sensitivity (87%) and specificity (89%) for self-report (Patrick *et al*., 1994). Our data have confirmed that actual smoking status is a predictor of both the number of follicles and the number of oocytes.

Age was a significant predictor of both the number of follicles ($P = 0.03$) and the number of oocytes ($P = 0.03$) in bivariate linear regression analyses. Interestingly, however, age was not an independent predictor in our study. This is in contrast with other studies (Ng *et al*., 2000) but may be explained by the inclusion of several other factors that are related to the biological age of the ovaries.

The importance of basal FSH levels remains controversial although it is well accepted that the patients with elevated basal FSH level exhibit diminished ovarian responsiveness. Our study shows that even in patients with normal basal FSH levels, FSH levels and the number of aspirated follicles were inversely related. Similar findings regard the basal E_2 levels as shown by Frattarelli *et al*. (2000) where patients with elevated E_2 levels were more likely to respond poorly to gonadotrophins. Although inhibin B was an independent predictor of the number of follicles, it accounted for only 3% of the variability of both the number of follicles and oocytes when analysed separately in bivariate linear regressions. The fact that our patient population was restricted to women with normal basal FSH levels, may account for this observation.

How do we implement the knowledge we have on predictive factors in clinical practice?

The problem is that 'standard' patients treated with 'standard' doses frequently do not exhibit 'standard' responses. The question is how, and to what extent, it is possible to avoid the inappropriate responses by administering appropriate dose of rFSH. The variability of the responses may be due to inherent biological mechanisms in relation to differences in the number of recruitable follicles, follicle sensitivity to rFSH and pharmacodynamics. On the other hand they may also be due to factors that may be predicted and at least partly controlled.

On the basis of our data but also taking into account clinical experience and safety considerations we would like to suggest an rFSH dosage normogram composed of the following variables: total number of antral follicles day 2–5, total Doppler score day 2–5, total ovarian volume day 2–5, age and smoking status (Table VII). This normogram is based on clinical and ultrasound data and doesn't include endocrine parameters.

Several suggestions for a normogram could be made. Our intention was to design a simple normogram, which would allow an easy individual approach to the determination of rFSH starting dose by taking a history and ultrasonography. The primary focus in the construction of the normogram was the number of oocytes and we therefore included the variables from the oocyte model.

Volume of the ovaries is an indirect marker of the ovarian responsiveness. The number of follicles was a better predictor than the ovarian volume and it may be used as a first method for predicting the ovarian response to gonadotrophins (Tomas *et al*., 1997). This is consistent with our findings as the number of antral follicles on day 2–5 independently predicted the number of both the follicles and oocytes, whereas the volume did not. However, ovarian volumetry by transvaginal sonography is accurate and easily performed in most women with very small intra- and interobserver variations (Goswamy *et al*., 1988; Higgins *et al*., 1990; Lass *et al*., 1997). In the situation where the quality of the ultrasound image may be impaired by the ovarian localization, obesity or other factors estimation of the antral follicle number may prove to be a difficult task especially the very small antral follicles with 2–3 mm in diameter. Ovarian volume could thus be considered as a safety variable.

Although age did not prove to be an independent predictor in the multiple regression it was significantly correlated to both the number of oocytes and follicles in the bivariate regressions. We have included age in the dosage normogram because evidence has accumulated over the years, which has shown that fertility declines with age (Lee *et al*., 1988; Scott *et al*., 1989; Toner *et al*., 1991; Faddy and Gosden, 1995; Rosenwaks *et al*., 1995).

One might criticise the normogram for not including any endocrine parameters although we have them in the model for both oocytes and follicles (Tables III and IV). Hormonal determinations may have inter-cycle variability, are expensive and are not commonly used in 'standard' patients, at least in Scandinavia.

The rFSH dosage normogram is easy to use in a clinical setting. To illustrate the use of the normogram a 36-year-old woman, non-smoker, with 10 antral follicles, ovarian volume

of 8 ml, and a Doppler score 4 should be given 220 IU/day of rFSH (20 + 0 + 90 + 90 + 20). A 28 year old woman, smoking 5 cigarettes per day, with 30 antral follicles, ovarian volume of 11ml and Doppler score 6 should be given 120 IU/day of rFSH (0 + 10 + 50 + 60 + 0).

The normogram has not been experimentally tested but a randomized trial comparing a standard dose of 150 IU/day of rFSH versus a normogram defined individual dose is presently under way.

References

Adena, M.A. and Gallagher, H.G. (1982) Cigarette smoking and the age at menopause. *Ann. Hum. Biol.*, **9**, 121–130.

Bancsi, L.F, Huijs, A.M., den Ouden, C.T., Broekmans, F.J., Looman, C.W., Blankenstein, M.A. and te Velde, E.R. (2000) Basal follicle-stimulating hormone levels are of limited value in predicting ongoing pregnancy rates after in vitro fertilization. *Fertil. Steril.*, **73**, 552–557.

Bancsi, L.F., Broekmans, F.J., Eijkemans, M.J., de Jong, F.H., Habbema, J.D. and te Velde, E.R. (2002) Predictors of poor ovarian response in in vitro fertilization: a prospective study comparing basal markers of ovarian reserve. *Fertil. Steril.*, **77**, 328–336.

Chang, M.Y., Chiang, C.H., Hsieh, T.T., Soong, Y.K. and Hsu, K.H. (1998) Use of the antral follicle count to predict the outcome of assisted reproductive technologies. *Fertil. Steril.*, **69**, 505–510.

Devroey, P., Tournaye, H., Van Steirteghem, A., Hendrix, P. and Out, H.J. (1998) The use of a 100 IU starting dose of recombinant follicle stimulating hormone (Puregon) in in-vitro fertilization. *Hum. Reprod.*, **13**, 565–566.

El-Nemr, A., Al-Shawaf, T., Sabatini, L., Wilson, C., Lower, A.M. and Grudzinskas, J.G. (1998) Effect of smoking on ovarian reserve and ovarian stimulation in in-vitro fertilization and embryo transfer. *Hum. Reprod.*, **13**, 2192–2198.

Engmann, L., Sladkevicius, P., Agrawal, R., Bekir, J.S., Campbell, S. and Tan, S.L. (1999) Value of ovarian stromal blood flow velocity measurement after pituitary suppression in the prediction of ovarian responsiveness and outcome of in vitro fertilization treatment. *Fertil. Steril.*, **71**, 22–29.

Evers, J.L., Slaats, P., Land, J.A., Dumoulin, J.C. and Dunselman, G.A. (1998) Elevated levels of basal estradiol-17beta predict poor response in patients with normal basal levels of follicle-stimulating hormone undergoing in vitro fertilization. *Fertil. Steril.*, **69**, 1010–1014.

Faddy, M.J. and Gosden, R.G. (1995) A mathematical model of follicle dynamics in the human ovary. *Hum. Reprod.*, **10**, 770–775.

Frattarelli, J.L., Bergh, P.A., Drews, M.R., Sharara, F.I. and Scott, R.T. (2000) Evaluation of basal estradiol levels in assisted reproductive technology cycles. *Fertil. Steril.*, **74**, 518–524.

Goswamy, R.K., Campbell, S., Royston, J.P., Bhan, V., Battersby, R.H., Hall, V.J., Whitehead, M.I. and Collins, W.P. (1988) Ovarian size in postmenopausal women. *Br. J. Obstet. Gynaecol.*, **95**, 795–801.

Higgins, R.V., van Nagell, J.R. Jr., Woods, C.H., Thompson, E.A. and Kryscio, R.J. (1990) Interobserver variation in ovarian measurements using transvaginal sonography. *Gynecol. Oncol.*, **39**, 69–71.

Jick, H. and Porter, J. (1977) Relation between smoking and age of natural menopause. Report from the Boston Collaborative Drug Surveillance Program, Boston University Medical Center. *Lancet*, **1**, 1354–1355.

Kupesic, S. and Kurjak, A. (2002) Predictors of IVF outcome by three-dimensional ultrasound. *Hum. Reprod.*, **17**, 950–955.

Lass, A. and Brinsden, P. (1999) The role of ovarian volume in reproductive medicine. *Hum. Reprod. Update*., **5**, 256–266.

Lass, A., Skull, J., McVeigh, E., Margara, R. and Winston, R.M. (1997) Measurement of ovarian volume by transvaginal sonography before ovulation induction with human menopausal gonadotrophin for in-vitro fertilization can predict poor response. *Hum. Reprod.*, **12**, 294–297.

Latin-American Puregon IVF Study Group (2001) A double-blind clinical trial comparing a fixed daily dose of 150 and 250 IU of recombinant follicle-stimulating hormone in women undergoing in vitro fertilization. *Fertil. Steril.*, **76**, 950–956.

Lee, S.J., Lenton, E.A., Sexton, L. and Cooke, I.D. (1988) The effect of age on the cyclical patterns of plasma LH, FSH, oestradiol and progesterone in women with regular menstrual cycles. *Hum. Reprod.*, **3**, 851–855.

McKinlay, S.M., Bifano, N.L. and McKinlay, J.B. (1985) Smoking and age at menopause in women. *Ann. Intern. Med.*, **103**, 350–356.

Meyerowitz, C.B., Fleischer, A.C., Pickens, D.R., Thurman, G.B., Borowsky, A.D., Thirsk, G. and Hellerqvist, C.G. (1996) Quantification of tumor vascularity and flow with amplitude color Doppler sonography in an experimental model: preliminary results. *J. Ultrasound. Med.*, **15**, 827–833.

Münster, K., Schmidt, L. and Helm, P. (1992) Length and variation in the menstrual cycle—a cross-sectional study from a Danish county. *Br. J. Obstet. Gynaecol.*, **99**, 422–429.

Noci, I., Biagiotti, R., Maggi, M., Ricci, F., Cinotti, A. and Scarselli, G. (1998) Low day 3 luteinizing hormone values are predictive of reduced response to ovarian stimulation. *Hum. Reprod.*, **13**, 531–534.

Ng, E.H., Tang, O.S. and Ho, P.C. (2000) The significance of the number of antral follicles prior to stimulation in predicting ovarian responses in an IVF programme. *Hum. Reprod.*, **15**, 1937–1942.

Out, H.J., Lindenberg, S., Mikkelsen. A.L., Eldar-Geva, T., Healy, D.L., Leader, A., Rodriguez-Escudero, F.J., Garcia-Velasco, J.A. and Pellicer, A. (1999) A prospective, randomized, double-blind clinical trial to study the efficacy and efficiency of a fixed dose of recombinant follicle stimulating hormone (Puregon) in women undergoing ovarian stimulation. *Hum. Reprod.*, **14**, 622–627.

Out, H.J., Braat, D.D., Lintsen, B.M., Gurgan, T., Bukulmez, O., Gokmen, O., Keles, G., Caballero, P., Gonzalez, J.M. and Fabregues, F., *et al*. (2000) Increasing the daily dose of recombinant follicle stimulating hormone (Puregon) does not compensate for the age-related decline in retrievable oocytes after ovarian stimulation. *Hum. Reprod.*, **15**, 29–35.

Out, H.J., David, I., Ron-El, R., Friedler, S., Shalev, E., Geslevich, J., Dor, J., Shulman, A., Ben-Rafael, Z., Fisch, B., *et al*. (2001) A randomized, double-blind clinical trial using fixed daily doses of 100 or 200 IU of recombinant FSH in ICSI cycles. *Hum. Reprod.*, **16**,1104–1109.

Pairleitner, H., Steiner, H., Hasenoehrl, G. and Staudach, A. (1999) Three-dimensional power Doppler sonography: imaging and quantifying blood flow and vascularization. *Ultrasound Obstet. Gynecol.*, **14**, 139–143.

Patrick, D.L., Cheadle, A., Thompson, D.C., Diehr, P., Koepsell, T. and Kinne, S. (1994) The validity of self-reported smoking: a review and meta-analysis. *Am. J. Public Health*, **84**, 1086–1093.

Rosenwaks, Z., Davis, O.K. and Damario, M.A. (1995) The role of maternal age in assisted reproduction. *Hum. Reprod.*, **10**, 165–173.

Scott, R.T., Toner, J.P., Muasher, S.J., Oehninger, S., Robinson, S. and Rosenwaks, Z. (1989) Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome. *Fertil. Steril.*, **51**, 651–654.

Seifer, D.B., Lambert-Messerlian, G., Hogan, J.W., Gardiner, A.C., Blazar, A.S. and Berk, C.A. (1997) Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. *Fertil. Steril.*, **67**, 110–114.

Syrop, C.H., Dawson, J.D., Husman, K.J., Sparks, A.E. and Van Voorhis, B.J. (1999) Ovarian volume may predict assisted reproductive outcomes better than follicle stimulating hormone concentration on day 3. *Hum. Reprod.*, **14**, 1752–1756.

Tinkanen, H., Blauer, M., Laippala, P., Tuohimaa, P. and Kujansuu, E. (1999) Prognostic factors in controlled ovarian hyperstimulation. *Fertil. Steril.*, **72**, 932–936.

Tomas, C., Nuojua-Huttunen, S. and Martikainen, H. (1997) Pretreatment transvaginal ultrasound examination predicts ovarian responsiveness to gonadotrophins in in-vitro fertilization. *Hum. Reprod.*, **12**, 220–223.

Toner, J.P., Philput, C.B., Jones, G.S. and Muasher, S.J. (1991) Basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. *Fertil. Steril.*, **55**, 784–791.

Treolar, A.E., Boynton, R.E., Behn, B.G. and Brown, B.W. (1967) Variation of the human menstrual cycle through reproductive life. *Int. J. Fertil.*, **12**, 77-120.

Van Voorhis, B.J., Dawson, J.D., Stovall, D.W., Sparks, A.E. and Syrop, C.H. (1996) The effects of smoking on ovarian function and fertility during assisted reproduction cycles. *Obstet. Gynecol.*, **88**, 785–791.

Zaidi, J., Barber, J., Kyei-Mensah, A., Bekir, J., Campbell, S. and Tan, S.L. (1996) Relationship of ovarian stromal blood flow at the baseline ultrasound scan to subsequent follicular response in an in vitro fertilization program. *Obstet. Gynecol.*, **6**, 779–784.

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