Antral follicle count and FSH concentration after clomiphene citrate challenge test in the prediction of ovarian response during IVF treatment

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BACKGROUND: We compared: (i) antral follicle count (AFC) in the early follicular phase, after the clomiphene citrate challenge test (CCCT) and before ovarian stimulation following pituitary down-regulation; and (ii) age of women, body mass index, basal and stimulated serum FSH concentrations and AFC in predicting the ovarian response of infertile women aged <40 years with basal FSH <10 IU/l on recruitment in their first IVF cycle.

METHODS: Two months prior to the treatment cycle, AFC and basal FSH concentration were determined on day 2–3 of a spontaneous period and on day 10 after CCCT. All women received a standard stimulation regimen. Ovarian response was represented by the number of oocytes, serum estradiol, the duration and dosage of gonadotrophins.

RESULTS: There was no significant difference between basal, stimulated and down-regulated AFC. AFC achieved the best predictive value in relation to the number of oocytes, followed by combined FSH concentration (sum of the two FSH concentrations) and age of women. Both basal AFC and combined FSH concentration were predictive factors of serum estradiol concentration, whereas stimulated FSH concentration was predictive of the total dosage of gonadotrophins.

CONCLUSION: Combined FSH concentration after CCCT provides additional information in predicting ovarian response.

Key words: antral follicle count/clomiphene citrate challenge test/FSH/ovarian response

Introduction

Recruitment and development of multiple follicles in response to gonadotrophin stimulation are the key factors leading to a successful outcome of the IVF treatment. Poor ovarian response may be associated with poor pregnancy rates and many of these cycles are cancelled without proceeding to oocyte retrieval (Keay et al., 1997; Tarlatzis et al., 2003). On the other hand, exaggerated ovarian response leads to an increased risk of ovarian hyperstimulation syndrome (OHSS) (Aboulghar and Mansour, 2003) and the resulting high serum estradiol (E2) concentrations may adversely affect the outcomes of the IVF treatment (Ng et al., 2000a).

Prediction of ovarian responses prior to stimulation is useful in counselling patients and may be helpful in tailoring the dosage of gonadotrophin to individual patients. Different hormonal and ultrasound markers for ovarian reserve have been examined to predict the ovarian response to gonadotrophins, including early follicular serum FSH (Scott and Hofmann et al., 1995; Sharara et al., 1998), serum inhibin B (Seifer et al., 1997; Tinkanen et al., 1999; Dzik et al., 2000), serum anti-Müllerian hormone (Seifer et al., 2002; van Rooij et al., 2002; Fanchin et al., 2003), ovarian volume (Syrop et al., 1995, 1999; Lass et al., 1997), antral follicle count (AFC) (Tomàs et al., 1997; Chang et al., 1998; Frattarelli et al., 2000; Ng et al., 2000b; Hsieh et al., 2001; Nahum et al., 2001; Bancsi et al., 2002) and ovarian stromal blood flow (Zaidi et al., 1996; Engmann et al., 1999; Kupesic and Kurjak, 2002; Kupesic et al., 2003; Popovic-Todorovic et al., 2003).

Early follicular FSH concentration is widely used in many IVF units to predict the ovarian response and is a better predictor of ovarian response than the age of women (Cahill et al., 1994; Sharif et al., 1998). In order to identify a greater number of women with decreased ovarian reserve, challenging the pituitary gland may be more likely to uncover an abnormality that would be missed by obtaining basal FSH concentration alone. Navot et al. (1987) first described the clomiphene citrate challenge test (CCCT), which consisted of measuring serum FSH concentrations on cycle day 3 (basal FSH) and then again on day 10 (stimulated FSH) after the administration of 100 mg of clomiphene citrate (CC) from day 5 to day 9. Subsequently, abnormal CCCT has been shown to be predictive of poor ovarian response, cycle cancellation and reduced pregnancy rate (Loumaye et al., 1990;
The significance of AFC in predicting ovarian response and pregnancy rate of IVF treatment has been demonstrated in many studies. We previously demonstrated that AFC achieved the best predictive value, followed by basal FSH concentration, body mass index (BMI) and age of women in a prospective study of 128 infertile patients undergoing the first IVF cycle using a standard regimen of ovarian stimulation (Ng et al., 2000b). In the majority of the above studies, AFC was determined after pituitary down-regulation and prior to ovarian stimulation in the treatment cycle. Bancsi et al. (2002) performed ultrasound assessment on day 3 of a spontaneous cycle prior to the IVF treatment. As the use of CC stimulates the growth of these antral follicles, perhaps it may be more accurate to determine AFC after CCCT. Moreover, there are very few studies in the literature comparing stimulated FSH concentration and AFC in relation to the prediction of ovarian responsiveness.

The objectives of this prospective study were to: (i) compare AFC in the early follicular phase, after CCCT and before ovarian stimulation following pituitary down-regulation, and (ii) compare age, BMI, basal and stimulated serum FSH concentrations and AFC in predicting the ovarian response of infertile women undergoing the first IVF cycle using a standard regimen of ovarian stimulation.

Materials and methods

Consecutive women attending the Department of Obstetrics and Gynaecology, University of Hong Kong between January 2002 and November 2003 for their first IVF treatment were recruited. Women who were aged ≥40 years at the time of treatment or whose basal FSH concentration on repeated testing on recruitment was ≥10 IU/l were advised against the IVF treatment according to the recruitment guideline issued by the Hospital Authority in Hong Kong. The current waiting time for IVF treatment was ~1 year after recruitment. The indications of conventional IVF treatment included tubal, male, endometriosis, unexplained and mixed factors. ICSI was performed for couples with severe semen abnormalities (<100,000 motile sperm recovered after sperm preparation) and surgically retrieved sperm from epididymis or testis in cases of obstructive azospermia. Poor visualization of ovaries because of abdominal position, presence of an ovarian cyst of ≥20 mm in diameter and the presence of polycystic ovaries on scanning (Adams et al., 1986) were excluded. Every woman gave a written informed consent prior to participating in the study, which was approved by the Ethics Committee, Faculty of Medicine, University of Hong Kong. They received no monetary compensation for participation in the study. The results of CCCT did not affect their IVF treatment.

Two months prior to the treatment cycle, they attended the clinic for a transvaginal ultrasound examination for AFC and a blood test for basal FSH concentration in the early follicular phase (days 2–4) of the cycle. They were asked to take clomiphene citrate (Clomid; Merrell, UK) 100 mg daily from day 5 to day 9. Transvaginal scanning for AFC and the blood test for FSH concentration were repeated on day 10.

The details of the long protocol of ovarian stimulation regimen at our centre have been previously published (Ng et al., 2000a). All women were pre-treated with buserelin (Suprecur; Hoechst, Germany) nasal spray 150 mg four times a day from the mid-luteal phase of the cycle preceding the treatment cycle. On the second day of the treatment cycle, transvaginal scanning was performed to determine AFC and blood was then taken for basal serum E2 concentration. When the ultrasound scanning showed no ovarian cyst and serum E2 concentrations were <200 pmol/l, hMG (Pergonal; Serono, Switzerland) injections were started at 300 IU daily for the first 2 days followed by 150 IU daily afterwards. The ovarian response was monitored by serial transvaginal scanning and the hMG dosage was increased if there was no follicle ≥10 mm after 7 days of stimulation. hCG (Profasi; Serono, Switzerland) was given i.m. when the leading follicle reached 18 mm in diameter and there were at least three follicles of ≥16 mm in diameter. Serum E2 concentration was measured on the day of hCG administration. Cycles were cancelled when the follicles remained <10 mm after 14 days of stimulation. Oocyte retrieval would be performed even when there was only one dominant follicle and was scheduled 36 h after the hCG injection and any visible follicles were aspirated during the procedure.

A maximum of three normally cleaved embryos was replaced into the uterine cavity 48 h after the retrieval and excess good quality embryos were frozen. All fresh embryos were cryopreserved if serum E2 on the day of hCG injection was >20,000 pmol/l in order to reduce the risks of ovarian hyperstimulation syndrome. Luteal phase was supported by two doses of hCG. A urine pregnancy test was done 16 days after embryo transfer; if positive, ultrasound examination was performed 10–14 days later to confirm intrauterine pregnancy and to determine the number of gestation sacs present. Only clinical pregnancies were considered and were defined by the presence of one or more gestation sacs or the histological confirmation of gestational product in miscarriages. Ongoing pregnancies were those pregnancies beyond 10–12 weeks of gestation, at which stage the patients were referred out for antenatal care.

AFC was determined during transvaginal scanning performed at 08:00–10:00 by EHYN (Ng et al., 2000b) using a 6.5 MHz vaginal probe (Aloka, Model SSD-5500; Aloka Co. Ltd, Japan). The intra-observer coefficient of variation (CV) for AFC was 7%. AFC in the early follicular phase of a spontaneous cycle, day 10 after taking clomiphene citrate and before ovarian stimulation following pituitary down-regulation, were termed as basal, stimulated and down-regulated respectively.

Serum FSH and E2 concentrations were measured using commercially available kits (Automated Chemiluminescence ACS-180 System; Bayer Corporation, USA). The FSH assay is standardized against the World Health Organization 2nd International Standard 94/632 reference material. The sensitivity of the FSH assay was 0.3 pmol/l and the intra- and inter-assay CV were 2.8 and 4.6% respectively. The sensitivity of the E2 assay was 36.7 pmol/l and the intra- and inter-assay CV were 8.1 and 8.7% respectively. FSH concentration on day 2 and day 10 were added together to give combined FSH concentration. CCCT was considered to be abnormal when either basal or stimulated FSH concentration was >10 IU/l.

Statistical analysis

The correlation coefficient between AFC and the number of oocytes obtained in the previous study (Ng et al., 2000b) was 0.36. Assuming that AFC and combined FSH concentration had similar correlation coefficients, the sample size required would be 107 to give a test significance of 0.01 and a power of 0.9 (Sigmastat; Jandel Scientific, USA).
The primary outcome measure was the number of oocytes obtained and the secondary outcome measures included the serum $E_2$ concentration on the day of hCG, the dosage and duration of hMG. Statistical tests were carried out by Friedman, Mann–Whitney U-tests, $\chi^2$ and Fisher’s exact tests, where appropriate. Multiple regression analysis with the least-squares regression was applied to evaluate the predictive values of different parameters on the ovarian response. Correlation was assessed by the Spearman rank method. Two-tailed $P < 0.05$ was taken as significant.

Results
A total of 300 eligible women undergoing the first IVF cycle during the study period was approached and 213 women agreed to participate in the study. After the first ultrasound examination, 68 women were excluded from the study: poor visualization of ovaries in 14 women, an ovarian cyst in 28 women and polycystic ovaries in 26 women. Five women did not return for the assessment on day 10 because they forgot to take clomiphene citrate. Two women became pregnant after taking clomiphene citrate and five women postponed their IVF treatment for personal reasons. Therefore, 131 women underwent ovarian stimulation and were included in the final analysis: 38 tubal factors; 15 endometriosis; 64 male infertility; eight unexplained and six mixed causes. Table I summarizes the demographic data and ovarian response. Four cycles did not proceed to oocyte retrieval because of premature luteinization in two cycles and absent follicular development in another two cycles. These patients were considered to have no oocyte obtained and oocytes were obtained in all planned retrievals. Failed fertilization was encountered in four cycles and in another three cycles embryos failed to cleave. Embryo transfer was postponed in three cycles because of the risk of OHSS. Embryo transfer was performed in 117 cycles and 31 clinical pregnancies resulted. The pregnancy rate was 23.7% per initiated cycle and 26.5% per transfer.

A paired analysis revealed no significant difference between basal (median: 11.0; range: 1–21), stimulated (median: 12.0; range: 3–28) and down-regulated (median: 11.0; range: 3–29) AFC (Figure 1). Basal AFC was negatively correlated with age of women ($r = -0.314; P < 0.001$), basal FSH concentration ($r = -0.324; P < 0.001$) and combined FSH concentration ($r = -0.252; P = 0.004$) but positively correlated with the number of oocytes aspirated ($r = 0.402; P < 0.001$) and serum $E_2$ on hCG day ($r = 0.327; P < 0.001$). Basal FSH concentration was positively correlated with age of women ($r = 0.202; P = 0.021$) and stimulated FSH concentration ($r = 0.348; P < 0.001$) but was negatively correlated with the number of oocytes aspirated ($r = -0.342; P < 0.001$) and serum $E_2$ on

![Figure 1. Box plot of antral follicle count determined in the early follicular phase of a spontaneous period (basal), day 10 after the clomiphene citrate test (stimulated) and prior to ovarian stimulation following pituitary down-regulation (down-regulated).](https://academic.oup.com/humrep/article/20/6/1647/748866/1649)
hCG day \((r = -0.293; P = 0.001)\). Stimulated FSH concentration was positively correlated with hMG duration \((r = 0.3; P < 0.001)\) and hMG dosage \((r = 0.328; P < 0.001)\) but was negatively correlated with the number of oocytes obtained \((r = -0.332; P < 0.001)\), the number of oocytes obtained \((r = -0.405; P < 0.001)\) and serum E \(_2\) on hCG day \((r = -0.291; P = 0.001)\). Combined FSH concentration was negatively correlated with basal AFC \((r = -0.252; P = 0.004)\), the number of oocytes obtained \((r = -0.405; P < 0.001)\) and serum E \(_2\) on hCG day \((r = -0.343; P = 0.001)\) but was positively correlated with hMG duration \((r = 0.313; P < 0.001)\) and hMG dosage \((r = 0.278; P < 0.001)\).

Age of women, BMI, basal AFC, basal/stimulated and combined FSH concentrations were entered in a stepwise fashion in the multiple regression analysis using the number of oocytes obtained as the dependent variable with a constant included in the equation. Basal AFC had the largest \(R^2\) change, which was followed by combined FSH concentration and age of women (Table II). When these parameters were entered in a stepwise fashion in the multiple regression analysis using serum E \(_2\) concentration on the day of hCG as the dependent variable, basal AFC had the largest \(R^2\) change, which was followed by combined FSH concentration (Table III). When these parameters were entered in a stepwise fashion in the multiple regression analysis with the dosage of hMG used as the dependent variable, stimulated FSH concentration was the only predictive parameter and all other parameters were excluded from the equation (Table IV).

Abnormal CCCT was encountered in 16 patients: basal FSH concentration >10 IU/l in eight patients and stimulated FSH concentration >10 IU/l in 13 patients. Eight patients with basal FSH concentration <10 IU/l had stimulated FSH concentration >10 IU/l. There were no significant differences in age of women, primary/secondary infertility, cause of infertility, duration of infertility, BMI, dosage/duration of hMG between women with normal and abnormal CCCT (Table V). Those with abnormal CCCT had significantly lower basal AFC, serum E \(_2\) concentration on the day of hCG and lower number of oocytes aspirated. Similar number of embryos transferred, pregnancy and implantation rates, multiple pregnancy rates and the pregnancy outcome were found in women with normal and abnormal CCCT (Table VI).

### Discussion

The present study compared AFC in the early follicular phase, after CCCT and before ovarian stimulation following pituitary down-regulation. In the majority of relevant studies, AFC was determined after pituitary down-regulation and prior to ovarian stimulation in the treatment cycle. The effects of pituitary down-regulation on AFC are still controversial in the literature, despite extensive use of GnRHa for pituitary down-regulation in IVF cycles. Sharara et al. (1999) and Hansen et al. (2003) showed that AFC did not change after pituitary down-regulation, whereas a significant decrease in AFC was observed after pituitary down-regulation by Jærela et al. (2003). In another group of patients, we have recently demonstrated that AFC, ovarian volume and ovarian power Doppler flow indices measured by three-dimensional ultrasound did not significantly change after pituitary down-regulation, both in patients with normal ovaries and with polycystic ovaries (Ng et al., 2004). In the present study, we could not demonstrate any significant difference between basal and down-regulated AFC.

Huang et al. (2001) demonstrated that AFC determined on day 6 or 7 after gonadotrophin stimulation was predictive of the ovarian response. Similarly, the combination of AFC on day 3 and day 7 had high positive and negative predictive values of ovarian response during IVF treatment (Durmusoglu et al., 2004). Both clomiphene citrate and gonadotrophins stimulate the growth of antral follicles and it is logical to postulate that CCCT might improve the assessment of AFC. To the best of our knowledge, this is the first

### Table II. Multiple regression analysis evaluating the values of different parameters in predicting the number of oocytes obtained

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(B) (95% CI)</th>
<th>(\beta)</th>
<th>(R^2) change</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>23.768 (13.319, 34.217)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Basal antral follicle count</td>
<td>0.335 (0.134, 0.536)</td>
<td>0.271</td>
<td>0.185</td>
<td>0.001</td>
</tr>
<tr>
<td>Combined FSH</td>
<td>-0.348 (-0.570, -0.126)</td>
<td>-0.247</td>
<td>0.062</td>
<td>0.002</td>
</tr>
<tr>
<td>Age</td>
<td>-0.411 (-0.674, -0.147)</td>
<td>-0.241</td>
<td>0.052</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Body mass index, basal and stimulated FSH concentrations were excluded in the equation.

Total number of oocytes \(= 23.768 + 0.335 \times \text{basal AFC} - 0.348 \times \text{combined FSH concentration} - 0.411 \times \text{age}\).

\(R = 0.547\). Adjusted \(R^2 = 0.283\).

### Table III. Multiple regression analysis evaluating the values of different parameters in predicting serum estradiol concentrations on the day of hCG

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(B) (95% CI)</th>
<th>(\beta)</th>
<th>(R^2) change</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>10,693.747 (5212.237, 16,175.258)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Basal antral follicle count</td>
<td>395.023 (153.431, 636.616)</td>
<td>0.278</td>
<td>0.119</td>
<td>0.002</td>
</tr>
<tr>
<td>Combined FSH</td>
<td>-365.128 (-643.523, -86.734)</td>
<td>-0.223</td>
<td>0.045</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Age, body mass index, basal and stimulated FSH concentrations were excluded in the equation.

Serum estradiol concentration \(= 10,693.747 + 395.023 \times \text{basal AFC} - 365.128 \times \text{combined FSH concentration}\).

\(R = 0.405\). Adjusted \(R^2 = 0.150\).
study comparing AFC determined in the early follicular phase and after CCCT. Our result indicated that there was again no difference between basal and stimulated AFC. Taking these results together, it can be concluded that the assessment of AFC for the prediction of ovarian response can be performed in the follicular phase either before or after pituitary down-regulation, and there is no additional advantage to count AFC again after CCCT.

Basal FSH concentration measured prior to the treatment cycle is widely used in many IVF programmes. A meta-analysis (Bancsi et al., 2003) showed that the performance of basal FSH concentration for predicting poor response was moderate and the performance for predicting no pregnancy was poor. Screening for elevated FSH concentrations is of no additional value in the prediction of fecundity in a general subfertility population with ovulatory menstrual cycles (van Montfrans et al., 2000). Therefore, a challenge test such as CCCT will be able to identify more women with impaired ovarian reserve than basal FSH screening alone. Women with abnormal CCCT were more likely to have poor ovarian response, higher cycle cancellation and reduced pregnancy rates (Loumaye et al., 1990; Tanbo et al., 1992; Hofmann et al., 1996; Kahraman et al., 1997; Gülekli et al., 1999; Van der Stege and Van der Linden, 2001; Csemiczky et al., 2002; Yanushpolsky et al., 2003; Hendriks et al., 2005). The utility of CCCT has also been validated in women among general infertile patients not receiving assisted reproduction methods (Scott et al., 1993, 1995).

Many studies on the prediction of ovarian response are retrospective in nature and patients received different stimulation regimens and different starting dose of gonadotrophins. Moreover, various factors affecting ovarian response such as age of women, BMI and AFC were not considered at the same time. In order to avoid the confounding variables, the study group consisted of women undergoing their first IVF cycle and receiving the same starting dose of gonadotrophin after a standard protocol of pituitary down-regulation for ovarian stimulation. All patients receiving ovarian stimulation would be offered oocyte collection, unless there was no follicular development after 14 days of stimulation. A multiple regression analysis was also applied to compare the values of these factors in the prediction of ovarian response. Ovarian response was represented by the number of oocytes aspirated, serum E2 on the day of hCG, and the duration and dosage of hMG, because we found that these measures are important in the counselling of patients undergoing IVF treatment. It is important to highlight that we examined a group of infertile women having relatively normal ovarian reserve as suggested by age < 40 years at the time of treatment and basal FSH concentration < 10 IU/L. Those patients who did not satisfy these criteria could have self-funded treatment cycles in a private unit.

In the present study, we showed that AFC achieved the best predictive value in relation to the number of oocytes aspirated, followed by combined FSH concentration and age of women. The result indicated that combined FSH concentration was a better predictor of the number of oocytes than either basal or stimulated FSH concentration. Unlike our previous finding (Ng et al., 2000b), BMI was not taken into the equation when combined FSH concentration was used. Both basal AFC and combined FSH concentration were predictive factors of serum E2 concentration on the day of hCG, whereas stimulated FSH concentration was predictive of

Table IV. Multiple regression analysis evaluating the values of different parameters in predicting the dosage of hMG used

<table>
<thead>
<tr>
<th>Parameters</th>
<th>B (95% CI)</th>
<th>β</th>
<th>R² change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>23.556 (19.579, 27.533)</td>
<td>0.214</td>
<td>0.119</td>
<td>0.014</td>
</tr>
<tr>
<td>Stimulated FSH</td>
<td>0.647 (0.133, 1.161)</td>
<td>0.214</td>
<td>0.119</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Age, body mass index, basal/stimulated and combined FSH concentrations were excluded in the equation.

hMG dosage = 23.556 + 0.647 × stimulated FSH concentration.

R = 0.214. Adjusted R² = 0.039.

Table V. Comparison of demographic data and ovarian responses between women with normal and abnormal clomiphene citrate challenge test (CCCT)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal CCCT (n = 115)</th>
<th>Abnormal CCCT (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of women (years)</td>
<td>35.0 (25.0–40.0)</td>
<td>35.0 (27.0–39.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Primary/secondary infertility</td>
<td>77/38</td>
<td>12/4</td>
<td>NS</td>
</tr>
<tr>
<td>Causes of infertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal</td>
<td>38</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Endometriosis</td>
<td>11</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Unexplained</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Infertility duration (years)</td>
<td>4.0 (1.0–11.0)</td>
<td>4.0 (2.0–8.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.5 (17.3–29.7)</td>
<td>21.0 (17.4–26.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Basal antral follicle count</td>
<td>12.0 (4.0–21.0)</td>
<td>7.0 (1.0–17.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>hMG dosage (IU)</td>
<td>1950 (1500–4950)</td>
<td>2025 (1500–3150)</td>
<td>NS</td>
</tr>
<tr>
<td>hMG duration (days)</td>
<td>11.0 (8.0–20.0)</td>
<td>11.5 (8.0–16.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum estradiol (pmol/l)</td>
<td>9578 (1687–31 862)</td>
<td>5538 (1503–11 590)</td>
<td>0.002</td>
</tr>
<tr>
<td>No. of oocytes obtained</td>
<td>8.0 (0.0–31.0)</td>
<td>4.0 (0.0–20.0)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data given as median (range).

aNo. of patients.
bOn the day of hCG.

NS = not significant.
Table VI. Comparison of treatment outcomes between women with normal and abnormal clomiphene citrate challenge test (CCCT)

| No. of embryos replaced | Normal CCCT  
\( (n = 115) \) | Abnormal CCCT  
\( (n = 16) \) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>9 (7.8)</td>
<td>5 (31.2%)</td>
</tr>
<tr>
<td>One</td>
<td>12 (10.4)</td>
<td>2 (12.5%)</td>
</tr>
<tr>
<td>Two</td>
<td>88 (76.5)</td>
<td>7 (43.8%)</td>
</tr>
<tr>
<td>Three</td>
<td>6 (5.3)</td>
<td>2 (12.5%)</td>
</tr>
<tr>
<td>Pregnancy rate/cycle</td>
<td>27/115 (23.8)</td>
<td>4/16 (25.0)</td>
</tr>
<tr>
<td>Pregnancy rate/transfer</td>
<td>27/106 (25.5)</td>
<td>4/11 (36.4)</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>33/206 (16.0)</td>
<td>4/22 (18.2)</td>
</tr>
<tr>
<td>Multiple pregnancy rate</td>
<td>7/27 (25.9)</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>Pregnancy outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First trimester miscarriage</td>
<td>7 (25.9)</td>
<td>0</td>
</tr>
<tr>
<td>Ectopic</td>
<td>2 (7.4)</td>
<td>0</td>
</tr>
<tr>
<td>Ongoing pregnancy</td>
<td>18 (66.7)</td>
<td>4 (100%)</td>
</tr>
</tbody>
</table>

Data given as number (%).
No differences were significant.

the total dosage of hMG used. We could not find any predictive factor for the duration of hMG.

Serum inhibin B concentration was not measured in this study. There is still controversy in the literature as to whether inhibin B is a useful marker for ovarian reserve. Seifer et al. (1997), Tinkanen et al. (1999) and Dzik et al. (2000) reported that women with inhibin B concentration < 45 pg/ml demonstrated a poor ovarian response during ART than those ≥45 pg/ml, whereas others (Hall et al., 1999; Corson et al., 1999; Creus et al., 2000) found no value in the prediction of ovarian response or pregnancy rate. Reduced production of inhibin B by granulosa cells of developing follicles during CCCT is responsible for the elevated FSH concentration in those with abnormal CCCT (Hofmann et al., 1998). Serum anti-Müllerian hormone appears to be an early marker for ovarian reserve (Seifer et al., 2002; van Rooij et al., 2002; Fanchin et al., 2003) but unfortunately was not checked in the present study.

There is still no consensus on threshold values for abnormal CCCT. We considered CCCT abnormal when either basal or stimulated FSH concentration was >10 IU/l. Such a definition was adopted by many groups (Kahraman et al., 1997; Gülkli et al., 1999; Van der Stege and Van der Linden, 2001; Csemiczky et al., 2002; Yanushpolsky et al., 2003) but other threshold values included stimulated FSH concentration >26 IU/l (Navot et al., 1987) and >12 IU/l (Tanbo et al., 1992) and combined FSH concentration >26 IU/l (Loumaye et al., 1990), >12 IU/l (Scott et al., 1995), >16 IU/l (Hofmann et al., 1996). In the present study, women with abnormal CCCT had significantly lower AFC, E2 concentration on the day of hCG and the number of oocytes obtained but similar pregnancy and implantation rates, when compared with those with normal CCCT. Our results were contradictory to those of others (Navot et al., 1987; Hofmann et al., 1996; Csemiczky et al., 2002; Hicks et al., 2003; Yanushpolsky et al., 2003) and did not support the conclusion in a meta-analysis that an abnormal CCCT result virtually confirmed that pregnancy would not occur with IVF treatment (Jain et al., 2004). In this study, CCCT was applied as a secondary screening test to women who had relatively normal ovarian reserve, i.e. <40 years old and serum FSH concentration on recruitment <10 IU/l. Women with normal CCCT had more oocytes obtained, and pregnancy rates from frozen–thawed embryo transfer cycles were not taken into account. Moreover, implantation and pregnancy rates of patients with poor ovarian responses were similar to those with normal response (Lashen et al., 1999; Biljan et al., 2000).

It is well known that basal FSH concentrations may vary from cycle to cycle (Scott and Hofmann, 1995). Significant cycle-to-cycle variability of the CCCT was also reported (Hannoun et al., 1998; Kwee et al., 2003). These may lead to variable results in the prediction of the ovarian response. On the other hand, there is only moderate intercycle variability in AFC and the pooled SD for intercycle variability up to a mean AFC of 15 was 3.0 (Hansen et al., 2003).

In conclusion, there was no significant difference between basal, stimulated and down-regulated AFC. AFC achieved the best predictive value in relation to the number of oocytes aspirated, followed by combined FSH concentration and age of women. Both basal AFC and combined FSH concentration were predictive factors of serum E2 concentration on the day of hCG while stimulated FSH concentration was predictive of the total dosage of hMG used. Women with abnormal CCCT had significantly lower AFC, E2 concentration on the day of hCG and the number of oocytes obtained but comparable pregnancy and implantation rates, when compared with those with normal CCCT. Although CCCT may add some information on the prediction of ovarian response, it may not be justified to screen our patients by CCCT, as only eight patients (6.1%) with normal basal FSH concentration were found to have abnormal CCCT and the pregnancy rate was not affected by the abnormal CCCT result.

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