BACKGROUND: Recent evidence supports a specific and broad role of androgen produced by theca cells in reproductive physiology. This pilot study evaluated the usefulness of hCG theca stimulation test in predicting ovarian response and pregnancy.

METHODS: Prospective cohort study including 80 infertile women treated with IVF/ICSI. On Day 3 of the menstrual cycle preceding the first IVF/ICSI cycle a blood sample was drawn to evaluate baseline FSH, estradiol (E₂), 17-hydroxy-progesterone, androstenedione and testosterone levels. All women then received 250 mg recombinant hCG s.c. and underwent a second blood sampling 24 h after hCG injection to measure steroid serum levels.

RESULTS: Percentage increment of E₂ but not its precursors was significantly higher in normo-responders and pregnancy cycles than in poor responders and non-pregnancy cycles (P = 0.03 and P = 0.02, respectively) diagnostic accuracy being 67 and 75%, respectively. The percentage increase in E₂ thus still fails in as many as 33 and 25% of patients in predicting ovarian response and pregnancy, respectively. In addition, E₂ concentrations are poorly reproducible and a wide range of variation in all serum steroids investigated—including E₂—after hCG injection was observed.

CONCLUSIONS: The predictive power of the hCG test is based on E₂ but not androgen response to hCG injection. This test cannot be recommended in routine clinical practice because it is too laborious for screening purposes, shows great variability in the response obtained and its overall accuracy is not better than that reported for other available markers of ovarian reserve. The use of the currently available markers, antral follicle count and anti-Müllerian hormone, is therefore recommended.

Key words: hCG test / IVF / ovarian reserve / poor responders / theca cell function

Introduction

Over the past three decades, especially in the context of assisted reproduction, investigation on the ovarian follicle has focused mainly on granulosa cells and their interaction with the oocyte, and the theca has been somewhat forgotten as a necessary part of the developmental process (Young and McNeilly, 2010). However, as recently reviewed (Young and McNeilly, 2010), theca cells are considered vital for folliculogenesis in the ovary. Thecal cells are specialized cells that are recruited to surround an activated follicle and provide structural support at first and then, by proliferating, differentiating and acquiring a capillary network, they have become essential components of developing follicles (Young and McNeilly, 2010). Also, several lines of evidence in the recent literature support a specific and broad role of androgen produced by theca cells in reproductive physiology as well as in the clinical setting (Hugues and Durnerin, 2005; Feigenberg et al., 2009). Thus, previous studies have reported that the basal serum androgen level can predict ovarian response to FSH and may...
be correlated with cycle outcome in the IVF setting (Frattarelli and Peterson, 2004; Barbieri et al., 2005; Frattarelli and Gerber, 2006; Hossein Rashidi et al., 2009; Qin et al., 2011).

The main function of theca cells is to synthesize androgens. During the human menstrual cycle, LH released by the pituitary gland provides the major endocrine drive to thecal androgen synthesis, which is the substrate for FSH-induced estrogen production in granulosa cells (the 2-cell, two-gonadotrophin concept), which is essential for the endocrine control of reproductive (Balasch and Fábrregues, 2002; Young and McNeilly, 2010). As the relative proportion of ovarian follicular cells decreases and that of stromal cell increases with age, it has been postulated that there may be changes in the contributions of these two cell compartments with regard to estrogen production (Piltonen et al., 2003). In fact, it has been reported that ovarian androgen capacity in response to hCG stimulation starts to decline as early as the age of 30 years despite a relatively large follicle pool in young healthy women (Piltonen et al., 2003).

On the above evidence, assessment of theca cell function prior to controlled ovarian stimulation (COS) in IVF cycles has been proposed using a GnRH agonist test (GAST) but results suggested that measurement of estradiol (£2), 17-hydroxy-progesterone (17-OHP) rather than androgens was a valuable, albeit indirect, method for assessing ovarian function (Hugues et al., 2010). Therefore, results of that study were limited and inconclusive.

This pilot study was undertaken to investigate the usefulness of an hCG stimulation test as a predictor of ovarian response and pregnancy in assisted reproduction technology (ART) cycles stimulated with GnRH agonist gonadotrophin treatment.

**Materials and Methods**

**Patients studied**

Between September and December 2009, a total of 80 consecutive women fulfilling the criteria reported below and undergoing their first cycle of IVF or ICSI treatment, thus avoiding possible bias from experience with previous cycles regarding ovarian response, were included in this study. The mean (± SD) age of the women was 36.2 ± 3.2 years (range, 25–42) and their BMI was 24.6 ± 3.3 kg/m² (range, 18–29.8). All patients were infertile but otherwise healthy premenopausal women, had both ovaries, no previous ovarian surgery and normal ovulatory function according to a regular menstrual pattern every 26–33 days and mild luteal phase determination. No patient had received any hormone therapy for ≥ 6 months before the study or had occult ovarian failure on the basis of their cycle day 3 FSH concentrations of ≤ 12 IU/l (range, 3.4–11.5 IU/l) measured in the cycle preceding IVF/ICSI. No women had polycystic ovary disease according to ultrasound examination of the ovaries or basal hormone measurement. Patient indications for IVF/ICSI included the following main diagnosis: male factor infertility (50% of patients), unexplained infertility (24%), endometriosis (15%) and tubal infertility (11%). All patients gave informed consent to participate in the study, which was approved by the ethics committee of the Hospital Clinic of Barcelona, Spain.

Sample size planning for a clinical study is based on an estimate from prior information and is performed in order to ensure the ability to detect a difference in outcome (Daya, 2006; Rohringer et al., 2010). A stressed above, the current investigation was planned as a pilot study of a subject that had not been studied previously; thus, sample size was not estimated and it was decided arbitrarily but in keeping with a recent report assessing for the first time theca cell function using a GAST instead of hCG injection as a predictor in IVF cycles (Hugues et al., 2010).

**hCG stimulation test**

The hCG test was performed on Day 3 of the menstrual cycle preceding the ART index cycle. A blood sample was drawn between 08:00 and 10:00 h to evaluate baseline hormone (FSH, £2), 17-OHP, androstenedione, testosterone levels. All women then received 250 µg recombinant hCG s.c. (Ovitrelle, Merck-Serono). Each woman underwent a second blood sampling 24 h after hCG injection for measurement of serum levels of £2, 17-OHP, androstenedione and testosterone. All collected sera were frozen and stored at −20°C until testing. Samples from each patient were examined in one run using the assays described below.

For the analysis of the main purpose of this study, we investigated the relative capacity of post-hCG serum 17-OHP, androstenedione, testosterone and £2 measurements for predicting ovarian response in an IVF cycle stimulated as described below. The secondary measure was the occurrence of pregnancy.

**Ovarian stimulation protocol**

All patients received standard ovarian stimulation with FSH under pituitary suppression with GnRH agonist according to a protocol used routinely (Penañubia et al., 2010). In all women, pituitary desensitization was achieved by s.c. administration of triptorelin acetate (Decapeptyl 0.1 mg, Ipsen Pharma, Barcelona, Spain) (0.1 mg daily, which was reduced to 0.05 mg after ovarian arrest was confirmed) started in the mid-luteal phase of the previous cycle. Gonadotrophin stimulation of the ovaries was started when serum £2 concentrations declined to < 50 pg/ml and a vaginal ultrasonographic scan showed an absence of follicles > 10 mm diameter. On Days 1 and 2 of ovarian stimulation, 450 IU and 300 IU/day of recombinant human FSH s.c. (Gonal-F, Merck-Serono S.A., Madrid, Spain), respectively, were administered. On Days 3 and 4 of ovarian stimulation, 150 IU per day of FSH were administered to each patient. From Day 5 onward, FSH was administered on an individual basis according to the ovarian response as assessed by sequential transvaginal ultrasonography and serum £2 measurements. The criteria for hCG administration (recombinant human hCG; 250 µg) (Ovitrelle; Merck-Serono S.A.) were the presence of ≥ 2 follicles ≥ 18 mm in diameter with ≥ 4 follicles measuring ≥ 14 mm in association with a consistent rise in serum £2 concentration. The cycle was cancelled when there were < 3 follicles with diameter ≥ 14 mm after 8–9 days of gonadotrophin therapy or after 4–5 additional treatment days without attaining, or the imminent prospect of attaining, the criteria for hCG administration. The cancellation of the cycle owing to insufficient follicular growth or collection of ≤ 3 oocytes at retrieval defined the poor responder patient, as recently recommended (Ferraretti et al., 2011).

Oocyte aspiration was performed with vaginal ultrasonography 35–36 h after hCG administration. Two to three days after oocyte retrieval up to three embryos per patient (depending on the age of the patient, the indication for IVF/ICSI, and the number and quality of embryos available per replacement) were replaced and the luteal phase was supported with vaginal micronized progesterone (600 mg/day given at 8-h interval) starting on the day following oocyte aspiration and continuing either up to menstruation, or if the patient became pregnant, for at least the first 3 weeks of pregnancy.

Pregnancy was diagnosed by increasing serum concentrations of β-hCG after embryo transfer, and the subsequent demonstration of an intrauterine gestational sac by ultrasonography.
Hormone analyses and ultrasonography

Hormones were measured using commercially available kits. FSH serum levels were determined using a chemiluminescent assay (ADVIA Centaur CP System; Siemens Healthcare Diagnostics, Deerfield, IL, USA). Data are expressed in terms of IS 94/632. The sensitivity of the assay was 0.1 IU/l for FSH and the inter-assay coefficient of variation (CV) was 2.7. E2 in serum was estimated by a competitive chemiluminescent assay (ADVIA Centaur CP System). The sensitivity was 10 pg/ml and the inter-assay CV was 5%. 17-OHP in serum was measured by radioimmunoassay (RIA) (MP Biomedicals, OH, USA). The lower limit of detection was 0.1 ng/ml. The intra- and inter-assay CVs were 4.2 and 8.3% respectively. Androstenedione was measured by a competitive RIA (Diagnostic Systems Laboratories, Inc., Webster, TX, USA); the sensitivity of the assay was 10 ng/dl and the inter-assay CV 12%. Serum levels of testosterone were determined using an electrochemiluminescent immunoassay (Roche Elecsys, Mannheim, Germany). The lower limit of detection was 8 ng/dl and the inter-assay CV was 9.5%. Total β-hCG was measured by an assay standardized against the Third International Standard 75/537 (ADVIA Centaur CP System) with a detection limit of 2 IU/l. The inter-assay CV was 5.8%.

Ultrasonas scans were performed using a Toshiba Eccocee SAA-340A/EF unit (Toshiba Co., Tokyo, Japan) equipped with a 5–7 MHz endovaginal probe (PVF-641VT).

Statistics

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) software (version 16; SPSS Inc., Chicago, IL, USA). All data were examined to determine whether they were distributed normally with the use of a 1-sample Kolmogrov–Smirnov test. To compare serum hormone levels between different groups of patients (<35 versus ≥35 years of age, poor responders versus normo-responders, pregnancy versus no pregnancy) at each time point, the Student t-test was used for normally distributed variables, and the non-parametric Mann–Whitney test was used for variables without a normal distribution. A Spearman’s r rank correlation was performed to correlate percentage changes in steroid levels after hCG administration and patient clinical characteristics, ovarian response to FSH in the ART cycle and embryological outcome. All statistical tests were evaluated at a significance level of 0.05. For normally distributed parameters the data are represented as mean (SD) and for data which did not show a normal distribution, as median (range).

Receiver operating characteristic (ROC) analysis was used to examine the diagnostic test performance (i.e. the discrimination attained between two study groups) (Hanley and McNeil, 1982; Zweig and Campbell, 1993). Sensitivity, specificity and the area under the ROC curve (AUROC) were obtained when appropriate and compared using the method of Hanley and McNeil (1982). Areas of 1.0 and 0.5 denote no overlapping and no discrimination, respectively, between groups.

Table I  Serum levels of steroids before (T0) and 24 h after (T24) the hCG stimulation test in 80 patients undergoing IVF.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T0 (n = 80)</th>
<th>T24 (n = 80)</th>
<th>Percentage change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-OHP (ng/ml)</td>
<td>0.61 ± 0.3</td>
<td>1.06 ± 0.4</td>
<td>86.6 (−25,530)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.33 ± 0.18</td>
<td>0.39 ± 0.21</td>
<td>6.5 (−51.9, 95.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>1.67 ± 0.55</td>
<td>1.97 ± 0.7</td>
<td>9 (−90, 53.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>33.7 ± 25.8</td>
<td>58.2 ± 28.4</td>
<td>55.1 (−23.5, 87)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median (range). 17-OHP, 17-hidroxi-progesterone; E2, estradiol.

Results

As summarized in Table I, the hCG test resulted in a significant increment in all mean serum steroid levels within the first 24 h. The largest increase was observed in 17-OHP serum values. However, when statistical correlations between percentage change in steroid levels after hCG injection and main patient clinical characteristics, ovarian response and embryological outcome were investigated, overall results were not significant (Table II). Only E2 serum levels on the day of hCG injection in the ART index cycle showed a positive but poor correlation with percentage changes in androstenedione serum levels after the hCG stimulation test.

Table III shows serum steroid levels investigated at baseline and after the hCG stimulation test in patients younger than 35 years (n = 20) and in those ≥35 years (n = 60). No significant differences were found in the responses in these two age groups. Also, the mean age was similar in normal (36.55 ± 3.41 years) (n = 63) and poor (37.35 ± 2.29 years) (n = 17) responders. However, when normal and poor responders were compared with respect to hormonal response to hCG injection, the mean percentage increment in E2 but not 17-OHP, androstenedione and testosterone serum levels was significantly higher among the normal responders group (Table III). The same was true when pregnancy versus non-pregnancy groups were compared. There were no differences in the mean age in pregnancy (35.92 ± 3.61 years) and non-pregnancy (36.38 ± 3.01 years) groups. There were three miscarriages at 7–9 weeks of gestation after demonstration of fetal cardiac activity. Two patients carrying twins delivered at 36 and 38 weeks of gestation and four live births were obtained.

To analyze the diagnostic accuracy of E2 percentage increment 24 h post-hCG to discriminate between normal and poor responder cycles as well as pregnancy versus non-pregnancy groups, the AUROC values for both comparisons are shown in Fig. 1 (upper panel and lower panel). Using a level of estimated probability of 0.5 as a cutoff value, a sensitivity, specificity and diagnostic accuracy of 55.8, 78.6 and 67%, respectively, were attained for E2 percentage increase after an hCG test in discriminating between normal and poor responder cycles. The corresponding figures when pregnancy and non-pregnancy groups were compared were 76.5, 67.5 and 75%, respectively. E2 percentage increment was a better predictor of both ovarian response and pregnancy than woman’s age, basal FSH and basal E2 (Table IV).

Discussion

Traditionally, methods used to assess ovarian reserve have mainly consisted of baseline evaluation of hormonal and also morphometric
markers. However, a number of provocative tests have been devised to assess ovarian reserve indirectly and unmask patients who might not be detected by basal screening alone (Bukman and Heineman, 2001; Tarlatzis et al., 2003; Broekmans et al., 2009).

Evaluation of ovarian reserve and prediction of ovarian response in IVF cycles focused, until recently, on markers reflecting mainly granulosa cell function. However, at present, the possibility that androgen treatment directly (Fábregues et al., 2009) or indirectly through the use of an aromatase inhibitor (which induces a temporary accumulation of intraovarian androgens) (Lee and Ledger, 2011), or recombinant human LH (considering that androgens are a direct secretory product of LH action on thecal cells) (Durnerin et al., 2008), may amplify the FSH effects on the ovary, improving follicular recruitment, is a matter of great interest and research. In fact, a group of experts has emphasized that androgens and drugs that increase ovarian androgens, such as letrozole, may become important adjuncts for patients with low prognosis IVF (Meldrum et al., 2009). In this context, assessment of theca cell function prior to ovarian stimulation for IVF seems warranted.

Recently, the usefulness of androgen measurement following stimulation of theca cell function in clinical work-up for ART has been investigated for the first time (Hugues et al., 2010). In that report (Hugues et al., 2010), serum 17-OHP, androstenedione, testosterone and E2 were measured before and 24 h after a GAST. A comparative analysis of hormonal data between normal (n = 50) and poor responders (n = 20) led the authors to conclude that serum androgen levels following GAST are correlated with ovarian response to FSH but serum testosterone is a less sensitive marker of theca cell function than 17-OHP. They proposed that measurement of 17-OHP following a GAST may be considered as a valuable method for assessing ovarian function prior to COS for IVF (Hugues et al., 2010); however, analysis of IVF outcome according to the hormonal test was not provided. On the other hand, the GAST is dependent on the pituitary production of gonadotrophins and the test causes a temporary increase in pituitary secretion of both LH and FSH (Bukman and Heineman, 2001). This stimulation causes FSH sensitive antral follicles to increase the production of paracrine signaling (through factors such as inhibin B) that up-regulates LH-stimulated thecal P450c17α mRNA expression and androgen synthesis (Hillier, 2000; Broekmans et al., 2009).

The current investigation prospectively investigated for the first time the hCG test as a predictor of ovarian response and pregnancy in IVF cycles using an observational cohort study in which the ovarian reserve markers were prospectively obtained without influencing management of the patient. Several features deserve comment in this study. First, all patients included underwent their first ART cycle thus avoiding possible bias from experience with previous cycles regarding ovarian response to gonadotrophin treatment. Second, all patients received ovarian stimulation using a step-down regimen of gonadotrophin and may overcome patient variability in FSH thresholds, metabolic clearance and ovarian sensitivity to FSH as well as too low residual LH concentrations existing in some women once pituitary suppression has been achieved (Porchet et al., 1994; Ben-Rafael et al., 1995; van Santbrink et al., 1995; Balasch et al., 2001; Peñarrubia et al., 2003) and may overcome patient variability in FSH thresholds, metabolic clearance and ovarian sensitivity to FSH as well as too low residual LH concentrations existing in some women once pituitary suppression has been achieved (Porchet et al., 1994; Ben-Rafael et al., 1995; van Santbrink et al., 1995; Balasch et al., 2001; Peñarrubia et al., 2003). Third, we used hCG as a surrogate for LH to investigate E2 ovarian basal and gonadotrophin stimulated capacity to secrete androgens. In addition, E2 precursors were investigated 24 h after hCG injection, the time point when a decreasing tendency with age in responses of 17-OHP, androstenedione and testosterone to hCG injection was observed (Piloton et al., 2003). Therefore, overall, this study included a homogeneous study population which, in addition, was managed in a homogeneous way. Finally, the definition of poor responsive cycle used in our study (≤3 oocytes) is in agreement with a very recent consensus on the subject (Ferraretti et al., 2011).

With our study design, we found that the hCG injection on menstrual cycle day 3 elicited a significant rise in mean serum levels of estrogen precursors and E2. Overall, however, no correlation was found between percentage change from baseline values in steroid serum levels 24 h after the hCG stimulation test and patient clinical

### Table II Correlation between percentage change from baseline values in steroid serum levels 24 h after the hCG stimulation test and patient clinical characteristics, ovarian response and embryological outcome.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>17-OHP percentage change</th>
<th>Androstenedione percentage change</th>
<th>Testosterone percentage change</th>
<th>E2 percentage change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>0.08 (0.4)</td>
<td>0.1 (0.4)</td>
<td>0.06 (0.5)</td>
<td>−0.1 (0.2)</td>
</tr>
<tr>
<td>Day 3 FSH (IU/l)</td>
<td>−0.1 (0.1)</td>
<td>0.07 (0.5)</td>
<td>0.02 (0.8)</td>
<td>−0.2 (0.08)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.1 (0.2)</td>
<td>0.06 (0.6)</td>
<td>−0.07 (0.5)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>E2 (pg/ml) (hCG day)</td>
<td>0.2 (0.07)</td>
<td>0.2 (0.03)</td>
<td>0.09 (0.04)</td>
<td>0.1 (0.2)</td>
</tr>
<tr>
<td>No. follicles ≥14 mm (hCG day)</td>
<td>0.01 (0.9)</td>
<td>0.2 (0.1)</td>
<td>0.02 (0.8)</td>
<td>−0.08–0.08 (0.6)</td>
</tr>
<tr>
<td>No. of oocytes</td>
<td>0.03 (0.7)</td>
<td>0.06 (0.6)</td>
<td>−0.08 (0.5)</td>
<td>0.2 (0.2)</td>
</tr>
<tr>
<td>No. of metaphase II oocytes</td>
<td>0.05 (0.6)</td>
<td>−0.2 (0.4)</td>
<td>−0.03 (0.6)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>No. of fertilized oocytes</td>
<td>0.1 (0.2)</td>
<td>0.01 (0.3)</td>
<td>0.1 (0.5)</td>
<td>0.02 (0.4)</td>
</tr>
<tr>
<td>No. of embryos per patient</td>
<td>−0.1 (0.3)</td>
<td>−0.03 (0.7)</td>
<td>−0.01 (0.9)</td>
<td>0.09 (0.5)</td>
</tr>
</tbody>
</table>

Figures are correlation coefficients (r) with probabilities shown in parentheses.

*Refers to the IVF index cycle.
Table III  Response of serum 17-OHP, androstenedione, testosterone and E₂ to the hCG stimulation test according to women's age, ovarian response and pregnancy.

<table>
<thead>
<tr>
<th></th>
<th>17-OHP (ng/ml)</th>
<th>Androstenedione (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>E₂ (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T24</td>
<td>Percentage change</td>
<td>T0</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 35 year (n = 20)</td>
<td>0.72 ± 0.4 1.17 ± 0.3</td>
<td>49.09 (15, 130)</td>
<td>1.97 ± 0.5 2.13 ± 0.6</td>
<td>6.8 (-19.1, 116)</td>
</tr>
<tr>
<td>≥ 35 year (n = 60)</td>
<td>0.54 ± 0.2 0.97 ± 0.4</td>
<td>98.7 (-25, 471)</td>
<td>1.53 ± 0.6 1.86 ± 0.6</td>
<td>9.6 (-47.3, 86.2)</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td><strong>Ovarian response</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normo-responders (n = 63)</td>
<td>0.62 ± 0.3 1.1 ± 0.2</td>
<td>97.5 (-3.1, 530)</td>
<td>1.67 ± 0.6 1.95 ± 0.7</td>
<td>9.7 (-30, 116)</td>
</tr>
<tr>
<td>Poor responders (n = 17)</td>
<td>0.42 ± 0.2 0.7 ± 0.3</td>
<td>5 2 (-25, 291.6)</td>
<td>1.61 ± 0.2 1.91 ± 0.4</td>
<td>16.7 (-47.3, 76.2)</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td><strong>Pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy (n = 28)</td>
<td>0.66 ± 0.3 1.14 ± 0.3</td>
<td>92 (-3.09, 471.4)</td>
<td>1.83 ± 0.5 2.54 ± 0.2</td>
<td>3.9 (-30, 705)</td>
</tr>
<tr>
<td>Non-Pregnancy (n = 52)</td>
<td>0.48 ± 0.4 0.88 ± 0.8</td>
<td>88 (4.1, 366.6)</td>
<td>1.50 ± 0.5 1.68 ± 0.7</td>
<td>2.2 (-27.7, 73)</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median (range).
characteristics, ovarian response or embryological and ART outcome. In addition the responses of all steroids to hCG was similar in women <35 years of age and in older subjects. Remarkably, however, as many as 92.5% of the patients in our study were older than 30 years. Therefore, our results do not conflict with those in a previous report showing a decreasing tendency with age in the responses of all steroids to hCG but statistically significant differences occurring only when women >30 years of age were compared with those 30 years or younger (Piltonen et al., 2003). In this cohort as many as 60 (75%) women were ≥35 years and 56 (70%) of them were aged 35–40 years. This may explain why age and basal FSH were poor predictors of both ovarian response and pregnancy in IVF cycles.

In contrast, the percentage increment of E₂ but not its precursors 24 h post-hCG injection was significantly higher in normo-responders and pregnancy cycles than in poor responders and non-pregnancy cycles, respectively. In fact, significant AUCs were obtained only for E₂ percentage increment 24 h post-hCG when ROC plots were used to discriminate between normo-response and poor response or pregnancy and non-pregnancy IVF cycles (Table IV). The predictive capacity of E₂ but not estrogen precursors may be explained on the basis of neuroendocrine changes associated with ovarian aging.

The primary endocrine function of the ovaries is to secrete sex steroids—E₂ and progesterone—that are essential to prepare the reproductive tract for pregnancy. Co-ordinated effects of FSH and LH on ovarian granulosa and thecal cells are required for steroid hormone synthesis, according to the two hormone-two-cell theory of steroidogenesis. In thecal cells, LH stimulates the synthesis of androgens (especially androstenedione) from cholesterol. These diffuse across the basal lamina to granulosa cells, where FSH stimulates their conversion, via aromatase, to estrogens. Androstenedione is converted to estrone by aromatase and is subsequently converted to E₂ (the major naturally occurring form of estrogen and end-product in women of reproductive age) by 17β-hydroxysteroid dehydrogenase (Fauser and Van Heusden, 1997).

Interestingly, E₂ secretion is preserved in women of advanced reproductive age in the face of decreased androstenedione concentrations and decreased follicle number (Piltonen et al., 2003; Welt et al., 2006). Decreasing androgen secretion capacity with age in association with unchanged or increased E₂ concentrations in older compared with younger women of reproductive age suggests that compensatory mechanisms are needed to maintain optimal estrogen biosynthesis. Higher estrogen levels associated with ovarian aging are not related to increased substrate availability as indicated by studies investigating the relative concentration of androstenedione and estrone that can be used to approximate aromatase function (Welt et al., 2006; Hall, 2007). The lower androstenedione-to-estrone ratio across the follicular phase in older cycling women is consistent with increased aromatase activity that may be caused by gradually rising early follicular phase FSH levels which compensate for the declining ovarian capacity to synthesize steroids (Piltonen et al., 2003; Welt et al., 2006; Hall, 2007; Broekmans et al., 2009).

On the other hand, experimental studies have revealed that in the presence of minimal stimulation by LH (and thus androstenedione, a direct secretory product of LH action on theca cells), FSH is able to...
activate paracrine signaling (granulosa on theca signaling mediated by insulin-like growth factors and inhibins), which sustains thecal androgen synthesis and thereby explains why treatment with FSH alone is capable of stimulating ovarian estrogen synthesis in many clinical situations (Hillier, 2000, 2001). In fact, in the clinical setting, it is accepted that the amount of LH activity actually necessary for normal follicle and oocyte development is very low, since <1% of follicular LH receptors need to be occupied in order to elicit a maximal steroidogenic response (Chappel and Howles, 1991).

Taken together, the above facts support the notion that E2 rather than its precursors directly reflects overall follicular activity and ovarian steroidogenic responsiveness to gonadotrophin stimulation. Also, they are in keeping with previous studies from our group indicating that LH supplementation does not increase ovarian response and implantation rates in older patients of reproductive age stimulated with recombinant human FSH under pituitary suppression (Fábregues et al., 2006, 2011).

The above notwithstanding, the percentage increase in E2 still falls in as many as 33 and 25% of patients to predict ovarian response and pregnancy, respectively, in IVF cycles. In addition, E2 concentrations are poorly reproducible thus precluding the definition of reference values for daily practice. In fact, non-parametric tests were used in our study because of the very wide range of variation in levels of all serum steroids investigated—including E2 after hCG injection. Therefore, despite the endocrine rationale of the hCG stimulation test, it cannot be recommended in routine clinical practice because, like other challenge tests, it is too laborious for screening purposes and its overall accuracy is no better than single basal tests, mainly antral follicle count (AFC) and anti-Müllerian hormone (AMH). The ovarian AFC and serum levels of AMH correlate with the ovarian primordial follicle number, even after adjustment for chronological age, the correlation between AFC and the ovarian primordial follicle count being greater than that of AMH with the ovarian primordial follicle count (Hansen et al., 2011). The AFC has been considered the test of first choice by some (Verhagen et al., 2008; Broekmans et al., 2009; Domingues et al., 2010). Unfortunately, AFC is not available in this study but AFC is simpler and cheaper than the hCG stimulation test, and easily repeatable with intercycle variability and observer reproducibility having no major effects upon the prediction of outcome, in terms of response or pregnancy after IVF, when a standardized approach according to practical recommendations is used (Broekmans et al., 2010).

Authors’ roles

All authors provided a substantial contribution to the conception of the paper. J.B. and F.F.: study design, execution, analysis, manuscript drafting and critical discussion. F.C., A.I. and R.C.: study design, execution, analysis of data and critical discussion.

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Conflict of interest

None declared.

References


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