The value of human chorionic gonadotropin stimulation test in predicting ovarian response during in-vitro fertilization

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Abstract

Purpose To assess the predictive value of human Chorionic Gonadotropin (hCG) theca-stimulation test for ovarian reserve in women undergoing in-vitro fertilization (IVF) treatments.

Methods 39 women were included in the study. All participants received a single hCG 10000 IU injection on cycle day 2–3. Serum levels of estradiol, testosterone, androstenedione and 17-OH progesterone were measured prior to the injection and on days 1, 3 and 7 following the injection. hCG-induced hormone levels were compared with ovarian response during the subsequent IVF cycle.

Results There were 11 good responders (>10 oocytes) and 22 low responders (<3 oocytes). Before hCG stimulation serum E2 levels were higher in low responders compared to good responders (370.3 ± 443 vs. 138.3 ± 54 pmol/ml). Following hCG stimulation, day 3 androstendione levels showed an increase in good responders compared to low responders. The ratio between day 3 androstendione and day 0 estradiol was significantly different between the two groups ($p=0.03$). ROC analysis of this test revealed area = 0.837 (good prediction), which was much better than day 3 FSH (area = 0.635, poor prediction).

Capsule Androstenedione production in response to hCG theca stimulation test was a good predictor of ovarian response in a subsequent IVF treatment

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Conclusions These preliminary results demonstrate the potential use of the hCG theca stimulation test in differentiating between good and poor responders. Larger series are needed for further verification of the test in routine clinical application.

Keywords Ovarian reserve · IVF · Androgens · hCG theca stimulation test

Introduction

In an era of delayed childbearing, and a consequently increased population of older women undergoing in-vitro fertilization (IVF), there is a growing need for a reliable method to assess ovarian reserve. Until recently, evaluation of ovarian reserve and prediction of IVF success focused on hormonal parameters that mainly reflect granulosa cell function. While most available tests are based on measuring baseline levels, such as those of FSH [1, 2], anti-Müllerian hormone [AMH, 3–6], inhibin B [7–9], basal estradiol—E₂ [10–12] or antral follicle count [11, 13–15], some dynamic tests were developed to predict ovarian reserve by its response to certain stimuli, such as clomiphene citrate challenge test [CCCT – 16–19], exogenous FSH ovarian reserve test [EFORT – 19–21] and gonadotropin releasing hormone agonist stimulation test [GAST – 22–25]. However, inconsistencies in the prognostic value for IVF success in these tests limit their clinical applicability.

Recently, the assessment of theca cell function as a predictor of IVF success has gained attention. Two studies [26, 27] showed that testosterone levels correlated well with several parameters such as oocyte number and peak E₂ levels, and were negatively associated with gonadotropin dosage and the number of stimulation days required for

response. In these studies, low serum testosterone level was predictive of poor cycle outcome. This association has motivated some authors to investigate the possibility of androgen supplementation in the treatment of poor responders to gonadotropins in order to achieve a better response to gonadotropin treatment [28, 29].

In view of the data regarding the possible value of serum androgens levels in predicting the response to gonadotropin treatment, performing a stimulation test to examine ovarian theca reserve could theoretically be of significant value. The only thecal stimulation test reported in the literature was conducted by Piltonen et al. [30] on 44 female volunteers aged 20–44 years. All subjects underwent hCG stimulation (10,000 I.U) 2–5 days after spontaneous menstrual bleeding. The ovarian capacity to secrete estrogen precursors in response to hCG stimulation was significantly decreased in women older than 30 years. This study suggested that the first sign of ovarian aging may be a reduced ability to produce androgens in response to hCG stimulation. Whether such a test can predict IVF outcome has not yet been determined. The aim of this study was to determine the predictive value of hCG theca cell stimulation test and IVF outcome, as a possible measure of ovarian reserve.

Materials & methods

Patients and hCG stimulation test

This prospective study was approved by the Hadassah Medical Center – Hebrew University Institutional Review Board. Women who participated in our IVF program and met our inclusion criteria were approached and 39 agreed to participate in this study. Patients were excluded if they had polycystic ovary syndrome, known allergy to hCG or if they had undergone ovarian stimulation of any type in the past 3 months. After obtaining a complete informed consent, blood sample was drawn for baseline estradiol (E_2), FSH, androstenedione (A), testosterone (T) and 17-hydroxy-progesterone (17-OHP) on day 2–3 of a spontaneous menstrual cycle. hCG thecal stimulation test was then performed by intramuscular injection of 10,000 IU hCG (Pregnyl®; NV Organon, Oss Holland) and collection of blood samples for E_2 , A, T and 17-OHP on days 1, 3 and 7 post stimulation. All collected sera were frozen and stored at -20°C until testing.

IVF cycle

In the following month, all women underwent an IVF cycle with a protocol tailored to their age. Women under 35 years were treated by long luteal phase GnRHa protocol. A single

daily dose (0.1 mg) of triptorelin (Decapeptyl®; Ferring, Sweden) was injected subcutaneously from the mid-luteal phase of the previous cycle. Once pituitary desensitization was achieved, a fixed daily dose (150 IU) of recombinant human FSH (Gonal-F®; Merck Serono, S.A, Switzerland) was administered for 5 days. Ovarian response was evaluated by ultrasound and estradiol level, and the dose was adjusted accordingly. When two follicles reached a diameter of ≥ 18 mm, 250 μg of recombinant hCG (Ovitrelle®; Merck Serono, S.P.A, Bari, Italy) was administered 36 h before oocyte pickup.

Women above 35 years, or patients who responded poorly previously, were treated by a short (“flare-up”) GnRHa protocol. From day 1 of the cycle, a single daily dose (0.1 mg) of triptorelin (Decapeptyl®) was injected subcutaneously. Ovarian stimulation was optimized according to the patient’s response starting with 300 IU of recombinant human FSH (Gonal-F®) and the criteria for hCG administration were the same as in the long protocol. Based on the number of oocytes retrieved, patients were categorized as good responders (≥ 10 oocytes), intermediate responders (4–9 oocytes) or poor responders (≤ 3 oocytes).

Hormonal assays

Estradiol, FSH, total testosterone and androstenedione levels were measured using a competitive chemiluminescent enzyme immunoassay (Immulate 2500®, Siemens Healthcare Diagnostics, IL, USA). 17α -OH Progesterone was measured using radioimmunoassay (Coat-A-Count®, Siemens Healthcare Diagnostics, IL, USA). The assays were performed according to the manufacturer’s recommendations. The sensitivities of the assay for estradiol, FSH, total testosterone, androstenedione and 17α -OH Progesterone were 55 pmol/L, 0.1 mIU/ml, 0.5 nmol/L, 1.0 nmol/L and 0.21 nmol/L, respectively.

Based on a previous study showing maximal response of androgens to hCG on day 3 after stimulation [30], our primary outcome was the proportional change in androgen levels on day 3 (A_3) post hCG challenge as compared to baseline (A_0). Since androgens are estrogen precursors, we normalized our outcome to baseline E_2 levels (E_0). Thus, we evaluated the response to our test as:

$$\text{hCG – Thecal stimulation test value (hCG TSTV)} = \left(\frac{A_3/A_0}{E_0} \right) * 100$$

We hypothesized that good responders would achieve higher values in this test when compared to poor responders because of better theca cell function and lower baseline estradiol levels. For the purpose of our study, we analyzed only the two extreme groups: good responders and poor responders.

Statistical analysis

In order to assess the significance of change between two time points within a study group, the paired *t*-test and the Wilcoxon sum-rank test were applied. The non-parametric Mann–Whitney test was used to compare variables between two independent groups. The Pearson correlation coefficient was utilized to estimate the strength of the association between two variables. ROC curve analysis was applied in order to find a cutoff point based on a specific variable, which best would discriminate between poor and good responders.

All tests applied were two-tailed, and a *p*-value <0.05 was considered statistically significant.

Results

Thirty-nine women participated in the study between January 2005 and July 2009. Table 1 summarizes patients' baseline characteristics and IVF outcome. Based on oocyte number, 11 subjects were defined as good responders and 22 as poor responders. Six patients were categorized as intermediate responders and were excluded from analysis. Good responders were more likely to be younger than poor responders (*p*<0.001). Basal day 3 FSH tended to be lower in good responders as compared to poor responders, but did not reach significance (*p*=0.059). Day 3 levels of estradiol, testosterone, androstenedione and 17-OH-progesterone in both poor and good responders were significantly higher than baseline levels. However, day 3 levels did not differ between the groups (Fig. 1). These levels then decreased toward day 7 except for estradiol in poor responders, which remained high even on day 7, and may reflect the sustained increased testosterone level in that group.

Evaluating hCG TSTV for androstenedione, testosterone and 17-OH progesterone demonstrated that only androstenedione differed significantly: hCG TSTVs for androstenedione were significantly higher in good responders as compared to poor responders (1.55±1.01 vs., 0.79±0.58 respectively, *p*=0.03, Fig. 2).

Applying Pearson correlation coefficient test, hCG TSTV was compared to basal day 3 FSH as predictors of the number of aspirated oocytes. Day 3 FSH was found to poorly correlate with the number of oocytes (*r*=−0.262, *p*=0.20) while hCG TSTV demonstrated a good correlation (*p*=0.016; *r*=0.543; Figs. 3, 4).

Comparing the different tests for ovarian reserve by area under ROC curve revealed that baseline serum FSH level was a poor predictor (area = 0.590) compared to the good prediction of thecal stimulation test (area = 0.818). A TVST cutoff of 0.87 demonstrated a sensitivity and specificity of 85% and 73%, respectively, in distinguishing between good and poor responders.

Discussion

This is the first study to assess ovarian reserve by hCG theca cell stimulation performed in the early follicular phase. Our study demonstrates that the ratio between the increase in androstenedione following hCG stimulation to estradiol baseline levels (TSTV) correlates significantly with the number of oocytes retrieved following gonadotropin stimulation. Recently, Hughes and colleagues [25] examined androgens levels after GAST and found that 17-OH progesterone and androstenedione increase after stimulation, but only the former correlated with ovarian response. Similarly, in this study we found that 17-OH progesterone had the highest increase (5 times of baseline) but only androstenedione could distinguish between good and poor responders. The difference between the results could be explained by the fact that we excluded patients with polycystic ovaries syndrome, the fact that GAST induces the secretion of both LH and FSH while we used hCG (LH alone) and finally it may be that this work's smaller sample size could not discern differences in 17-OH progesterone levels between the groups.

These results support the two-cell two-gonadotropin theory and the fundamental role of theca cells in folliculogenesis. Theca cell function is necessary for normal folliculogenesis and a minimal threshold level of LH is

Table 1 Demographic data of good and poor responders

	Good responders (<i>N</i> =11)	Poor responders (<i>N</i> =22)	<i>p</i>
Age	29.6±5.6	39.6±4.7	<i>p</i> <0.001
Day 3 FSH (mIU/ml)	7.3±3.3	13.35±13	<i>p</i> =0.059
Basal estradiol (pmol/L)	138±151	263±268	<i>p</i> =0.26
Oocytes retrieved	16.18±6.9	1.05±1.0	<i>p</i> <0.001
Mature oocytes (MII)	11.0±4.1	2.7±3.2	<i>p</i> <0.001
Fertilized oocytes	7.3±3.4	2.3±2.9	<i>p</i> =0.004
Pregnancy rate	5/11	1/22

Fig. 1 Serum steroid levels before and 1, 3 and 7 days after hCG stimulation test from poor and good responders (mean $\pm$ SE)

needed for theca cell stimulation and production of androgens to improve folliculogenesis, as was demonstrated in hypogonadotropic hypogonadism patients [31].

Several tests that examine granulosa cell function were developed to evaluate ovarian reserve before ART: day 3 FSH, day 3 estradiol, inhibin B, CCCT, EFORT, and GAST [32]. Most of these tests have shown only a modest predictive value and consequently, have not gained wide clinical acceptance and application. Even AMH and AFC, which have a relatively good prediction of ovarian

response to gonadotrophin stimulation, have failed to predict the occurrence of conception. Therefore, none of these markers should be used to deny couples from proceeding with IVF treatment. Factors such as female age, parity, duration of infertility, ovarian response in the first IVF attempt and embryo quality are employed for counseling and individualization of a treatment protocol [33, 34].

Ovarian aging mechanisms include decreased ovarian stromal and perifollicular blood flow [35, 36]. Granulosa cells are the first to be affected by a decreased blood flow, as their supply depends on oxygen diffusion from the theca cell layer. Deterioration of theca cell function probably represents a progression in ovarian aging. As a consequence, the results of the hCG stimulation test have the potential to predict ovarian reserve as well as the extent of the aging process. Normal results in poor responders may indicate a relatively preserved (residual) thecal reserve, which can be stimulated by LH/hCG during or before an IVF attempt. A low androgen response to the stimulation may suggest a more advanced stage of ovarian aging, and these patients may potentially benefit from androgen supplementation, to potentiate stimulation for folliculogenesis by FSH [37, 38].

Our study has several limitations. First, the sample size is relatively small, which may limit our ability to draw

Fig. 2 Box plot of the ratio of the proportional increase in androstenedione at day 3 to baseline estradiol ($A3/A0/E0$) in poor and good responders

Fig. 3 Pearson correlation coefficient of FSH and hCG-stimulation test to the number of oocytes retrieved

definitive conclusions about the clinical benefit of the test. However, the significant differences found in our study support the hypothesis regarding the role of androgen production on folliculogenesis. These encouraging results should serve as a preliminary proof of concept and an extended adequately powered study should follow. Secondly, patients were treated with different IVF protocols based on the physicians' judgment. Specifically, older patients, or those with a history of poor response, were treated differently from young patients. However, this should not interfere with our conclusions regarding ovarian reserve since all patients received adequate and aggressive gonadotropin stimulation. There was also a selection bias of 2:1 ratio of poor responders to good responders. This may reflect the particular interest of older patients or poor responders in innovative treatments, but does not affect the significance of our results.

The strengths of this study stems from the fact that the groups studied were preselected to exclude conditions of

hyperandrogenism and strict criteria of ovarian response were employed to categorize the two groups.

Fig. 4 Areas under the ROC curves evaluating the prediction of low responders by hCG stimulation test and day 3 FSH

In summary, this preliminary observation shows that hCG thecal stimulation test by hCG administration on days 2–3 of the menstrual cycle and measurement of androstenedione and estradiol before and 3 days after treatment, may predict ovarian reserve and IVF results. Its potential application in clinical practice needs further validation.

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