Clomiphene Citrate Challenge Test and Prediction of Outcome in IVF

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Received October 2007; Revised and accepted December 2007

Abstract

Objective: The aim of this study was to assess the accuracy of ovarian reserve tests, mainly basal and clomiphene stimulated follicle stimulating hormone (FSH) concentrations.

Materials and methods: A prospective cohort study was conducted on 120 infertile patients referring to Mirza Koochak Khan Hospital for IVF from 2004 to 2006. All the patients underwent clomiphene citrate challenge test (CCCT) and then in vitro fertilization (IVF) was performed. We evaluated predictive power of CCCT to find IVF outcome in women with decreased ovarian reserve in comparison to those who have appropriate ovarian reserve. SPSS 12 was used to analyze the data of this study via student t test, Fisher exact, Chi -square tests and logistic regression analysis.

Results: IVF success rate was 11.7% and CCCT sensitivity and specificity were 30.2% and 92.9% while its positive and negative predictive values were 14.9% and 97% respectively.

Conclusion: CCCT is a good test in estimating ovarian reserve. Due to its high negative predictive value, lower chance of pregnancy is expected in the cases with abnormal results.

Keywords: Clomiphene Citrate Challenge test, Pregnancy, IVF, Infertility

Introduction

Infertility is generally described as one year of unprotected intercourse without conception. Approximately 85-90% of healthy young couples conceive within 1 year. Infertility therefore, affects approximately 10%-15% of couples and is an important part of clinical practice for many clinicians. With improvements in Assisted Reproductive Technology, we encounter increasing number of infertile couples to seek this kind of treatment. All of these procedures are expensive, so finding a cheap, available, and rapid way to select patients with low ovarian reserve as a preliminary measure to perform the best ART protocol is of great importance (1).

One of the tests for evaluating ovarian reserve is clomiphene citrate challenge test (CCCT). This test in comparison with other tests, has acceptable clinical reliability, specificity and sensitivity and can be used as a screening test because it is cheap, available, easy and can be performed in all clinical centers (1).

This study was designed to evaluate predictive power of CCCT to find IVF outcome in women with decreased ovarian reserve in comparison to those who have appropriate ovarian reserve.

Materials and methods

This cohort study consisted of 120 infertile women that were referred to IVF center of Mirza Koochak Khan Hospital in Tehran from 2004 to 2006. All the procedures were performed under patients' consents and Helsinki's protocol in medical ethics.

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After gathering demographic information from these patients, CCCT was performed on all of them. At first, FSH level was evaluated on the third day of menstruation cycle, and then clomiphene citrate was given to these patients during 5th to 9th days of cycle. FSH was re-evaluated on the 10th day. FSH value more than 10 IU/lit was considered abnormal. All samples were tested by radioimmunoassay and in a single approved laboratory by an expert immunologist.

After CCCT, all women underwent IVF cycle based on "long protocol". Vaginal ultrasound was performed on 21st day of menstruation cycle, and then GnRH-a (bucerelin acetate, 500 µg/daily subcutaneously) was prescribed. Second vaginal ultrasound was performed during first three days of the next cycle.

Thereafter, if ovarian suppression signs were observed (thin or <4 mm endometrial thickness, inactive ovaries, no cysts, and estradiol or E2 level more than 50 picogram/ml), hMG (75-450 U/daily IM) was prescribed and the dose of bucerelin acetate was reduced to 300 µg/daily.

On the 7th day of the cycle, another ultrasound was scheduled and if there was at least 3 follicles with more than 18 mm in diameter and E2 level more than 300-500 picogram/ml, hCG (10000 units IM) was injected. Thirty six hours later, puncture was performed transcervically with ultrasound guidance. After puncture, patients received 100 mg progesterone daily (IM) till the day of embryo transfer. After 72 hours, and in 4-cell stage, embryos were transferred transcervically under ultrasonographic guidance. Luteal phase was supported by progesterone suppository (400 mg twice daily). When heart rate of embryo was detected, luteal phase support was continued with HCG injections for 12 weeks (10000 units/every 4 days).

Main outcome of this study was pregnancy occurrence. The gold standard to measure this effect was observation of gestational sac in ultrasonography.To assess CCCT sensitivity and specifity; results were compared with this gold test.

Gathered data were analyzed by t-test (for quantitative variables), Chi-square and Fisher's exact test (for qualitative variables) with 95% significance level by SPSS software version 12. We used logistic regression test to find the true effect of different variables on CCCT.

P-value < 0.05 was considered for statistical significance.

Results

Table 1 shows the descriptive and analytic data of this research. Half of the patients were under 34 years and the oldest one was 48 years old. Eighty three patients had regular menstruation and 28 women had irregular menstruation, and 2 (1.8%) patients had amenorrhea. Hirsutism was detected in 33 (30.6%) and galactorrhea in 10 (9.2%) patients. Infertility was primary in 89 (74.2%) patients and secondary in others (31 patients, 25.8%). Mean infertility duration was 7 years. The most common causes of infertility were male factor, tubal factor and PCOS. Unexplained infertility was seen in 27% of the patients (37.5% was for patients' ≤ 35 years of age).

Mean age of spouses was 38.9±0.8. Totally 28 cases (23.3%) had azospermia. Success rate of IVF was 11.7% (14 cases of pregnancy). CCCT was abnormal in 33 (27.5%) patients. In comparison to gold standard (GS in Ultrasonography) CCCT specificity and sensitivity, to predict pregnancy were 92.9% and 30.2% respectively. Positive predictive value (PPV) and negative predictive value (NPV) were 14.9% and 97% respectively. This study showed 4.9 times chance of pregnancy in those women with normal CCCT. Mean FSH level was 16.6±5.96 mu/ml in abnormal CCCT patients and 6.8±2 in the other group.

Regression analysis (table 2) showed significant effect of increased number of mature oocytes, transferred embryos, regularity of menses and lower level of 10th FSH (independently to BMI, age, cause of infertility and 3rd day FSH) on the CCCT result.

Discussion

CCCT was described by Navot in 1987. During following years, predictive value of this test for evaluating ovarian reserve has been showed (2). In another study which was designed to measure predictive ability and clinical liability of CCCT and repeated CCCT in outcome of IVF, basic markers of ovarian reserve were compared. Sixty three infertile women who underwent IVF treatment for the first time considered for taking part in that prospective study. After measuring basic markers (FSH, Inhibin B) at the 3rd day of menstruation cycle, CCCT was performed. FSH and Inhibin B were measured at the 10th day again. After menstruation period, another CCCT was performed as well, and in all patients IVF was performed after the latter test. Both single and repeated CCCTs predicted weak response to IVF. Although this study did not prove that predictive

Table 1: CCCT results in the study (n=120)

	CCCT results		P-Value*
	Normal	Abnormal	
Age (year)	34.0 ±6.18	38.3 ±6.29	NS
Spouse age (year)	38.35 ±7.4	40.27 ±5.8	NS
BMI (kg/m^2)	29.5 ±6.25	29.5 ±6.00	NS
Menstruation			
Regular	61 (75%)	22 (73%)	NS
Irregular	22 (25%)	8 (27%)	NS
Clinical manifestations			
Hirsutism	27 (81.8%)	6 (18.2%)	NS
Galactorrhea	6 (60%)	4 (40%)	NS
Type of Infertility			
Primary	66 (74.2%)	23 (25.8%)	NS
Secondary	20 (64.6%)	11 (35.4%)	NS
Infertility duration (year)	6.85 ±4.5	8.43 ±4.8	NS
Infertility factors			
Male	23 (74.2%)	8 (25.8%)	0.007
Tubal	27 (90%)	3 (10%)	0.007
Other female factors	22 (58%)	16 (42%)	0.007
3rd day FSH (IU/mL)	5.9 ±7.3	11.1 ±5.9	<0.001
10th day FSH (IU/mL)	6.8 ±2.0	16.6 ±5.9	<0.001
Retrieved oocytes (n)	6.7 ±3.8	3.3 ±2.6	<0.001
Transferred embryos (n)	2.8 ±1.2	1.8 ±1.2	<0.001

* P-value < 0.05 is considered significant.
Values are presented as Mean±SD or n (%).

Table 2: Regression analysis model of effective factors on CCCT results

Variable	B	SE	β	P-Value	$CI_{95\%}$
Oocytes N.	-0.12	0.15	-0.66	0.009	0.05-0.12
Transferred embryos	-0.21	0.02	-0.41	0.012	0.30-0.11
10th day FSH	3.3E-0.2	0.0006	-0.66	0.01	0.18-0.058
Mense regularity	7019-6E-0.2	0.011	0.072	0.021	0.02- 0.11

ability and clinical liability of CCCT was higher than FSH or inhibin B levels significantly. Therefore the authors of the study could not recommend CCCT as a single test to predict IVF outcome (3).

In our study, we showed the high value of prediction of CCCT in IVF outcome when there is weak response to IVF. Although many studies in recent years showed the high value of CCCT in predicting IVF outcome (4, 5).

One of the most important factors in IVF outcome in our study was the number of transferred oocytes, and lack of similarity between two groups which can make bias for this study. Therefore we suggest that other studies would be designed to overcome these biases. Also we suggest comparative studies between CCCT and other tests for evaluating ovarian reserve and predicting IVF outcome with the best and most available tests for each group of patients.

As the final conclusion it was shown that CCCT is a good test in estimating ovarian reserve. Due to its high negative predictive value, lower chance of pregnancy is expected in the cases with abnormal results.

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