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Serum Estradiol Level as a Predictor of Ovarian Response and Pregnancy Outcome During Controlled Ovarian Hyperstimulation in Women from Gaza Strip

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Abstract

***Background**: Currently, controlled ovarian hyperstimulation (COH) is monitored by serum estradiol (E2) levels which are believed to primarily detect functional activity of follicles.*

Objective: To evaluate estradiol level as a predictor of ovarian response and pregnancy outcome during COH in women from Gaza Strip.

***Methods:** This prospective cohort study consisted of 75 women attending in vitro fertilization (IVF) at Al-Basma Fertility Center in Gaza City. Blood withdrawal for E2 hormone measurement was performed in all the patients and the number of oocytes and embryos were recorded for each female and the occurrence of pregnancy was followed for three months. Obtained data were computer analyzed using SPSS statistical package version 18.*

***Results**: The mean age of the study population was 29.2±5.9 years. Questionnaire interview showed that the cause of infertility was mostly referred to husbands. More than half of women seeking IVF had no children and had repeated IVF. The mean level of E2 showed the highest value of 2194.4 (pg/ml) at age group 26-35 years. However, the difference in E2 levels among the age groups was not significant (F=0.940 and P=0.395). When related to the number of oocytes retrieved, E2 level showed general increase with increase ovarian response, recording values of 1642.7, 1665.1, 2156.8 and 1798.7 pg/ml with <4, 4-8, 9-16 and >16 oocytes, respectively, but this change was not significant (F=0.219 and P=0.883). The mean level of E2 showed its maximum value of 2143.6 pg/ml in positive pregnancy. However the difference in E2 levels among the different categories of IVF outcome was not significant (F=0.423 and P=0.656). The numbers of total and mature oocytes, and embryo were significantly increased with increased levels of E2, showing a good response at E2 level=1000-2000 pg/ml. The number of mature oocytes showed positive correlations with E2 and number of embryo (r= 0.159, P= 0.177 and r= 0.890, P=0.000, respectively) and negative correlation with age (r=-0.276, P=.017).*

***Conclusion:** Estradiol level 1000-2000 pg/ml at mean age of 27.8±4.9 years, could be a predictor of ovarian response and pregnancy outcome during COH. Consequently, for women to undergo a more likely successful IFV program, it is recommended to optimize E2 level at 1000-2000 pg/ml.*

***Keywords:** Estradiol, Controlled ovarian hyperstimulation, In vitro fertilization, Gaza Strip.*

Introduction

Controlled ovarian hyperstimulation is a standard practice in assisted conception programs. Monitoring of COH in IVF aims to evaluate whether the response to exogenous gonadotropins is sufficient to obtain an adequate number of mature oocytes. The second aim is to determine the optimal time for induction of final oocyte maturation by human chorionic gonadotropin (hCG) administration. Currently, COH is monitored by repeated pelvic ultrasonography or serum E2 measurement. It is believed that ultrasonographic findings reflect growth, whereas serum E2 levels primarily detect functional activity of follicles[1]. Estradiol is the 2nd predominant steroid sex hormone present in females when naturally produced, synthesized from cholesterol and produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex[2]. There are two peaks of secretion: one just before ovulation and one during the mid-luteal phase. In blood stream, 98% of estrogen circulates bound to sex hormone binding globulin[3,4]. Estrogens facilitate the growth of the ovarian follicles and increase the motility of the uterine tubes. They increase uterine blood flow and have important effects on the smooth muscle of the uterus[5]. Globally several investigators have been focused on serum E2 level in COH to improve the outcome of IFV[1,6,7,8]. In the Gaza Strip only one study speculates the role of antimullerian hormone as a predictor of ovarian reserve and

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ovarian responses in IVF candidates[9]. However, there were no previous studies on the role of serum E2 in COH or even in the IVF process. Therefore, this study is the first to assess the E2 levels during COH influence the ovarian response and pregnancy outcome in IVF women from Gaza Strip.

Materials and Methods

This prospective cohort study consisted of 75 women undergoing COH program and aged between 20-40 years without history of other diseases. The subjects were recruited from Al-Basma fertility Center in Gaza City in the period September 2011 to November 2011. Each patient gave informed consent to participate in the study. The criteria for inclusion were as follows: (i) age 20–40 years, (ii) regular menstrual cycle, (iii) not on hormone therapy for three months and (iv) have not been subject to surgical operation in the reproductive system. This study was approved by the Ethical Committee of the institution. All participants were guaranteed confidentiality, and only the principal investigator has full access to the data.

Sonography, Blood Sampling and IVF Protocol

The vaginal sonography was performed in the second day of the menstrual cycle. Blood samples were collected on the third day of the menstrual cycle. Serum samples were then separated by centrifugation at 3500 rpm for 10 minutes and stored at -20°C until use. All serum samples were submitted to E2 determination using Diagnostic Systems Laboratories Inc ELISA kit for E2. There were two protocols used during ovarian hyperstimulation, the first one is long protocol, which relies on pre-stimulation pituitary down regulation using GnRH agonists in daily intermittent or depot formulations, the second one is short protocols using GnRH antagonist during the late follicular phase of the stimulation cycle have been utilized. Adding recombinant LH to recombinant FSH protocols, when starting antagonists, as a strategy to increase oocyte yield and improve pregnancy rates. Human chorionic gonadotropin (hCG) was injected at a dose of 5000 or 10,000 IU. Oocyte retrieval for IVF was then typically scheduled for 30–34 hr thereafter. Then the fertilized oocyte was placed in G1 media for 3-4 days then in G2 media before rewind.

Statistical analyses

Simple distributions of the study variables and cross tabulation were applied. One-way ANOVA test was used for evaluating the relation between hormone level and qualitative variables. Person correlation test was applied. The results in all the above mentioned procedures were accepted as statistically significant when the p-value was less than 5% ($p<0.05$).

Results

The study population comprised 75 females who had seeking in IVF at Al-basma fertility Center in Gaza City, Gaza Strip. Age classification of the study population revealed that more than half of woman 42 (56.0%) were 26-35 years old. The age was ranged from 20-42 years with mean of 29.2±5.9 years (Table I). Clinical data showed that most of the interviewed woman 46 (61.3%) claimed that their husbands were the main cause of infertility. Forty six (61.3%) women do not have children. Around half of population 38 (50.7%) had undergone repeated IVF; out of them 30 (40.0%) had repeated IVF 2-4 times. The result of IVF was positive in 27 (36.0%) of the study population.

Table I

Clinical data of the study population (n=75)

Clinical characteristics	n	%
Age (year)		
< 25	20	26.7
26-35	42	56.0
>35	13	17.3
Mean ±SD (years)	29.2 ± 5.9	
Range (years)	20-42	
Cause of infertility		
Husband	46	61.3
Wife	11	14.7
Idiopathic	14	18.7
Both partner	4	5.3
Number of children		
No children	46	61.3
One child	19	25.3
Two children	7	9.3
Three children	2	2.7
Four children	1	1.3
Repeated in vitro fertilization		
Yes	38	50.7
No	37	49.3
Number of IVF operation		
2-4	30	40.0
4-6	8	10.7
IVF result*		
Positive	27	36.0
Negative	45	60.0
No cleavage	2	2.7

* One case had no sperm

As indicated in table II, the mean levels of E2 showed the highest value of 2194.4 (pg/ml) at age group 26-35 years. However the difference in E2 levels between the age groups was not significant (F=0.940 and P=0.395). According to the number of oocytes retrieved upon stimulation by menotrophin (FSH 75IU, LH 75 IU), the study population was divided into poor, normal, good and high responders. Fifteen (20%), 21 (28%), 32 (42.6%) and 7 (9.3%) women gave <4, 4-8, 9-16 and >16 oocytes during ovarian stimulation of IVF program respectively. When related to E2, its level was generally increased with increased ovarian response, showing <4, 4-8, 9-16 and >16 oocytes at mean E2 levels of 1642.7, 1665.1, 2156.8 and 1798.7 pg/ml, respectively, but this change was not significant (F=0.219 and P=0.883). The mean level of E2 showed its maximum value of 2143.6 pg/ml in positive pregnancy (indicated by â-hCG hormone test). However, the difference in E2 levels among the different categories of IVF outcome was not significant (F=0.423 and P=0.656).

Table III relates the three groups of E2 levels (group A: <1000 pg/ml, group B: 1000-2000 pg/ml and group C: >2000 pg/ml) to mean age, number of oocytes and embryo. Estraddiol level was inversely related to woman age (F=8.709 and P=0.000). The mean numbers of total and mature oocytes, and embryo were significantly increased with increased levels of E2 (P=0.000) showing a good response in group B (E2 level=1000-2000 pg/ml) where the mean numbers of total and mature oocytes, and embryo were 10.49±4.7 and 7.89±3.5, and 4.03±1.3, respectively.

Table II

Estradiol level in relation to investigated parameters study population (n=75)

	Variable	n	Estradiol (E2) (pg/ml)	F	P-value
Age (year)	(<25)	20	1750.0	0.940	0.395
	(26- 35)	42	2194.4		
	(> 35)	13	1113.6		
Ovarian response	<4 oocytes(Poor responders)	15	1642.7	0.219	0.883
	4-8 oocytes(Normal responders)	21	1665.1		
	9-16 oocytes(Good responders)	32	2156.8		
	> 16 oocytes(High responders)	7	1798.7		
IVF outcome	Positive	27	2143.6	0.423	0.656
	Negative	36	1791.7		
	No cleavage	2	578.0		

All values are expressed as mean ±SD.
P- value less than 0.05 was considered for statistical significance.
F: ANOVA test, E2: Estradiol.
Positive: pregnancy occurred, Negative: no pregnancy

Table III

Estradiol level in relation to age, number of oocytes and embryo

Variable	Group A (E2<1000) (pg/ml)	Group B (E2 1000-2000) (pg/ml)	Group C (E2>2000) (pg/ml)	F	P - value
Age (year)	33.9±5.9	27.8±4.9	27.8±5.6	8.709	0.000
Total number of oocytes	3.47±2.5	10.49±4.7	11.47±5.4	17.761	0.000
Number of mature oocytes	2.88±1.97	7.89±3.5	7.5±3.89	14.216	0.000
Number of immature oocytes	0.71±0.99	3.0±2.62	4.5±2.5	12.230	0.000
Number of embryos	2.1±1.2	4.03±1.3	3.8±1.4	13.676	0.000

All values are expressed as mean ±SD.
P- value less than 0.05 was considered for statistical significance.
F: ANOVA test E2: Estradiol.

As illustrated in Table IV, the number of mature oocytes showed positive correlations with E2 level and number of embryos (r=0.159, P=0.177 and r=0.890, P=0.000) and negative correlations with age (r=-0.276, P=0.017).

Table IV

Correlation between the number of mature oocytes, and age, estradiol level and number of embryo

Variable	r	P-value
Age	-0.276	0.017
E2 (pg/ml)	0.159	0.177
Number of embryos	0.890	0.000

P- value less than 0.05 was considered for statistical significance.
r: Correlation coefficient.
E2: Estradiol.

Discussion

In the Gaza Strip, women seek IVF mostly when the man had fertility problems and the process of pregnancy is delayed. Data on IVF are rare in Gaza Strip. There is only one recent study on the role of antimullerian hormone as a predictor of ovarian reserve and ovarian response in IVF candidates in Gaza Strip[9]. The present study is the first to assess serum estradiol level and it is possible role as an indicator of ovarian response and pregnancy outcome during COH in women from Gaza Strip. The results presented in this study dealt with 75 women enrolled in IVF programs. The mean age of women (29.2 years) was close to that reported in Egyptian (29.0 years) and Iranian (29.1 years) studies[10,11]. But lower than that reported from the Maryland and South Korea (34.0 years)[8,12]. The younger age of women seeking IVF in developing countries, including Gaza Strip, could be explained in the context of social habits where most families have the desire to have children immediately after marriage and they encourage women to undergo IVF. This is supported by the finding that more than half of the study population had no children and had undergone repeated IVF. The outcome of IVF was positive in 27 (36.0%) of the study population. This finding was in agreement with the results of studies from the Netherlands and South Korea which showed that 36.0% and 32.5% of women undergoing IVF achieved viable pregnancy[8,13]. However, the pregnancy rate among patients who had an IVF trial in different settings was 23.1%. Nevertheless, IVF treatment outcome is highly variable and difficult to predict[10]. When E2 related to the age of the study population, estradiol showed the highest mean level of 2194.4 (pg/ml) in the age group of 26-35 years, indicating the best ovarian response at this age interval. Such finding is in agreement with that previously reported[7,8,14]. Pearson's correlation test revealed negative and positive significant correlations between number of mature oocytes and age and number of embryo, respectively. Similar results were observed [8,9,12,16]. This coincides with the prevailing concept of female reproductive aging suggesting that the decline of the oocytes pool determines the age-dependent loss of female fertility[16]. However, chronological age at the final stage of reproductive aging (i.e., the occurrence of menopause) shows a considerable individual variation, it is most likely that women differ with regard to the status of the oocytes pool at a given age. Regarding ovarian response in relation to estradiol levels, the present finding illustrated that the mean levels of E2 showed a generally increase parallel to the total number of oocytes in poor, normal, good and high responders with maximum level in good response (2156.8 pg\ml) which coincide with E2 level at age 26-35. Such E2 level at this particular age (26-35 years) could by used as primary indicator of good ovarian response. Similar successive increase in the total number of oocytes as a result of increasing E2 levels in responder women attending IVF [6,8,12,17]. E2 level showed its maximum value of 2143.6 pg/ml in positive pregnancy. This reinforced the previous finding that such E2 level, which was postulated to be used as primary indicator of good ovarian response, could enhance the chance of pregnancy. Higher levels of E2 in pregnant women undergone IVF were recorded [9,7,13, 18,19]. At E2 level 1000-2000 pg/ml, the highest number of mature oocytes and embryo were detected. Similar result was reported [6,8,12]. From clinical point of view, one can say that at E2 level of 1000-2000 pg/ml, good ovarian response and high chance of pregnancy could be achieved. Consequently, for women to undergo a more likely successful IFV program, it is recommended to optimize E2 level at 1000-2000 pg/ml.

Conclusion

Estradiol level 1000-2000 pg/ml at mean age of 27.8±4.9 years, could be a predictor of ovarian response and pregnancy outcome during controlled ovarian hyperstimulation. Consequently, for women to undergo a more likely successful IFV program, it is recommended to optimize E2 level at 1000-2000 pg/ml.

Conflict of Interest: The authors have no relevant conflicts of interest to declare.

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