The Effect of Serum Estradiol Level at the Time of HCG Injection on the ICSE Outcome

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Abstract

Background: In addition to multi-follicular development, controlled stimulation of ovaries is associated with a high serum level of estradiol (E2). The increased serum E2 level during COS may therefore be associated either with an increased chance of pregnancy or an impaired reproductive outcome secondary to changed endometrial receptivity.

Objective: To assess the effect of serum estradiol level at the day of hCG administration on the success of intracytoplasmic sperm injection (ICSI) cycles

Patients and Method: A retrospective study, in which a total of 235 women who were undergo a single ICSI cycle were introduced in this retrospective study, All patients were received gonadotrophin agonist either short or long protocol. The patients were classified at the day of hCG administration into three groups according to serum E2 level group A <1000pg/ml, group B 1000-3000pg/ml and group C >3000pg/ml.

Results: The mean number of retrieved oocytes and embryos obtained in group C were more than in group A and B but clinical pregnancy rate was higher in group B than in group A and group C which is statistically significant.

Conclusion: Although there is no high quality evidence to support a positive correlation between serum E_2 levels and IVF-ICSI products, the E2 level in the serum may has a level –dependent effect on the success of pregnancy. The optimal range of serum E2 is 1000-3000pg/ml is associated with higher rate of clinical pregnancy.

Keyword: *IVF-ICSI, Ovarian hyperstimulation syndrome, E2.*

Introduction

In contemporary IVF procedure, there is a positive correlation between retrieved oocytes and rate of live birth. Therefore, the live birth rate which based on obtaining enough mature follicles which contains critically well oocytes, may reflect the success rate [(1, 2)].

There are many method can be used to obtain a large numbers of follicles. In the past decade, many centers use the ovulation induction regimens, mainly those using gonadotropins and GnRH analogues. COH lead to achieve multiple oocytes, that result in supraphysiologic E_2 levels and this has a direct effect on endometrial implantation. Increased E2 concentration on the day of hCG injection have a positive effects on IVF-ICSI results or a low outcome, caused by endometrial receptivity disruption [(3-6)].

Many studies have reported that supraphysiological E_2 concentrations may affect the chance of pregnancy. On the other hand, an other studies suggest that high serum E_2 level have no effect on endometrial receptivity[(5,7,8)]. In the bases of these results, the effectiveness of high

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E_2 levels on the day of hCG administration have controversial association, which in turn affect IVF-ICSI outcome. So we need to assess the association between E_2 levels on the day of hCG administration and IVF-ICSI outcome, in which GnRH analogues were used for down regulation

The aim of the study: This retrospective study was performed to assess the effect of serum estradiol level at the day of hCG administration on ICSI patients who were undergone controlled ovarian stimulation (COS).

Patients and Method

This study was performed in the fertility center of Al-Sader teaching hospital in Al-Najaf city.

From February 2018 to February 2019, (235) couples underwent ICSI cycles because of tubal, ovarian, male and unexplained infertility. Exclusion criteria for the study included the presence of known intrauterine anomalies and ovarian stimulation other than GnRH agonist protocol (short & long).

In cycle day 2-3 hormonal assay in form of (FSH, LH, Prolactin,and E2) done along with vaginal ultrasound was carried to identify the antral follicle amount and to eliminate the existence of ovarian cysts or other pelvic pathology and seminal fluid analysis for the partner reviewed then according to above results, stimulation protocol was selected. Patients were screened and were negative for infection with HIV, hepatitis B and C.

Stimulation Protocol: Gonadotrpin releasing hormone (GnRH) agonist used for all women. In the current study we used two main protocols;

1. **Long Protocol:** GnRh agonist (3.7 mg) was administered to suppress the pituitary gland on day 21 of the menstrual cycle (middle luteal phase). Vaginal ultrasound performed on day 2 or 3 of the next menstrual cycle to confirm the absence of any functional ovarian cyst larger than 10 mm. Gonadotropin stimulation consisted of 1-2 ampoules per day of recombinant FSH (Gonal-F^R, 75 IU). The initial dose was individualized according to the patient›s age, the reserve ovarian test was adjusted according to its response after the first 4-5 days of stimulation.
2. **Short Protocol or Flare-up protocol:** Involves agonist treatment (decapeptyl 0.1mg S.C daily) for 10-14 days started one day before or concurrently with ovarian gonadotropines stimulation and the dose of gonadotropines adjusted as the long protocol(as described above).

For both protocols, follicular development was examined by vaginal ultrasonography and serum E2 level starting from cycle day 8 or 9 and then every other day. At the day of human chorionic gonadotrophine (hCG) administration, serum levels of E2 were measured.

Serum concentration of estradiol (E2) was measured by (VIDAS measurement – ELFA technique (Enzyme Linked Fluorescent Assay) bio Meriexu sa/France).

Serum E2 level at the day of hCG administration were classified into three groups:-

A. (<1000pg/ml),
B. (1000-3000pg/ml),
C. (>3000pg/ml).

thirty- four to 36 hours following 10,000 IU of hCG administration, oocyte retrieval was performed under general anesthesia and by guided of transvaginal ultrasound. After aspiration, oocytes were screened and injected (ICSI). The fertilization rate calculated as (number of embryos obtained per number of injected oocytes). After embryo transfer, all patients were given luteal phase support using progesterone vaginal pessaries 400mg twice a day(Cyclogest ^{R}) Started at day of ovum pick up for 16 days and was continued if pregnancy occurred up to 10-12 weeks. After 2 weeks the serum level of βhCG was checked after embryo transfer, and clinical pregnancy was confirmed two weeks later by sonographic

Detection of the gestational sac and positive heart beat. Clinical pregnancy rate calculated as the number of women with clinical pregnancy per number of women that did embryo transfer.

Embryos were classified according to the number of blastomeres, percentage of fragmentation & blastomere appearance as type 1, 2, 3 or 4.

Regarding the equality of embryos, good quality embryos are those of grade 1 and 2, while bad quality embryos are those of grade 3 and 4.

Statistical Analysis: This study was analyzed statistically by using SPSS (statistical package for social science) version 20. In which we use chi sequare (x^2)

For categorical data and independent sample T-test for measurement data. We set P value <0.005 as significant.

Results

Table (1) shows demographic characteristic of the women according to E2 level in three groups were no significant difference in their age, BMI, endometrial thickness and hormonal assay (FSH,LH, E2 and Prolactin) hormone.

Table(1): Demographic characteristics according to E2 at HCG level in the studied group.

Characteristic	E2 at HCG			P value
	<1000 Mean±SD	1000-3000 Mean±SD	>3000 Mean±SD	
Age/years	30.45±6.4	30.5±5.7	28.6±5.6	0.104
BMI(kg/m^2)	27.7±3.1	27.3±3	27.1±3.4	0.576
FSH(IU/L)	4.2±2.3	4.1±2.7	4.2±1.7	0.716
LH(IU/L)	2±1.4	2.7±1.8	2.3±4	0.319
E2(pg/ml)	27.8±14.3	35.6±16.4	45.6±22.5	<0.001
Prolactin(ng/ml)	23.5±8.6	26.7±23.7	24.3±8.2	0.514

Table (2) shows the mean number of retrieved oocytes (p=0.001), no. metephase II oocytes (p=0.001) and number of embryos obtained (p=0.001) were significantly higher in group C than in groups A & B.

Percentage of metaphase were not statistically different among three groups (p=0.284).

Fertilization rate (number of embryos obtained per number of injected oocytes) was higher in group A (0.6067± 0.2469) than in group B (0.5170±0.2615) and group C(0.4330±0.2865) but it was not statistically significant (p=0.088).

Table (2): Demonstrates the comparison of the outcome according to E2 at HCG level.

Characteristic	E2 at HCG			P value
	<1000 Mean±SD	1000-3000 Mean±SD	>3000 Mean±SD	
No. of oocyte	6.1±3.6	9.4±4.8	13.1±6.2	<0.001
No. of M2	2.9±2	4.4±2.1	5.8±2.6	<0.001
No. of embryo	2±0.9	2.5±0.7	2.7±0.5	<0.001
Percentage of M2	52.7±23.4	50.1±17	47.3±14.1	0.284
Fertilization rate	0.606±0.246	0.517±0.261	0.433±0.286	0.088

Table (3) shows the types of protocols and type of infertility were not significantly different among the studied group. There is significant difference between <1000 and 1000-3000 and <1000 and >3000 while there is no significant difference between 1000-3000 and >3000

Table (3) The type of protocol and indication of ICSI according to level of E2 at HCG

Variable		E2HCGG			P value
		<1000	**1000-3000**	**>3000**	
Protocol	Long	924.3%	2156.8%	718.9%	0.385
	Short	3517.7%	10653.5%	5728.8%	
Indication	Female	1719.3%	4955.7%	2225.0%	0.980
	Male	2418.3%	7053.4%	3728.2%	
	Unexplained	318.8%	850.0%	531.2%	

Table (4) shows clinical pregnancy rate (calculated per embryo transferred) was higher in group B 49/127(37.8%) than in groups A 5/44(9.1%) and C 20/64 (29.7%). It was statistically significant (p=0.002).

Table (4): Relation between E2 HCG level and clinical pregnancy rate.

		Fertilization		P value
		Negative	**Positive**	
E2HCGG	<1000	39	5	0.002
		90.9%	9.1%	
	1000-3000	78	49	
		62.2%	37.8%	
	>3000	44	20	
		70.3%	29.7%	
Total		**161**	**74**	
		69.8%	**30.2%**	

Table (5) regarding the quality of embryos, good quality embryos were more frequent in group B (113) than group C (57) and group A (38) this difference was not statistically significant.

Table (5): shows the comparison of the quality of embryos among three groups.

		Grading		P value
		Good quality	**Bad quality**	
E2HCGG	<1000	38	6	0.833
		86.4%	13.6%	
	1000-3000	113	13	
		89.7%	10.3%	
	>3000	57	7	
		89.1%	10.9%	

Discussion

IVF-ICSI is tried in the world very widely and controlled stimulation of the ovaries is very beneficial to achieve these outcomes. COH may improve the success of fertilization and lead to large number of embryos that can be transferred to mother to be happy with a nice and acceptable success rates [9.10].

In the present study the number of retrieved oocytes and number of mature follicles (M II) is higher in group C (E2>3000pg/ml) than group A (E2<1000pg/ml) and B (E21000-3000pg/ml), this mainly depends on appropriate follicular development in patients with high E2 level. [11]

The embryos, number that obtained was higher in B and C than in group A which is statistically significant, this result agree with Jones et al.,1983; and Dor et al., 1986.[12, 13]

Regarding the rate of fertilization there is no statistical significant difference were found in between group A, B and C although it is decrease with higher peak E2 level this also shown by previous study [6,13]. This result disagree with Chenentte et al., study in 1990 who found that the fertilization rate to be unrelated to the serum E2 concentration or the number of oocytes retrieved. [14]

Regarding pregnancy rate this study demonstrate that the pregnancy rate increase as serum E2 level increase up to 3000pg/ml and start to decrease when concentration rise >3000pg/ml, this outcomes are also obtained by Blazar et al., 2004[15] who conducted that the rate of pregnancy is become higher with increment in the peak of serum E2 and continue until plateau level is reached at nearly 2500pg/ml. this also showed by Mitwally et al., 2006.[16]

The is a study performed by Simon et al., 1995 and is found that there is impairment of implantation occur with a high serum estradiol concentration in spite of the presence of large number of oocytes. [17] This may caused by direct negative effect of increased estradiol concentration on endometrial receptivity by tissue and cellular damage because of alteration in the ratio of estrogen to progesterone as shown in recent study were serum E2level significantly correlated with the number of oocytes obtained (p=0.001).

There is evidence that optimizing serum E2 level on hCG administration may improve the treatment outcome after ICSI but that low or high E2level have negative impact. So, a good estrogenization of the uterus is important for preparing the uterus to implant the emberyo.

It also caught our attention that, in case with decreased E2 level on the day of hCG administration (<1000pg/ml) had pregnancy rate significantly lower than in group B and C respectively, this agree with study made by Ng et al.[12], this attributed to lower number of retrieval oocytes, number of metaphase II and ultimately embryos transferred as shown in table (2) is a possible cause for lower pregnancy rate.

The effects of E2 level on endometrial receptivity should be further evaluated in future studies.

Conclusion

We can conclude that the success of ICSI cycle is affected by serum E2 level. However, the extreme of serum E2 level associated with poor ICSI outcome.

Recommendation: Since the concentration of estradiol are at the ends of extreme level can affect pregnancy outcome, so we advocate the possibility of canceling such cycle and frozen embryo for transfer in subsequent fresh cycle. Nevertheless, larger randomized studies were recommended with large sample size and for longer duration of follow up to define appropriate protocols in clinical practice of ICSI cycle.

Conflicts of Interest: No

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