Causes of fluctuation of estradiol level during ICSI cycle

اسباب تذبذب مستوى الاسترادايول خلال دورة الحقن المجهري للحيمن

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Abstract :

Eighty eight subfertile women involved in the study . All of them were subjected to controlled ovarian hyperstimulation during intracytoplasmic sperm injection cycle(ICSI) . Measurement of serum estradiol (E2) level was done at day 2 of their menstrual cycles .Second reading was done at day of HCG injection (where ovum size reach 16-22 mm).Third reading of serum E2 was done at the day of pickup of the ovum (before the pickup) and at the time of pickup follicular fluid(FF) E2 was also measured. The results show that E2 in blood in day 2 is significantly higher in those with BMI≥25. The result show significant difference in E2 in blood which is higher in polycystic ovarian syndrome (PCO) in day2 of cycle and higher in non PCO group in day of HCG. There was significant positive correlation between the level of E2 in blood in day of pickup and in the FF .There reveal highly significant difference in the E2 level in blood in different days. The result show highly significant difference in the E2 level in different days in PCO group. The result show highly significant difference in the E2 level in different days in non PCO group. Correlation between serum E2 in day of pickup and E2 in follicular fluid among PCO group show significant positive correlation between E2 in day of pickup and E2 in FF. Correlation between serum E2 in day of pickup and E2 in FF among non PCO group show significant positive correlation between E2 in day of pickup and E2 in FF.

الخلاصة:

ثمانية و ثمانون امراة عقيمة اشتركن في الدراسة. جميع النساء خضعن لتنشيط المبيض ضمن برنامج الحقن المجهري للحيمن.قياس تركيز هرمون الاسترادايول في الدم تم في اليوم الثاني للدورة الحيضية للنساء و في اليوم الذي يصل حجم البيوض 16-22 ملم و في اليوم الذي يتم فيه سحب البيوض .بعد سحب البيوض قياس تركيز الهرمون في السائل الحويصلي المبيضي . النتائج اظهرت زيادة معنوية في تركيز الاسترادايول في الدم في اليوم الثاني للدورة الحيضية عند النساء ذوات الوزن الاعلى و زيادة معنوية في نفس اليوم عند النساء ذوات التكيس المبيضي و زيادة معنوية عند النساء غير ذوات التكيس المبيضي في يوم الذي يصل حجم البيوض 16-22 ملم . كذلك اظهرت النتائج علاقة معنوية بين تركيز هرمون الاسترادايول في الدم مع تركيز الهرمون في السائل الحويصلي المبيضي في يوم سحب البيوض في كل المجاميع.

Introduction :

Estrogens are hormones that are important for sexual and reproductive development, mainly in women. They are also referred to as female sex hormones. The term "estrogen" refers to all of the chemically similar hormones in this group, which are estrone, estradiol (primary in women of reproductive age) and estriol (1,2). Estrogen in women is produced mainly in the ovaries, but it is also produced by fat cells and the adrenal gland. Estrogen is involved in the onset of puberty and development of secondary sex characteristics, such as breasts, and pubic hair. Estrogen also helps regulate the menstrual cycle, controlling the growth of the uterine lining during the first part of the cycle (2,3). If the woman's egg is not fertilized, estrogen levels decrease sharply and menstruation begins. If the egg is fertilized, estrogen works with progesterone, another hormone, to stop ovulation during pregnancy. Estrogen controls lactation and other changes in the breasts in adolescence and during pregnancy. During pregnancy, the placenta produces estrogen, specifically the hormone estriol (2,4). Estrogen is instrumental in bone formation, working with vitamin D, calcium and other hormones to effectively break down and rebuild bones according to the body's natural processes. Estrogen also plays a role in blood clotting, maintaining the strength and thickness of the vaginal wall and the urethral lining, vaginal lubrication. It affects skin, hair,

mucous membranes and the pelvic muscles (3,5). The hormone also affects the brain .Men produce estrogen as well, but at lower levels than women. In men, estrogen is thought to affect sperm count .Estrogen levels fall after menopause, or when a woman stops menstruating. This reduction in estrogen production can cause symptoms such as hot flashes, vaginal dryness and loss of sex drive. Other conditions that can cause estrogen levels to drop include hypogonadism(1,2,5). Extreme exercise and anorexia can also cause a decrease in estrogen levels because women with low body fat may not be able to produce adequate amounts of estrogen. Estrogen levels also decrease after childbirth(1,2).

The three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). Estradiol is the predominant estrogen during reproductive years .

During menopause, estrone is the predominant circulating estrogen and during pregnancy estriol is the predominant circulating estrogen(2,5). Though estriol is the most plentiful of the three estrogens it is also the weakest, whereas estradiol is the strongest with a potency of approximately 80 times that of estriol. Thus, estradiol is the most important estrogen in non-pregnant females who are between the menarch and menopause stages of life. Another type of estrogen called estetrol (E4) is produced only during pregnancy(6). All of the different forms of estrogen are synthesized from androgens, specifically testosterone and androstenedione, by enzyme aromtase. Estrogens, in females, are produced primarily by the ovaries, and during pregnancy FSH stimulates the ovarian production of estrogens by the granulsa cell of the ovarian follicles and corpora lutea(4,5,6) . Some estrogens are also produced in smaller amounts by other tissues such as the liver, adrenal glands, and the breasts. These secondary sources of estrogens are especially important in postmenopausal women. Fat cells produce estrogen as well. In females, synthesis of estrogens starts in theca interna cells in the ovary, by the synthesis of androstenedione from cholesterol(5). Androstenedione is a substance of weak androgenic activity which serves predominantly as a precursor for more potent androgens such as testosterone as well as estrogen. This compound crosses the basal membrane into the surrounding granulosa cells, where it is converted either immediately into estrone, or into testosterone and then estradiol in an additional step (7,8). The conversion of androstenedione to testosterone is catalyzed by 17β-hydroxysteroid dehydrogenase , whereas the conversion of androstenedione and testosterone into estrone and estradiol, respectively is catalyzed by aromatase, enzymes which are both expressed in granulosa cells. In contrast, granulosa cells lack 17α-hydroxylase and 17,20-lyase, whereas theca cells express these enzymes and 17β-HSD but lack aromatase(6,7). Hence, both granulosa and theca cells are essential for the production of estrogen in the ovaries. Estrogen levels vary through the menstrual cycle, with levels highest near the end of the follicular phase just before ovulation (7,8). The actions of estrogen are mediated by the estrogen receptor (ER), a dimeric nuclear protein that binds to DNA and controls gene expression. Like other steroid hormones, estrogen enters passively into the cell where it binds to and activates the estrogen receptor. The estrogen:ER complex binds to specific DNA sequences called a hormone response element to activate the transcription of target genes .Since estrogen enters all cells, its actions are dependent on the presence of the ER in the cell. The ER is expressed in specific tissues including the ovary, uterus and breast. The metabolic effects of estrogen in postmenopausal women has been linked to the genetic polymorphism of the ER(9,10,11).

Aim of the study: is to detect the cause of fluctuation of estradiol level during ICSI cycle .

Material and method:

Eighty eight subfertile women with ovulatory cause of infertility were involved in the study . These women were selected randomly from those attending fertility centre in Al-Sader Teaching Hospital in Al-Najaf city during the period from January 2014 to October 2015. The mean age of women was 28.9±6 years (range 19-42). Measurement of serum E2 level was done at day 2 of their menstrual cycles before the start with controlled ovarian hyperstimulation. All women participated in this study were subjected to controlled ovarian hyperstimulation method using the short protocol (antagonist cycles) by daily dose GnRH antagonist alongside with gonadotrophin stimulation. Transvaginal ultrasound scan was performed to measure the size of the leading follicles

and to estimate when it will reach (16-22) mm in diameter, then hCG injection to trigger ovulation , the second reading of E2 done before hCG injection . Ovum collection was performed under general anesthesia under ultrasound guide. The ova and their FF were taken and perform isolation of ova and transported into special media,5 milliliters of FF were collected . Measurements of blood and FF E2 done using Elisa technique . At the day of pickup of oocytes blood and follicular fluid level of estradiol measured . Enzyme linked immunosorbent assay (ELISA) system and kits for hormone measurement, kits used for ELISA technique for detection of estradiol level.

Statistical analysis :

Statistical analysis was done by using SPSS(statistical package for social science) version 20. In which we use independent sample T-test, paired T-test and pearson correlation coefficient accordingly. We set (p value ≤ 0.05) as significant

Result:

Comparison between different parameters according to BMI. Table 1 show no significant difference in E2 level between those with BMI<25 and ≥25 in all measures except E2 in blood in day 2 which is significantly higher in those with BMI≥25. (p value ≤ 0.05)

Table(1) Comparison between different parameters according to BMI.

Parameter	BMI<25(n=22)	BMI≥25(n=66)	P value
Age/years	27.36±6.9	29.48±5.7	0.156
E2bd1(pg/ml)	37.2±9.3	46.3±17.8	0.025
E2bd2(pg/ml)	2273.5±748.4	2148.8±953.5	0.578
E2bd3(pg/ml)	416.9±221.7	387.06±218.4	0.582
E2ff(pg/ml)	2884.09±214.7	2767.7±628.5	0.398

E2bd1:serum estradiol at day 2 of menstrual cycle
E2bd2: serum estradiol at day of HCG injection
E2bd3: serum estradiol at day of pickup of ova
E2ff: follicular fluid estradiol at day of pickup

Comparison between different parameters according to ovarian texture. Table 2 show significant difference in E2 in blood which is higher in PCO in day2 and higher in normal group in day of HCG. No significant difference in E2 in day of pickup in blood and in follicular fluid. (p value ≤ 0.05)

Table(2) Comparison between different parameters according to ovarian texture.

Parameter	PCO(n=44)	Normal (n=44)	P value
E2bd1(pg/ml)	48.15±17.65	40.07±14.43	0.021
E2bd2(pg/ml)	1936.13±996.08	2423.8±734.9	0.011
E2bd3(pg/ml)	403.6±251.96	385.45±181.2	0.699
E2ff(pg/ml)	2719.9±739.2	2873.6±257.4	0.196

E2bd1:serum estradiol at day 2 of menstrual cycle
E2bd2: serum estradiol at day of HCG injection
E2bd3: serum estradiol at day of pickup of ova
E2ff: follicular fluid estradiol at day of pickup

E2 level in blood in different days. In table 3 there was highly significant difference in the E2 level in different days. (p value ≤0.05)

Table(3) E2 level in blood in different days.

n=88		Mean	Std. Deviation	P value
	E2bd1(pg/ml)	44.1170	16.54103	<0.001
	E2bd2(pg/ml)	2180.0000	904.17669	
	E2bd1(pg/ml)	44.1170	16.54103	<0.001
	E2bd3(pg/ml)	394.5273	218.38652	
	E2bd2(pg/ml)	2180.0000	904.17669	<0.001
	E2bd3(pg/ml)	394.5273	218.38652	

E2bd1:serum estradiol at day 2 of menstrual cycle
E2bd2: serum estradiol at day of HCG injection
E2bd3: serum estradiol at day of pickup of ova
E2ff: follicular fluid estradiol at day of pickup

E2 level in blood in different days among PCO group. In table 4 there was highly significant difference in the E2 level in different days in PCO group. (p value ≤0.05)

Table(4)) E2 level in blood in different days among PCO group.

PCO group E2 n=44		Mean	Std. Deviation	P value
	E2bd1(pg/ml)	48.1555	17.65912	<0.001
	E2bd2(pg/ml)	1936.1364	996.08640	
	E2bd1(pg/ml)	1936.1364	996.08640	<0.001
	E2bd3(pg/ml)	403.6000	251.96068	
	E2bd2(pg/ml)	48.1555	17.65912	<0.001
	E2bd3(pg/ml)	403.6000	251.96068	

E2bd1:serum estradiol at day 2 of menstrual cycle
E2bd2: serum estradiol at day of HCG injection
E2bd3: serum estradiol at day of pickup of ova
E2ff: follicular fluid estradiol at day of pickup

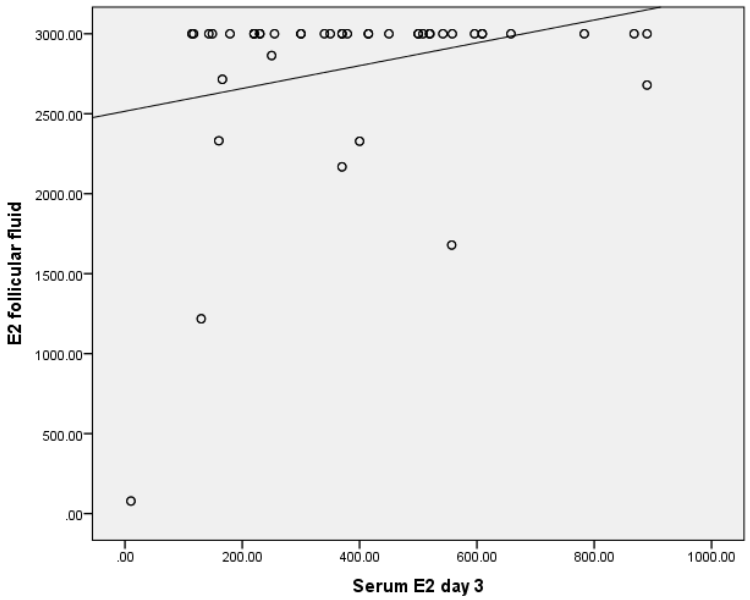
E2 level in blood in different days among non PCO group In table 5 there was highly significant difference in the E2 level in different days in non PCO group. (p value ≤0.05)

Table(5) E2 level in blood in different days among non PCO group

Non PCO group E2 n=44		Mean	Std. Deviation	P value
	E2bd1(pg/ml)	40.0786	14.43446	<0.001
	E2bd2(pg/ml)	2423.8636	734.97643	
	E2bd1(pg/ml)	2423.8636	734.97643	<0.001
	E2bd3(pg/ml)	385.4545	181.22316	
	E2bd2(pg/ml)	40.0786	14.43446	<0.001
	E2bd3(pg/ml)	385.4545	181.22316	

E2bd1:serum estradiol at day 2 of menstrual cycle
E2bd2: serum estradiol at day of HCG injection
E2bd3: serum estradiol at day of pickup of ova
E2ff: follicular fluid estradiol at day of pickup

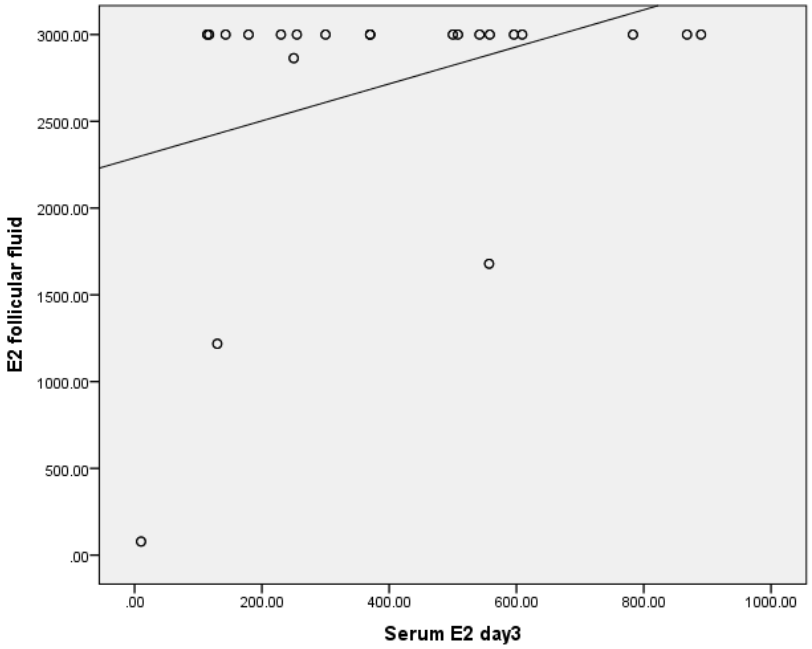
Correlation between E2 in blood and follicular fluid at day of pickup. There was significant positive correlation between the level of E2 in blood in day of pickup and in the follicular fluid as in figure 2.



r=0.280 P=0.008

figure(2) Correlation between E2 in blood and follicular fluid at day of pickup.

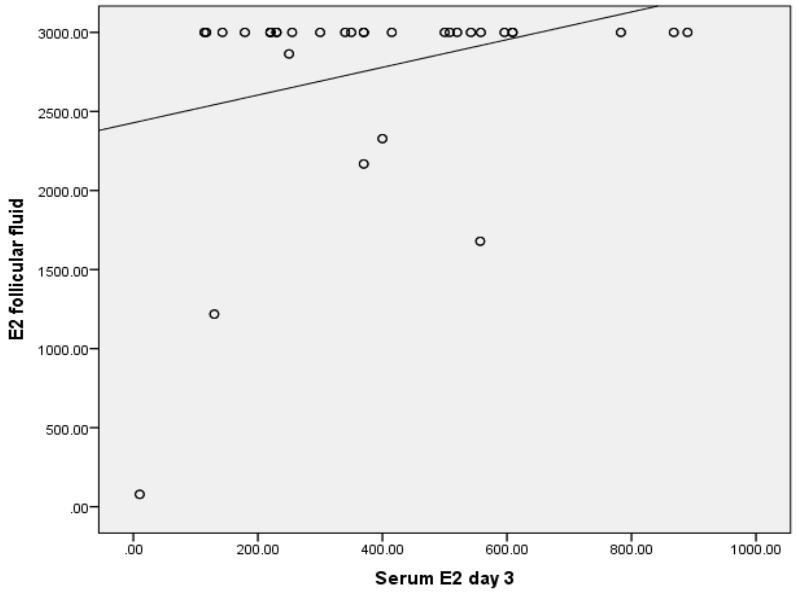
Correlation between serum E2 in day of pickup and E2 in follicular fluid among PCO group in figure 3 there is significant positive correlation between E2 in day of pickup and E2 in follicular fluid.



r=0.363 P=0.015

figure(3) Correlation between serum E2 in day of pickup and E2 in follicular fluid among PCO group

Correlation between serum E2 in day of pickup and E2 in follicular fluid among non PCO group in figure 4 there is significant correlation between E2 in day of pickup and E2 in follicular fluid



figure(4) Correlation between serum E2 in day of pickup and E2 in follicular fluid among non PCO group

Discussion:

The result show that E2 level in blood in day 2 of the cycle is higher in women with BMI ≥25 Kg/m2 . Fat cells produce estrogen, so that obese female may produce more estrogen than normal or underweight women . Also most of overwieght women have polycystic ovaries which produce more estrogen than non polycystic ovaries . Adipose tissue possesses aromatase, an enzyme that converts androstenedione to estrone and testosterone to estradiol and the excess of adipose tissue in obese patients create excess androgens and estrogens . Also PCOS is associated with obesity and hyperandrogen state (12,13) .

The results show significant difference in E2 in blood which is higher in PCO in day2. Polycystic ovaries which produce more estrogen than non polycystic ovaries , due to production of multiple small follicles in early stages of menstrual cycle. Also, increase in estradiol in women with polycystic ovarian syndrome due to enhanced peripheral aromatization of androgens to estrogens (mainly testosterone to E_2) in extraglandular tissues in the presence of androgen excess(14,15) .

E2 is higher in normal group in day of HCG. This could be due to that normal ovaries respond well to ovarian stimulation and produce more number of ova that enlarge well in preparation for ovulation (16,17).

There was highly significant difference in the E2 level in different days in PCO group and non PCO group .These results are due to that estradiol is produced in low level in the start of the menstrual cycle and increase progressively to reach its higher level near ovulation ,and this is true for polycystic and non polycystic ovaries . This effect is exaggerated due to effect of ovarian induction of ovulation . Before LH surge , estradiol level increase progressively in a manner proportional to the activity of aromatase system in granulosa cells(18,19).

There was significant correlation between the level of E2 in blood in day of pickup and in the follicular fluid both in PCO group and non PCO group .So that when E2 is some women is higher than other women so it is higher both in blood and FF .Also E2 in the period prior to ovulation decease in blood while increase sharply in FF in all groups of women . This could be due to metabolism of E2 . Its accumulation in FF can be of benift for ovulation .The estrogens synthesized

by follicular cells during the process of follicle maturation accumulate in follicular fluid , so that there is association between the hormonal content of FF and the degree of oocyte maturity . FF is a product of both the transfer of blood plasma constituents that cross the blood follicular barrier and of the secretary activity of granulosa and thecal cells .The analysis of FF components may also provide information on metabolic changes of the blood . After the onset of the ovulatory LH surge ovarian estradiol secretion decrease abruptly and consequently estradiol level in plasma are markedly decrease which is mainly due to inhibition of the synthesis of androgen precursors by thecal cells due to desensitization of these cells to this gonadotropic stimulus(20,21) .

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