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ORIGINAL ARTICLE

Serum Estradiol as a Predictor of Success of In Vitro Fertilization

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Abstract

Aim The aim of this study was to assess the role of total serum estradiol on the day of injection HCG, estradiol per mature follicle, and estradiol per oocytes retrieved (OR) on clinical pregnancy rate (CPR) and oocyte/embryo quality in assisted reproduction.

Materials and Methods A retrospective review of 342 in vitro fertilization cycles with normal ovarian reserve in women who underwent long GnRH agonist protocol was included. The outcomes assessed are number of OR, number of mature oocytes (MO), number of oocytes fertilized (FO), fertilization rate, number of embryos cleaved (EC), cleavage rate (CR), number of Grade I embryos (E), number of cryopreserved embryos (CPE), and CPR. The Estradiol/follicle ratio (E2/fol) was defined as estradiol level per mature follicle >14 mm in diameter. Estradiol/oocyte (E2/O) ratio was defined as estradiol level per OR. These two ratios were categorized by the 25th percentile into four groups.

Results A positive correlation was seen between E2/fol and OR ($r = .334$, p value $= .0001$), MO ($r = .335$, p value $= .0001$), FO ($r = .222$, p value $= .002$), and CPE ($r = .289$, p value $= .0001$). Increased CPR was seen in Group C (E2/fol = 200–299.99) compared to Group A, B, and D (p value $= .033$). With E2/O ratio, negative correlation was seen between E2/O and OR ($r = -.281$, p value $= .002$), MO ($r = -.296$, p value $= .008$), FO ($r = -.220$, p value $= .003$), EC ($r = -.211$, p value $= .004$), Grade 1 embryo ($r = -.216$, p value $= .001$), and CPE ($r = -.206$, p value $= .005$). No difference in FR, CR, or CPR was seen. No difference was seen in CPR with total serum estradiol.

Conclusions In conclusion, serum estradiol is an important determinant of IVF success. While total serum estradiol does not exert any positive or negative influence on IVF outcome, estradiol per mature follicle and retrieved oocytes do have an impact. Pregnancy rate is better when E2/fol is between 200 and 299.99 pg/ml. Also, increasing serum E2/fol positively correlates with better oocytes and embryo quality. In contrast, E2/O negatively correlates with oocytes and embryo quality parameters.

Keywords Clinical pregnancy rate · Estradiol · Oocytes · Follicle · In vitro fertilization · Assisted reproduction

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Introduction

The success of in vitro fertilization (IVF) depends on controlled ovarian hyperstimulation resulting in multi-follicular response. The follicles contain granulosa cells which secrete hormone estradiol (E2). Serum estradiol (E2) plays an important role in oocytes/follicular maturation and preparation of the uterus for implantation [1]. Controversy

surrounds the role of total estradiol value as well as estradiol/follicle ratio (E2/fol) and estradiol/oocytes (E2/O) ratio in relation to IVF outcome.

In a systemic published review [2], correlating total serum estradiol with IVF outcome, two studies (1,286 patients) showed decreased probability of pregnancy with high serum estradiol, while in five studies (1,875 patients), no detrimental effect of high serum E2 was seen on pregnancy rate. In two studies (191 patients), increased probability of pregnancy was seen with higher serum estradiol levels.

The aim of this study was to correlate the role of total serum estradiol as well as E2/mature follicle and E2/retrieved oocytes with clinical pregnancy rate (CPR) and oocytes/embryo quality. We aim to find the optimal estradiol value for best oocytes/embryo quality and higher pregnancy rate.

Materials and Methods

We retrospectively reviewed all patients who underwent IVF cycle in our institution from January 2008 to October 2010. Patients with a normal ovarian reserve who received long GnRH agonist protocol were included in the study. Patients with a poor ovarian reserve or apparent endometrial pathology were excluded from the study. A total of 342 cycles were retrospectively included from our IVF database.

Stimulation Protocol

The long agonist protocol was used for controlled ovarian hyperstimulation. In the long agonist protocol, pituitary desensitization with daily SC administration of 1 mg leuprolide acetate (lupride 4, Sun Pharmaceuticals, Gujarat, India) began in the mid-luteal phase of the previous menstrual cycle. This dose was continued until ovarian quiescence was confirmed by ultrasound examination in next menstruation and estradiol values <50 pg/ml, after which the dose of lupride was halved and stimulation with recombinant FSH was started until the day of human chorionic gonadotropin injection. The dosage of recombinant FSH was decided based on the patient's age, antral follicle count, basal hormonal levels, and response to previous stimulation. Dosages were adjusted according to the response seen on vaginal ultrasound examination. Human chorionic gonadotropin was administered SC when at least two leading follicles reached a mean diameter of 18 mm. Transvaginal oocyte retrieval was scheduled 34–36 h after HCG injection. Embryo transfer was performed 2–3 or 5–6 days after oocyte retrieval via the vaginal route.

Venous blood sample was collected on the morning of human chorionic gonadotropin injection for serum estradiol (E2) estimation. The serum E2 levels were analyzed using an Autoanalyzer E-170 system (Roche Diagnostics, USA) with the electrochemiluminescence immunoassay technique.

Fertilization was assessed after 18 h and embryo cleavage 24 h thereafter.

Embryo morphology was studied on days 2 and 3 (48–72 h), taking into account the number of cells and percentage of fragmentation. The luteal phase was supported with intramuscular progesterone, 100 mg daily.

The outcomes assessed are the number of oocytes retrieved (OR), number of mature oocytes (MO), number of oocytes fertilized (FO), fertilization rate (FR), number of embryos cleaved (EC), cleavage rate (CR), number of Grade I embryos (E), number of cryopreserved embryos (CPE), and CPR. The E2/fol was defined as estradiol level per mature follicle >14 mm in diameter. E2/O ratio was defined as estradiol level per OR. These two ratios were categorized by the 25th percentile into four groups. On the basis of E2/fol, the four groups categorized were Group A(E2/fol ≤139.99); Group B (E2/fol 140–199.99); Group C (E2/fol 200–299.99); and Group D (E2/fol ≥300). In E2/O, the four groups were Group I (E2/O ≤199.99); Group II (E2/O 200–299.99); Group III (E2/O 300–449.99); and Group IV (E2/O ≥450).

The statistical analyses were performed using SPSS for Win. Ver. 15.00 (SPSS, Chicago, IL, USA). Chi square test was used to examine whether the predictive variables were associated with pregnancy outcome. A Kruskal–Wallis non-parametric variance test was used for the comparison between groups, followed by the Mann–Whitney test for inter-group comparison if an overall level of significance of p .05 was reached. Results are presented as mean ± SD. A p value of less than .05 was considered significant.

Results

A total of 342 IVF cycles from our database were studied. The reasons for infertility included tubal factor (33.2 %), male factor (23 %), polycystic ovaries (18.4 %), and endometriosis (11 %), with more than one factor in 14 % patients.

The mean age of the patients studied was 31.56 ± 3.98 years and was comparable in all four categories. The mean cycle parameter in all 342 cycles is as shown in Table 1. The mean serum estradiol level on the day of HCG was 3,876 ± 2,950 pg/ml, while mean E2/fol and E2/O were 281.79 ± 215 and 361.38 ± 304.64 pg/ml, respectively. The mean OR, MO, fertilized, and EC were respectively 11.68 ± 8.53, 9.4 ± 5.71, 7.99 ± 5.66, and

Table 1 Cycle parameter in all patients

Parameter	Mean	SD
Day 2 FSH	6.29	2.09
Day 2 LH	6.71	3.24
Inhibin B	50.5	12.8
AMH	3.96	2.7
Total dose of FSH	2,694.8	987.24
Total days of stimulation	10.02	1.64
E2 on HCG day	3,876	2,950
Oocytes retrieved	11.68	8.53
Mature oocytes	9.4	5.7
Oocytes fertilized	7.991	5.662
Fertilization rate	72.41	24.8
Embryos cleaved	7.45	5.65
Cleavage rate	67.91	27.06
Embryos	7.35	5.63
Cryopreserved embryos	4.06	4.965
E2/mature follicle	281.79	215
E2/oocytes	361.38	304.6

7.45 ± 5.65. The fertilization and CR were 72.41 ± 24.8 and 67.91 ± 27.06, respectively.

The mean number of embryos was 7.35 ± 5.63 with E being 6.29 ± 4.89 and the number of embryos cryopreserved was 4.06 ± 4.965. A total of 77 clinical pregnancies occurred in 342 cycles with a CPR of 22.52 %.

We first correlated total serum estradiol (E2) on the day of HCG with the outcome parameters. No difference was seen in CPR with serum estradiol. But, there were increased no. of OR, MO, no. of FO, number of EC, E, and CPE (p value = .0001) as the serum estradiol increase. There was no difference in fertilization and CR.

Based on the 25th percentile, serum estradiol was divided into four categories—Group 1: ≤1,700 pg/ml; Group 2: 1,701–2,700 pg/ml; Group 3: 2,701–5,600; and Group 4 ≥5,600 pg/ml. In all these four categories, the outcome parameters were assessed. There was no difference in CPR in these subgroups. The NO, MO, FO, EC, E, and CPE increased as serum estradiol value increased from Group 1 to 4. No difference in fertilization and CR was seen in the four subgroups.

Then, we correlated the outcome parameters with the ratio of serum estradiol per mature follicle.

A positive correlation was seen between E2/fol and OR ($r = .334$, p value = .0001), no. of MO ($r = .335$, p value = .0001), no. of FO ($r = .222$, p value = .002), and number of CPE ($r = .289$, p value = .0001). No difference in FR, EC, CR, number of E, and CPR was seen. Then, the E2/fol was subdivided into four groups as mentioned before.

In the subgroup analysis of E2/fol with the outcome parameters (Table 2), no difference was seen in the four groups in terms of OR, MO, FO, FR, EC, CR, number of E, and number of CPE. Increased CPR was seen in Group C (E2/fol = 200–299.99) compared to Group A, B, and D (p value = .033).

With E2/O ratio, negative correlation was seen between E2/O and OR ($r = -.281$, p value = .002), MO ($r = -.296$, p value = .008), FO ($r = -.220$, p value = .003), EC ($r = -.211$, p value = .004), Grade 1 embryo ($r = -.216$, p value = .001), and CPE ($r = -.206$, p value = .005). No difference in FR, CR, or CPR was seen.

Table 2 Effect of E2/mature follicle on IVF outcome

Parameter	Group A ≤139.9 $N = 92$	Group B 140–199.99 $N = 82$	Group C 200–299.99 $N = 88$	Group D ≥300 $N = 80$	p value
Total estradiol	2,121.44 ± 1,556	3,011 ± 1,710.29	4,762 ± 2,722	5,256.94 ± 3,645	NS
No. of oocytes retrieved	11.14 ± 9.50	11.12 ± 6.37	12.0 ± 7.156	12.35 ± 10.15	NS
No. of mature oocytes	9.3 ± 7.65	9.45 ± 7.92	10.4 ± 8.2	10.8 ± 8.8	NS
No. of oocytes fertilized	7.38 ± 5.233	7.74 ± 5.59	8.75 ± 5.62	8.46 ± 6.17	NS
Fertilization rate	68.49 ± 27.36	69.545 ± 25.49	72.51 ± 24.33	71.0 ± 25.93	NS
No. of embryos cleaved	7.18 ± 5.20	7.37 ± 5.83	8.36 ± 5.69	7.58 ± 5.8	NS
Cleavage rate	81.45 ± 30.03	81.79 ± 29.52	82.01 ± 25.5	78.05 ± 27.04	NS
Grade I embryos	7.14 ± 5.24	7.37 ± 5.83	8.07 ± 5.63	7.54 ± 5.7	NS
Cryopreserved embryos	3.64 ± 4.76	3.72 ± 4.89	4.7 ± 4.66	4.96 ± 5.52	NS
E2/follicle	104.50 ± 26.27	165.8 ± 17.57	245.9 ± 29.89	697 ± 592	–
E2/oocyte	239.43 ± 180.26	284.9 ± 109	421 ± 160.05	506 ± 238.06	–
Clinical pregnancy	17	14	29	17	.033
Clinical pregnancy rate	18.4 %	17.07 %	32.95	21.25	.039

Table 3 Effect of E2/oocytes ratio on IVF outcome

Parameter	Group I ≤199.9 $N = 78$	Group II 200–299.99 $N = 86$	Group III 300–449.99 $N = 82$	Group IV ≥450 $N = 96$	P value
Total estradiol	2,191.16 ± 1,495.85	3,386.98 ± 2,210.58	3,992.61 ± 2,510.35	5,374.94 ± 3,586	–
No. of oocytes retrieved	14.73 ± 10.78	13.61 ± 8.49	11.02 ± 6.49	8.65 ± 5.62	.002
No. of mature oocytes	12.2 ± 7.65	11.45 ± 8.62	9.4 ± 8.2	7.8 ± 4.8	.008
No. of oocytes fertilized	9.2 ± 4.89	9.48 ± 6.898	7.82 ± 4.54	6.59 ± 5.34	.003
Fertilization rate	71.12 ± 21.42	67.365 ± 25.60	72.65 ± 24.91	74.65 ± 24.41	NS
No. of embryos cleaved	8.59 ± 4.30	9.07 ± 7.07	7.39 ± 4.42	6.16 ± 5.508	.004
Cleavage rate	84.45 ± 20.125	75.84 ± 32.98	81.6 ± 25.26	86.07 ± 23.64	NS
Grade I embryos	8.59 ± 4.307	9.07 ± 7.08	7.07 ± 4.28	6.1 ± 5.51	.001
Cryopreserved embryos	4.83 ± 5.04	5.98 ± 6.075	3.68 ± 4.66	2.98 ± 4.37	.005
E2/follicle	229.59 ± 363.29	384.16 ± 620.45	262 ± 243.81	334.03 ± 123.46	–
E2/oocyte	159.13 ± 35.57	247.48 ± 26.94	357.37 ± 44.38	636.2 ± 175.98	–
Clinical pregnancy	14	21	24	18	NS
Clinical pregnancy rate	17.9 %	24.4 %	29.3 %	18.8 %	NS

In the analysis of difference in between the four groups (Group 1–4) in E2/O ratio, the significant difference seen was among Group I, II, and IV (Table 3). In patients in Group IV (E2/O ≥450), decreased no. of retrieved oocytes, less MO, fertilized oocytes, EC, E, and CPE were seen. Table 4 shows pairwise comparison between all four groups on the outcomes assessed. As can been seen from Table 3, as E2/O ratio reached ≥450 pg/ml, the number of OR as well as the quality of oocytes assessed by number of MII oocytes with no abnormal features and FO significantly deteriorated. Although no difference was seen in CPR, the best pregnancy rate (29.3 %) was seen when E2/O was in between 300 and 449.99 pg/ml.

Table 4 Correlation (Z) and p value of cycle parameters in between subgroups

Parameter	Groups	II		III		IV	
		Z	p	Z	p	Z	p
OR	I	−.256	.798	−1.674	.094	−3.253	.001
	II			−1.455	.146	−3.082	.002
	III					−1.796	.073
MO	I	−.234	.749	−1.54	.086	−2.896	.001
	II			−1.432	.135	−3.08	.002
	III					−1.782	.058
FO	I	−.298	.765	−1.376	.169	−3.058	.002
	II			−.830	.406	−2.426	.015
	III					−1.911	.056
EC	I	−.456	.648	−1.461	.144	−3.234	.001
	II			−.745	.456	−2.479	.013
	III					−2.108	.035
E	I	−.456	.648	−1.801	.072	−3.248	.001
	II			−1.033	.302	−2.554	.011
	III					−1.901	.057
CPE	I	−.834	.404	−.922	.357	−2.207	.027
	II			−1.732	.083	−2.737	.006
	III					−1.309	.190

Discussion

The quality of oocytes and embryo quality are important determinants of the success of IVF. Serum estradiol as a factor affecting these parameters has been studied in only a few publications [3–11, 19, 20]. Also, serum estradiol on the day of HCG as a factor in IVF success is controversial with both positive as well as negative relations quoted in the literature.

In this study, we correlated both total serum estradiol and E2 per mature follicle and E2 per OR with cycle parameters as well as IVF outcome. The cycle parameters studied were number of OR, MO, FO, FR, number of EC, CR, number of good quality embryos, and CPR.

It has been reported that 17 β estradiol induces cytoplasmic maturation of GV oocytes through increase in intra-cytoplasmic calcium concentration [12]. This has been associated with better fertilization and CRs.

In our study, we found that with increase in serum estradiol, an increased number of oocytes were retrieved, more number of MO were seen, and an increased number of embryos were cryopreserved. But, no difference in overall pregnancy rate was seen.

Through multiple studies, it has been shown that an increased number of oocytes are retrieved with increasing serum estradiol on the day of HCG. But, there is conflicting

evidence regarding its impact on oocytes/embryo quality and pregnancy rate. In the studies by Simon et al. [5] and Yu Ng et al. [10], increased total serum estradiol was correlated with poorer pregnancy rate. This has been associated with normal oocytes and embryo quality as similar pregnancy rates were seen in normal and high responders in donor oocytes cycle. In other studies by Sharara et al. [4, 7] and Papageorgiou et al. [14] in which 106 and 905 IVF cycles were studied, respectively, no detrimental effect of high estradiol was seen on oocytes/embryo quality or pregnancy rate. On the other hand, in the studies by Loutradis et al. [13] and Pena et al. [9, 17], higher serum estradiol on the day of HCG was associated with a larger number of oocytes and embryos and high-grade embryos for transfer/cryopreservation. In our study, no correlation of serum estradiol with pregnancy rate was seen. Increased number of mature and fertilized oocytes might be due to an increased number of retrieved oocytes with increasing estradiol value, and this does not reflect on oocytes or embryo quality. To reflect the role of estradiol better, we correlated the ratio of E2/mature follicle and E2/oocytes with cycle parameter outcomes.

On continuous correlation of E2/fol, an increased number of OR, and increase in no. of MO and FO were seen with increasing E2/fol. Hence, a positive correlation of better oocytes quality was seen with increase in E2/fol. On the 25th percentile categorization of E2/fol, maximum pregnancy rate was seen when E2/fol was in between 200 and 299.99 pg/ml.

This is in accordance with a study by Ozdegirmenci et al. [15] in which a positive correlation was seen in OR, MO, and FO with E2/fol ratio. In this study, no difference in pregnancy rate was seen.

In the correlation of serum E2 per OR, negative correlation was seen between E2/O and OR ($r = -.281$, p value = .002), MO ($r = -.296$, p value = .008), FO ($r = -.220$, p value = .003), EC ($r = -.211$, p value = .004), Grade 1 embryo ($r = -.216$, p value = .001), and CPE ($r = -.206$, p value = .005). In the subgroup analysis of four groups, it was seen that as E2/O ratio reached $\geq$450 pg/ml, the number of OR as well as the quality of oocytes assessed by number of MII oocytes with no abnormal features and FO significantly deteriorated. Although no difference was seen in CPR, the best pregnancy rate (29.3 %) was seen when E2/O was in between 300 and 449.99 pg/ml.

Similarly, in the studies by Ozdegirmenci et al. [15] and Yang et al. [16, 18], higher E2/O ratio correlated with poorer pregnancy rate and lower no. of retrieved oocytes. In the study by Yang et al. [18], E2/O ratio $\geq$350 pg/ml was associated with poorer pregnancy rate (17.9 vs. 32.8 %).

In the study by Ozdegirmenci et al. [15], while no difference in pregnancy rate was seen with increase in E2/O, the number of retrieved, mature, and fertilized oocytes decreased. This is similar to our study in which with increasing E2/o ratio, NO, MO, FO, EC, and E decreased. This negative effect of higher E2 level might lead to arrested maturation of oocytes, leading to immature oocytes being picked up at the time of pickup. The exact mechanism by which high serum estradiol per oocyte picked exerts this negative influence on oocytes' quality needs to be further studied.

In conclusion, serum estradiol is an important determinant of IVF success. While total serum estradiol does not exert any positive or negative influence on IVF outcome, estradiol per mature follicle and retrieved oocytes do have an impact. Pregnancy rate is better when E2/fol is between 200 and 299.99 pg/ml. Also, increasing serum E2/fol positively correlates with better oocytes and embryo quality. In contrast, E2/O negatively correlates with oocytes and embryo quality parameters. Further research is needed to address the exact mechanism of this negative influence at molecular and receptor level.

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