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Role of Serum Estradiol Levels on the Day of Human Chorionic Gonadotropin Injection Administration in the Prediction of Results of Assisted Reproductive Techniques

Authors
Fessy Louis .T[1], Sagar S Mane[2], Ramesh P[3*], Gopinathan KK[4]
[1]Senior consultant, Department of Reproductive Medicine and Surgery, Amrita Institute of Medical Sciences, Kochi, Kerala
[2]Shree Radhakrishna hospital and IVF centre, Phalthan, Maharashtra
[3]MCh Resident, Department of Reproductive Medicine and Surgery, Amrita Institute of Medical Sciences, Kochi
[4]Head of the department of Reproductive Medicine, CIMAR, Edappal, Kerala
*Corresponding Author
Dr Ramesh P
MCh Resident, Dept of Reproductive Medicine and Surgery, Amrita Institute of Medical Sciences, Kochi, India
Phone- 09249426575, Email: *drrameshp07@gmail.com*

Abstract
We need to find out factors which can predict the outcome of In vitro fertilization/ Intracytoplasmic sperm injection cycles so that couple can take correct decision before opting for assisted reproductive techniques. Our objective was to evaluate the role of serum estradiol level on the day of human chorionic gonadotropin administration as a potential predictive marker of outcome of IVF/ICSI cycles. This was a prospective study done in a tertiary care centre. Serum estradiol (E_2) levels were obtained on the day of hCG administration. After 14 days of embryo transfer, serum beta hCG level was estimated and a value of 50 IU/L was taken as cut-off of positive cycle. The biochemical pregnancy rate was 31%. The mean value of serum estradiol was 1970.16 ± 1140.64 pg/ ml in the non pregnant group whereas that in the pregnant group was 2129.31 ± 787.91pg/ ml. (p= 0.34). The study population was divided into 5 groups based on the serum estradiol level (pg/ml) on the day of hCG administration. Number of follicles >14mm was significantly higher in groups with E2 values 3000-4000 and >4000 pg/ml (p<0.001). There was no significant difference in the endometrial thickness in the groups (p=0.131). The pregnancy rate was not found to be significantly different among the groups (p=0.34). The number of MII oocytes and the number of transferable embryos were significantly less in the groups with E2 level <1000 and 1000-2000 pg/ ml.
Therefore, serum estradiol level on the day of hCG administration was positively correlated with number of follicles with size more than 14mm obtained, metaphase II oocytes obtained, number of transferable embryos obtained. There was no association of serum estradiol level on the day of hCG administration with endometrial thickness on day of hCG and IVF outcome.
Keywords: *Estradiol, Day of trigger, ART outcome, Pregnancy rate.*

Introduction

The first child resulting from IVF was born in 1978[1]. After that the technology of assisted reproductive technology (ART) has enormously improved and expanded to a greater extent to optimize the outcomes of ART procedures (mainly IVF/CSI). IVF has revolutionized the treatment of infertility. Considering the serious complications of IVF which can endanger life of female partner like ovarian hyper stimulation syndrome, high expenditures involved, stress, anxiety & emotional factors of couples involved & also part of pre-procedure counseling, one needs to find out factors which can, at least to a certain extent, predict the outcome of IVF/ICSI cycle so that couple can take correct decision before opting and also physicians to help take decisions to make IVF/ICSI cycle successful.

Accordingly, several factors have been tried upon. Factors, on which we can predict the IVF/ICSI outcome, can be known before start of cycle or during cycle or even at nearing the completion of cycle. Major Prognostic factors during cycle are response to gonadotropins stimulation (number of follicles developing, serum estradiol levels on the day of hCG administration, endometrial receptivity evidenced by endometrial thickness, endometrial blood flow by Doppler)[2]. Some studies suggested that pregnancy rates are negatively associated with estradiol levels on the day of hCG administration[3,4] whereas, few other studies suggested that pregnancy rates are not associated[5-8] or positively associated[9-10] with serum estradiol levels on the day of hCG administration. There is lack of clear consensus regarding the actual role of serum estradiol in this aspect. Our objective was to evaluate the role of serum estradiol level on the day of human chorionic gonadotropin administration as a potential predictive marker of outcome of IVF/ICSI cycles.

Material and Methods

This was a prospective observational study involving 212 couples who underwent IVF/ ICSI treatment at a tertiary care hospital in Kerala, India. The duration was from January 2014 to December 2015. A detailed history with respect to nature, duration and etiology of infertility, previous treatments and surgeries were obtained. Approval from institutional ethics committee was obtained. Sample size was estimated on a previous publication (Ng E.H.Y. et al. 2000[4]) with 5 % allowable error, 95% confidence and 80% statistical power.

Inclusion criteria comprised of those undergoing first ART cycle, female partner age <35 years, Day 2 FSH values < 8 IU/L. All the ART cycles were done with standard long Protocol. The subjects visited our department in the 3rd week of their menstrual cycle. Trans-vaginal ultrasound was done using LOGIQ PRO 5 ultrasound machine (GE) with 8MHz transvaginal probe. Female partner was put on long luteal phase hypothalomo-pituitary ovarian axis down regulation with gonadotropin releasing hormone agonist-leuprolide acetate 0.5mg subcutaneously once daily.

The couple was asked to report on day 2/ day 3 of next menstrual cycle. The female partner was subjected to transvaginal ultrasound with assessment of ovarian volume, antral follicle count and endometrial thickness. On the same day, basal hormonal assays-serum estradiol, progesterone, Follicle stimulating hormone, Leutinizing Hormone were obtained.

Controlled ovarian stimulation with daily intramuscular injections of exogenous gonadotropins [recombinant FSH(r-FSH) or human menopausal gonadotropin (HMG) 150-300IU] was done. Couple was advised to report after 7 or 8 days for evaluating response to gonadotropin therapy (serum estradiol level estimation, ultrasonographic assessment of endometrial thickness and follicular growth). Exogenous gonadotropins along with leuprolide acetate were continued till at least 1 or 2 follicles attained size of 17-18mm and endometrial thickness became more than 7mm. Then, for final oocyte maturation, intramuscular injection of

human chorionic gonadotropin (hCG) 10000 units was given.

Serum estradiol (E_2) levels were obtained on the day of hCG administration. Measurement was done by electrochemiluminescence method with competitive principle using Elecsys 2010 machine. Transvaginal ultrasound guided oocyte retrieval was done under intravenous anaesthesia after 36hours of hCG injection. IVF/ICSI was done with retrieved oocyte. Embryos of good quality were transferred to uterine cavity under transabdominal ultrasound guidance on day 3. Luteal phase support was started with micronized progesterone, estradiol tablets and human chorionic gonadotropin hormone injections. After 14 days of embryo transfer, serum beta hCG level was estimated and a value of 50 IU/L was taken as cut-off of positive cycle. Pregnancy, location and viability were confirmed by tranavaginal ultrasound done four weeks after embryo transfer.

Statistical analysis was done using IBM SPSS software (version 19). Results on continuous measurements are presented on Mean ± SD and results on categorical measurements are presented as number (%). Significance was assessed at 5% level. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale, Chi-square/Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Results

The mean age of the study population was 31.86 ± 1.86 years. The mean duration of married life was 6.29 ± 0.76 years. The baseline characteristics are depicted in Table 1. The mean duration of infertility was 6.29± 1.1 years. The ART outcome as evidenced by serum beta hCG> 50 IU/L is also shown in table 1, indicating a biochemical pregnancy rate of 31%.

The stimulation drug used was recombinant FSH (r-FSH) in 46% and the remaining 54% received human menopausal Gonadotropin (HMG). Table 2 shows the ART outcome as compared with the basal variables. The biochemical pregnancy was positive in 41% of those who received r-FSH as opposed to 21% of those who took HMG (p= 0.038).

The mean value of serum estradiol was 1970.16 ± 1140.64 pg/ ml in the non pregnant group whereas that in the pregnant group was 2129.31 ± 787.91pg/ ml. (p= 0.34). The mean endometrial thickness in the study population was 8.68 ± 1.17mm. The mean number of metaphase II (MII) oocytes and transferable embryos (TE) was 6.41 ± 1.35 and 5.27 ± 1.03 respectively.

The study population was divided into 5 groups based on the serum estradiol level (pg/ml) on the day of hCG administration. (Table 3). Number of follicles >14mm was significantly higher in groups with E2 values 3000-4000 and > 4000 pg/ml (p<0.001). There was no significant difference in the endometrial thickness in the groups (p=0.131). The pregnancy rate was also not found to be significantly different among the groups (p=0.34). The number of MII oocytes and the number of transferable embryos were significantly less in the groups with E2 level <1000 and 1000-2000 pg/ ml.

Table 1 Basal characteristics (n= 212)

Parameter		Number	Percentage
Age (Years)	≤ 25	8	3.8 %
	26-30	48	22.6 %
	>30	156	73.6 %
Duration of Married life (Years)	≤ 5	104	49.1 %
	6-10	72	34.0 %
	>10	36	16.9 %
Type of Infertility	Primary	136	64.2 %
	Secondary	76	35.8 %
Cause of infertility	Combined factor	56	26.4%
	Female factor	106	50.0%
	Male factor	50	23.6%
Type of Gonadotropin	HMG	114	54%
	r- FSH	98	46%
ART Outcome	Positive	65	31 %
	Negative	147	69 %

HMG- Human Menopausal Gonadotropin, r-FSH- Recombinant FSH,
ART- Assisted reproductive technology

Table 2 Comparison of basal parameters and ART outcome

Parameter		Positive	Negative	p value
Age (Years)	≤ 25	2 (25%)	6 (75%)	0.20
	26-30	13 (27%)	35 (73%)	
	>30	50 (32%)	106(68%)	
Duration of Married life (Years)	≤ 5	32(31%)	72(69%)	0.047*
	6-10	28 (39%)	44(61%)	
	>10	5 (14%)	31 (86%)	
Type of Infertility	Primary	40 (29%)	96 (71%)	0.98
	Secondary	25 (33%)	51(67%)	
Type of Gonadotropin	HMG	25(22 %)	89 (78 %)	0.038*
	r- FSH	40 (41 %)	58 (59 %)	

HMG- Human Menopausal Gonadotropin, r-FSH- Recombinant FSH

Table 3 Association of various outcome variables versus serum Estradiol level on the day of hCG

Parameter		E2 level on the day of hCG					p value
	Category	≤1000 (n=44)	1001-2000 (n=76)	2001-3000 (n=52)	3001-4000 (n= 24)	>4000 (n=16)	
Follicle >14mm	≤5 (n=86)	36	28	16	4	2	<0.001*
	6-10 (n=84)	8	40	24	12	0	
	11-15 (n=28)	0	8	11	5	4	
	>15 (n= 14)	0	0	1	3	10	
Endometrial thickness (mm) on day of hCG	≤8	0	8	4	8	4	0.131
	8.1-9	36	52	40	12	8	
	>9	8	16	8	4	4	
MII Oocytes	≤5	34	34	8	12	8	<0.001*
	5-10	9	33	40	6	0	
	10-15	1	9	4	6	4	
	>15	0	0	0	0	4	
Transferable embryos	≤5	40	44	24	16	8	<0.001*
	5-10	4	28	20	4	4	
	10-15	0	8	8	4	0	
	>15	0	0	0	0	4	
Pregnancy outcome	Negative	38	54	27	14	14	0.34
	Positive	6	22	25	10	2	

Discussion

We found no association between serum estradiol level on the day of hCG administration and IVF outcome.

The serum E2 values on the day of hCG (E2hCG) was classified into 5 groups (<1000, 1001-2000, 2001-3000, 3001-4000 and >4000 pg / ml).The number of follicles with size more than 14mm was significantly associated with elevated E2 values on day of hCG administration (E2hCG). This finding was in agreement with other studies by Chenette et al[9] and Gelety et al[10]. The endometrial thickness was not significantly different in the different groups of E2 values. Simon et al [3] and Ng et al[4] also had similar results. This implies that at least endometrial thickness, if not morphology and receptivity, is least affected by elevated levels of E2hCG.

The number of MII oocytes and the number of transferable embryos obtained were significantly high with high serum E2hCG. This finding was in agreement with other studies (Chennette et al[9], Gelety et al.[10]

The association between serum estradiol levels on the day of hCG administration with IVF outcome was not found to be statistically significant. This finding explains that pregnancy rates are not associated with E_2 levels on the day of hCG

administration. Our finding was in agreement with findings of other studies done by Mettler and Tavmergen[5], Doret al[6], Sharara et al[7], Chen et al[8] and Papageorgiou et al [11.] However, some studies have reported that pregnancy rates are positively associated with E_2 levels on the day of hCG administration. (Chenette et al[9], Gelety et al[10]). Some studies report that pregnancy rates are negatively associated with E_2 levels on the day of hCG administration. (Simon et al[3], Ng et al[4]). In theory, if there is a positive association between E2 levels on the day of hCG administration and pregnancy rates, then low E2 levels on that day should be considered as a bad prognostic factor for pregnancy. In this case, the cycle should either be cancelled or the follicular phase be prolonged in order to achieve higher E_2 levels, but avoiding the occurrence of ovarian hyper stimulation syndrome.

Conversely, if there is a negative association between E_2 levels on the day of hCG administration and pregnancy rates, then low estradiol levels on that day should be considered as a good prognostic factor for pregnancy and there is a need to assess the value of maintaining low E_2 levels during ovarian stimulation, in order to increase the chance of conception (Simon et al) [3].

Finally, if there is a lack of association between E_2 levels on the day of hCG administration and pregnancy achievement, then E_2 achievement, then E_2 assessment should not be incorporated in the criteria used for hCG administration and should only be performed to assess the risk of ovarian hyper stimulation syndrome. (D' Angelo et al)[12]. Therefore, our data is suggesting that E2hCG might not be an essential tool in predicting IVF outcome. The measurement of E2hCG need not be implemented as a universal practice. Since we did not have any cases with moderate to severe ovarian hyper stimulation syndrome, the role of E2hCG in this scenario could not be assessed.

Limitations

We took into consideration only agonist cycles. It would have been better to include antagonist protocols and other protocols as well. The serum progesterone was not considered and hence we could not do a subgroup analysis of E2 – Progesterone ratio. We also did not consider other aspects like ongoing pregnancy rate and live birth rate.

Conclusions

Serum estradiol level on the day of hCG administration was positively correlated with number of follicles with size more than 14mm obtained, metaphase II oocytes obtained, number of transferable embryos obtained. There was no association of serum estradiol level on the day of hCG administration with endometrial thickness on day of hCG and IVF outcome. Henceserum estradiol level on the day of hCG administration may not be used as predictor of cycle outcome in IVF/ICSI.

Grants: Nil

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