



## Predictive value of basal FSH, Endometrial thickness, Pattern and Serum E2 in In-vitro Fertilization embryo transfer cycles success

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### Abstract

To evaluate predictive value of basal FSH, serum estradiol (E<sub>2</sub>) and endometrial thickness and pattern in success of IVF-ET (In vitro fertilization- embryo transfer) cycles. A total of 211 patients under went long protocol which started in secretory phase (day 21) of cycle and success rate of IVF-ET cycle is determined on the basis of clinical pregnancy rate (CPR). All the patients were studied on the various parameters like age, type of infertility, cause of infertility, basal FSH, serum estradiol, endometrial thickness and pattern on the day of HCG injection, number of ampoules of Gonadotrophins used, no. of follicles, oocyte retrieved, embryo formed, embryo grade. Predictive value of basal FSH, serum estradiol and endometrial thickness and pattern were assessed in success of IVF-ET cycles. Success rate in present study is 37.44%. Overall mean age of study group is 32.49+<sub>-</sub>3.42 years. This study indicates that significant predictive factors are basal FSH, endometrial thickness, presence of triple line, peak estradiol level, and age, number of follicle, oocyte, and embryo formed and transferred has effect on CPR. Embryo grade is independent predictor of IVF success. Basal FSH is a marker of ovarian reserve and value >10 IU/ml indicate poor ovarian reserve with poor quality of oocyte as shown in the study. High serum E2 has a positive association on endometrial thickness and presence of triple line, individually both endometrial parameter are predictor of IVF-ET cycle success.

**Keywords:** Follicular stimulating hormone (FSH), clinical pregnancy rate (CPR), serum estradiol (E<sub>2</sub>), predictive value of basal FSH, endometrial thickness, pattern and serum E2 in In-vitro fertilization embryo transfer cycles success.

### Introduction

IVF involves a sequence of events which begin with different stimulation protocols with controlled ovarian hyperstimulation with exogenous gonadotrophin. Oocyte retrieval is done from the ovaries under the guidance of ultrasonography, fertilization in the laboratory, and transcervical transfer of embryos into the uterus. The first pregnancy resulting from IVF was reported in 1976 was ectopic. The first child resulting from IVF was born in 1978<sup>1</sup>.

Since the last two decades, ART has been used in treatment of infertile couples but the low implantation, pregnancy rate and high cost of treatment leads to the need to assess the predictors of success in IVF- ET cycles. Prognostic factors of IVF success in the study are basal FSH, serum estradiol, endometrial receptivity and embryo grading. Endometrial receptivity is one of the important factors of IVF outcome. It has been estimated that uterine receptivity accounts for about 31–64% of implantation<sup>2</sup>. Implantation of blastocyst in the endometrium takes place only during short duration called the window of implantation which exists for about 48 hours, beginning 6–10 days after the LH surge in a spontaneous cycle<sup>3</sup>. Embryo implantation is regulated by a multitude of factors. This endometrial receptivity can be assessed by ultrasound markers<sup>4, 5</sup>. Aim of the study was to assess endometrial receptivity by using following parameter like endometrial thickness,

endometrial pattern on day of HCG injection to predict pregnancy during IVF-ET cycles.

Serum estradiol plays a crucial role in the reproductive system, being involved in cervical mucus, in endometrial proliferation for embryo implantation, and in induction of midcycle LH surge in natural cycle. E2 synthesis is merely associated with dominant follicle development and plasma E2 concentration is an index to assess follicle maturity. During ART cycle E2 synthesis is directly related to follicle size and the contribution of the mature follicle to E2 outcome may be estimated about 200pg/ml<sup>6</sup>. For many years focus of research interest has been on the possible association between serum estradiol level and the IVF outcome on the day of HCG trigger. If E2 levels are high they might have better effect on IVF/ICSI outcome or might have an adverse effect cause by a decreased endometrial receptivity<sup>7-10</sup>. Several studies in the past have suggested that high physiological E2 concentrations affects the chances of pregnancy while other studies indicated that high E2 level didn't alter endometrial receptivity<sup>9, 11, 12</sup>. Because of this controversial result we aim to assess the association between E2 level on the day of HCG administration and IVF outcome.

In most of the IVF units, to select the patient for IVF-ET cycle's day 3 FSH is measured. For the first time, Muasher *et al* described that the cycle day 3 FSH level is one of the most common used tests for predicting success in ART treatment<sup>13</sup>.

Several studies showed that on day 3, elevated FSH level indicates that the patient is a poor responder to ovarian stimulation protocol which might lead to lower pregnancy rate in ART and this is independent of age<sup>14</sup>. However, in older women, the FSH concentration maybe an unreliable indicator of reproductive potential because FSH level gives an indirect assessment of ovarian reserve<sup>15,16</sup>. We therefore assessed its predictive value in IVF-ET outcome.

## Material and Methods

This hospital based prospective study was conducted in Jaipur fertility centre (ART division of department of Obstetrics and Gynecology at Mahatma Gandhi University of Science and Technology, Jaipur). The study was carried for a period of twelve month from Jan 2013 to Dec 2013. Study population is taken from patient attending the ART unit in the hospital. A total of 211 women requiring in vitro fertilization here enrolled in the study based on inclusion and exclusion criteria.

Inclusion criteria were age 25-37 year, BMI 19 –24.9 kg/m<sup>2</sup>, no history of irregular menstrual cycle ranging from 25-35 days. No relevant systemic disease, severe endometriosis or uterine and ovarian anomalies. No more than three previous failed IVF cycle. No previous IVF cycle with poor response or Ovarian Hyperstimulation Syndrome. Exclusion criteria were diminished ovarian reserve where basal FSH was >15mIU/ml and age >37 year, known endometrial pathology, cycles with donor oocyte and cryopreserved cycles. An informed consent was not taken from patients as all these test are done routinely at the centre. Initial assessment was carried out by taking a detailed history, clinical examination and routine blood investigation.

During history duration and type of infertility, menstruation and obstetrics history, past medical and surgical history, coital history, past infertility, treatment history or any investigation previously done were asked. General examination especially for thyroid enlargement, galactorrea, hirsutism, weight and height was done. Per abdominal, speculum, vaginal examination was done to rule out any local pathology and discharge. For male partner working condition, history of alcohol intake, smoking, past medical and surgical history was required.

Apart from routine investigation hormonal profile TSH, prolactine, baseline FSH, LH, serum estradiol and baseline ultrasonography was done. Husband's blood group, blood sugar, HIV, HbsAg, VDRL, semen analysis based on WHO criteria and semen culture were also done. The serum FSH, LH, serum estradiol was analyzed by using mini VIDAS method. All ultrasound examination was done by using 7.5 MHz probe of Toshiba.

In all cases pituitary down regulation was done with GnRH agonist started on 21<sup>st</sup> day of luteal phase of previous cycle and suppression is confirmed by using TVS showing no ovarian cyst, endometrial thickness < 4 mm and blood investigation in

which serum estradiol <80pg/ml, FSH< 10mIU/ml, and LH< 5mIU/ml. The dose is then reduces to half and continued till 1 day prior to HCG injection. Ovulation induction was done with HMG (human menopausal gonadotrophin) and r- FSH (recombinant follicular stimulating hormone) started on day 3 of cycle. Average starting dose is 150 IU and dose is adjusted according to response. Response is monitored by frequent estrogen level and pelvic ultrasound examination to measure follicle size, aim is to achieve 3-4 follicles that are > 17 mm in size. Ovulation trigger was done by HCG (human chorionic gonadotrophin) 10,000 IU IM injection. This causes the final maturation of egg and also stimulates ovulation to occur approximately 36 hours later. On day of HCG injection number of follicle, endometrial thickness and pattern was assessed by transvaginal ultrasound. Serum estradiol, serum progesterone and serum LH is also done. Ultrasound guided transvaginal egg retrieval is performed 32-35 hours after HCG injection under general anesthesia, by 17 G oocyte retrieval needle. After incubation for 3-4 hours eggs are inseminated with washed motile sperm prepared by swim up technique in Earle's balanced salt solution. After insemination embryo are cultured in G-IVF plus, G1 plus and G2 plus media. Fertilization was assessed at 19-21 hour (day1), 43-45 hour (day 2) and 68-70 hour (day 3) after insemination. Embryo where classified according to number of blastomere, percentage of fragmentation, and blastomere appearance as grade A,B,C,D. Up to three top quality embryo where transferred into the uterine cavity on day 4<sup>th</sup> or 5<sup>th</sup> after oocyte retrieval. All transfer where made by Wallace embryo replacement catheter number 23cm.

Luteal phase support is essential in these IVF-ET cycles as ovulation induction and egg retrieval may reduce subsequent production of progesterone by the ovary. Progesterone is important hormone help to prepare the lining of the uterus for implantation for this reason progesterone support is done we use either cap geston (micronized progesterone) 200mg thrice daily or injection geston intramuscular daily. The support is continued for 15 days following embryo transfer. Serum beta HCG is done after 15 days of embryo transfer if > 20m IU/ml pregnancy is confirmed with transvaginal sonography 15 days after serum beta HCG. Outcome is measured by clinical pregnancy rate which is defined as presence of one or more gestation sac. Cardiac activity was monitored at 6 week. Primer of biostatics adviser software was used as statistical method.

## Results and Discussion

Predicting chances of pregnancy after an IVF cycle can help to prevent overtreatment and to balance the probability of achieving a pregnancy after IVF against the probability of achieving a pregnancy through natural conception. Although many studies reported on potential predictors of pregnancy chances after IVF, there is no consensus to pinpoint which predictors are clinically most relevant and on what factors one should base the decision to start treatment or not. For table-2

when Chi test was applied for test of significance, a significant relationship is found between basal FSH and IVF success ( $p < 0.05$ ). According to chi-test significant association was found between endometrial thickness and success of IVF ( $p$  value  $< 0.05$ ) (table-3). According to chi-test significant association was found between endometrial pattern and success of IVF ( $p$  value  $< 0.05$ ) (table-4). For table-5 when chi-test was applied statistically significant ( $p < 0.05$ ) association was found

between pattern of endometrium and IVF success. For table-6  $p < 0.05$ , Peak estradiol level and endometrial thickness is positively correlated. For table-7  $p > 0.05$ , Among patients with peak estradiol  $> 3000$ pg/ml in 39 patients (70.91%) triple line is present and in remaining 16 patients (29.10%) triple line is not present. When chi-square test analysis is applied non significant ( $p > 0.05$ ) association is seen between peak serum estradiol and endometrial pattern.

**Table-1**  
**Baseline cycle characteristics**

	Variables	Pregnant	Non pregnant	p-value
1.	Age ( mean)	30.24+ <sub>-</sub> 2.90	33.46+ <sub>-</sub> 3.36	<0.01
2.	Type of infertility Primary Secondary	55/79=69.62% 24/79=30.38%	97/132=73.48% 35/132=26.52%	>0.05
3.	BMI kg/m <sup>2</sup>	22.62+ <sub>-</sub> 1.14	22.45+ <sub>-</sub> 1.29	>0.05
4.	Basal FSH mIU/ml <10 >10	71/79=89.87% 8/79=10.13%	92/132=69.7% 40/132=30.30%	<0.05
5.	No. of follicle >15 mm	11.86+ <sub>-</sub> 5.89	5.96+ <sub>-</sub> 2.92	<0.05
6.	ET mm	8.44+ <sub>-</sub> 1.35	7.05+ <sub>-</sub> 1.47	<0.05
7.	Triple line endometrium Present Absent	64/79=81.01% 15/79=18.99%	61/132=46.21% 71/132=53.79%	<0.05
8.	E2 level (mean) pg/ml	2294.6+ <sub>-</sub> 1245.06	1574.52+ <sub>-</sub> 1103.46	<0.01
9.	No. of ampoules of gonadotrophin	33.42+ <sub>-</sub> 3.72	34.36+ <sub>-</sub> 4.78	>0.05
10.	No. of oocyte retrieval	8.32+ <sub>-</sub> 3.67	4.97+ <sub>-</sub> 2.81	<0.05
11.	No. of embryo formed	5.72+ <sub>-</sub> 2.52	3.08+ <sub>-</sub> 1.78	<0.05
12.	No. of grade A embryo out of 3 transferred 2 or >2 1	70/79=88.61% 9/79=11.39%	50/132=37.88% 82/132=62.12%	<0.05
13.	No. of embryo transferred 1 2 3	2/70=2.53% 24/79=30.37% 53/79=67.09%	22/132=16.67% 61/132=46.21% 49/132=37.12%	<0.05

**Table-2**  
**Distribution of patients according to Basal FSH**

Basal FSH IU/ml	Successful		Unsuccessful		Total		P value <0.05
	No.	%	No.	%	No.	%	
<10	71/79	89.87	92/132	69.70	163/211	77.25	
>10	8/79	10.13	40/132	30.30	48/211	22.75	

**Table-3**  
**Distribution of patients according to endometrial thickness**

ET(mm)	Successful		Unsuccessful		Total	
	No.	%	No.	%	No.	%
< 7	17/79	21.52	45/132	34.09	62/211	29.38
7 – 9	41/79	51.90	81/132	61.36	122/211	57.82
> 9	21/79	26.58	6/132	4.55	27/211	12.80

**Table-4**  
**Distribution of patients according to endometrial pattern**

Triple line	Successful		Unsuccessful		Total		p <0.05
	No.	%	No.	%	No.	%	
Present	64/79	81.01	61/132	46.21	125/211	59.24	
Absent	15/79	18.99	71/132	53.79	86/211	40.76	

**Table-5**  
**Distribution of patients according to estradiol level on day of HCG injection**

Estradiol (pg/ml)	Successful		Unsuccessful		Total	
	No.	%	No.	%	No.	%
<1500	23/79	29.11	91/132	68.94	114/211	54.03
1500 – 3000	26/79	32.91	25/132	18.94	51/211	24.17
>3000	30/79	37.97	16/132	12.12	46/211	21.80

**Table-6**  
**Association of Estradiol on day of HCG and endometrial thickness.**

Endometrial thickness	Estradiol on day of HCG injection					
	<1500pg/ml		1500 – 3000pg/ml		> 3000pg/ml	
	No.	%	No.	%	No.	%
< 7mm	26/112	23.21	7/44	15.90	25/55	45.46
7 – 9mm	64/112	57.14	34/44	77.27	24/55	54.55
> 9mm	22/112	19.64	3/44	6.82	6/55	10.91

**Table-7**  
**Association of peak estradiol with endometrial pattern**

Endometrial pattern	Estradiol on day of HCG injection (pg/ml)					
	> 1500		1500 – 3000		> 3000	
	No.	%	No.	%	No.	%
Triple line present	72/112	64.29	32/44	72.73	39/55	70.91
Absent	40/112	35.71	12/44	27.27	16/55	29.10

Out of total 211 patients, 79 patients (37.44%) had successful IVF pregnancy which is comparable to the success rate of IVF cycles, according to SART (Society of assisted reproductive technology) which keep a data base of IVF clinic in North America which vary between 31%-46% in age <35 year and 13% in age > 40 year. Out of the successful patients, 36 patients (45.57 %) had delivered and 28 (35.44 %) have ongoing pregnancy of more than 26 weeks, 12 patients (15.18%) aborted. Three pregnancies resulted in ectopic (3.78%). Twelve patients had twin pregnancy and no triplet pregnancy occurred. Results were comparable to study by Mustafa kara et al 2012 21% clinical pregnancy were achieved<sup>17</sup>, by V.A Akande et al 2002 28.1% become pregnant<sup>18</sup>, and by Ahlam Al Ghamdi et al analyzed retrospectively CPR was 35.8%.<sup>19</sup> In study by Mustafa kara et al 2012 in total of 203 women undergoing IVF, 43 patients (21%) clinical pregnancy were achieved but delivery live baby rate was 15%<sup>17</sup>. In study by V.A Akande et al 2002 out of 1019 patients undergoing IVF 977 (96%) received embryo suitable for transfer and 275 (28.1%) become pregnant and 227 (23.2%) had a live birth.<sup>18</sup> Study by Ahlam Al Ghamdi et al 2009 A total of 2464 cycles were analysed retrospectively . Pregnancy rate (PR) was 35.8%<sup>19</sup>.

In present study mainly following predictors were taken into account basal FSH, serum estradiol, endometrial thickness and pattern on day of HCG injection, along with age, type of infertility, cause of infertility, number of ampoules of gonadotrophin used, number of follicles >15mm formed, number of oocyte retrieved, number of embryo formed, number of top quality embryo transferred .After IVF result patients were divided into successful and unsuccessful group and were compared to above factors. Success rate is high in our study as compare to other studies because we have taken good prognosis patients that are patients <37 year age, and top quality embryos were transferred.

**Basal FSH:** Seventy one patient (89.87%) in successful group had basal FSH <10mIU/ml whereas in unsuccessful group 92 (69.70%) patients have basal FSH <10mIU/ml. The mean FSH in study population is (7.39±2.41) when chi test was applied significant relationship was seen between basal FSH and success of IV. As basal FSH is taken as one of the marker of ovarian reserve the result are similar with below studies.

Ottosen et al and Sabatini et al., 2008<sup>20, 21</sup> dichotomized basal FSH into the categories 0–10 IU and >10 IU. In both studies, the chances of pregnancy were significantly higher in women with FSH <10 mIU/ml than in women with FSH concentrations of >10mIU/ml.

**Endometrial thickness and endometrial pattern:** In the study population the distribution of endometrial thickness was, (29.38%) patients having ET <7mm, (57.82 %) patients had ET between 7 – 9 mm and (12.80 %) patients had ET >9mm. Thus maximum percentage of the patients undergoing IVF had endometrial thickness in (7 – 9 mm) group. In the successful patients mean ET (8.44 ± 1.35) is higher than in the unsuccessful patients (7.05 ± 1.47). Study by Ahlam et al 2008 PR increased linearly (r = 0.864) from 29.4% among patients with a lining of less than or equal to 6 mm, to 44.4% among patients with a lining of greater than or equal to 17 mm. ROC showed that endometrial thickness is not a good predictor of PR, so a definite cut-off value could not be established (AUC = 0.55)<sup>19</sup>.

The association between the endometrial pattern and success of IVF was also studied in present study. In cases of successful IVF group triple line was present in (81.01%) of the cases whereas (46.21%) of the unsuccessful IVF cases had triple line pattern. According to chi test significant association is seen between endometrial pattern and success of IVF. Result is similar to below studies.

Chen et al 2010<sup>22</sup> Studied endometrial thickness and pattern together and found A no-triple line endometrial pattern with moderate endometrial thickness (7-14 mm) had a detrimental effect on pregnancy outcome and adequate endometrial thickness (>14 mm) seemed to mitigate the detrimental impact (high miscarriage rate) of no triple line pattern and thus concluded that a combined analysis of endometrial thickness and pattern on the day of HCG administration was a better predictor of the outcome of IVF/ICSI-ET and may be more helpful for patient counseling than the separate parameter.

In study by Neena et al 2012<sup>23</sup> The mean endometrial thickness was 8.1 mm .Overall, 27 (26.73%) patients conceived and in these women the endometrial thickness was between 6 and 12 mm With a thin endometrium (≤7 mm) and no-triple-line endometrial pattern coexisting in an *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) candidate, cryopreservation should be recommended. With a thin endometrium and a good texture (triple-line), other prognostic factors, such as embryo quality, should be taken into account.

**Serum estradiol:** Serum estradiol value was divided into three group <1500pg/ml, 1500-3000pg/ml and >3000pg/ml. Success rate increase with increase in estradiol level as the success rate in the three groups where (29.11%, 32.91% and 37.97%)

respectively in unsuccessful group the order is reversed (68.94%, 18.94%, 12.12%) in the three group.

In study by Ernest et al 2000<sup>24</sup> studied 1122 women <40year undergoing IVF, serum estradiol on day of HCG divided a<1000 pmol/ml, b>1000and <2000 pmol/ml, and c>2000 pmol/ml group a and c has significantly lower pregnancy and implantation rate as compare to b.

In our study mean peak estradiol in successful patients (2294.6 ± 1245.06) is higher than in unsuccessful patients (1574.52 ± 1103.46) and (p is < 0.01) therefore high peak serum estradiol have better result. Therefore serum estradiol on day of HCG is a strong predictor of IVF outcome similar to results by Chenette et al<sup>25</sup> and Gelety and Buyalos et al studies<sup>26</sup>. Serum peak estradiol and pregnancy outcome was also analyzed in different studies and variable results were found in study by Kosmos et al 2004<sup>10</sup> a systematic review was done and concluded that there is no high-quality evidence to support or deny the value of E<sub>2</sub> determination on the day of hCG administration for pregnancy achievement in IVF cycles, where pituitary down-regulation is performed with GnRH agonists.

## Conclusion

This study indicate that basal FSH level is a good predictor of ovarian reserve and success of IVF-ET cycles. When FSH is >10 mIU/ml the patients should be counseled for poor quality of embryo and thus may need donor oocyte. Endometrial thickness and pattern collectively is good predictor of IVF success. Peak estradiol level independently is a strong predictor of IVF success.

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