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Original Research Article

Serum estradiol concentrations as a predictor of successful outcome in artificial frozen-thawed embryo transfer cycles

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ABSTRACT

Background: The role of late follicular serum estradiol monitoring in artificial FET cycles remain unclear. The purpose of this study was to evaluate the correlation between serum estradiol levels on the day of starting progesterone supplementation with clinical pregnancy rates in FET cycle.
Methods: This was a non-interventional observational cohort study of patients undergoing ICSI followed by FET at Nadkarni hospital and test tube baby center, Killa-Pardi, Gujarat during the period of January 2021 to May 2021. Total 64 cycles were studied and serum estradiol levels were analyzed on the day of starting progesterone supplementation. They were divided into 3 groups based on serum E2 levels (0-25th centile, 25th-75th centile and >75th centile). Chi square/Fisher exact test were used to compare the clinical pregnancy and implantation rates between these groups.
Results: Clinical pregnancy and implantation rates in group A, B and C were 68.8%, 78.1%, 75% and 36.98±9.06, 32.03±4.48 and 29.69±5.69 respectively.
Conclusions: Serum estradiol levels before progesterone supplementation in FET cycles do not predict the outcome of FET cycle therefore making routine monitoring of serum estradiol in FET cycle of questionable value.

Keywords: Estradiol, FET, ICSI, Pregnancy

INTRODUCTION

With the improvement in cryopreservation techniques, there is an increasing trend towards frozen embryo transfer.[1] Frozen embryo transfer offers multiple advantages like prevention of ovarian hyper-stimulation syndrome, increasing the cumulative pregnancy rates etc. Various recent studies show improved pregnancy rates with elective frozen cycles as compared to fresh transfers.[2,3] For successful implantation to occur there should be precise timed synchronization between the implanting embryo and the endometrium. Estradiol plays a critical role in the development of the endometrium and induction of progesterone receptors, hence different protocols for endometrial preparation in FET cycles have been developed.[4] These protocols can largely be divided into natural and artificial cycles. In natural cycles no pharmacological intervention is done for endometrial growth and the natural cycle is monitored via ultrasound prior to ovulation. Meanwhile, in artificial cycles down regulation is achieved by exogenous estrogen administration and the dose and duration of estradiol varies depending on the endometrial response.[5] However studies in the past have shown that it is the days of exposure that matters rather than the serum estradiol levels for an adequate endometrial response.[6] There is no

consensus on one method being better than the other.[1,7] Studies in the past have shown comparable pregnancy rates in artificial versus natural cycles. Once there is sufficient endometrial proliferation with estradiol, progesterone supplementation is started to promote the final phase of endometrial preparation prior to the transfer. The detrimental effect of elevated progesterone prior to embryo transfer is well established.[8] However the correlation between serum estradiol levels before embryo transfer and successful implantation is poorly defined in the literature thus requiring further evaluation. Some studies in the past have shown decreased endometrial receptivity with increasing estradiol levels.[9] The purpose of the present study was to investigate the effect of serum estradiol levels before initiating progesterone supplementation prior to embryo transfer in FET cycles on pregnancy outcomes.

METHODS

Study population

The study was performed in a rural area at Nadkarni hospital and test tube baby center, Killa-Pardi, India. Patients undergoing frozen embryo transfer after ICSI cycle from January 2021 to May 2021 were recruited in the study.

Inclusion criteria

The inclusion criteria were patient undergoing frozen embryo transfer with self or oocyte donation, patients with endometrial thickness of more than 8 mm on the day of transfer and patients receiving oral estradiol valerate or estradiol hemihydrate for endometrial preparation.

Exclusion criteria

Patients with abnormal endometrial cavity or high progesterone levels on the day of trigger were excluded from the study.

Endometrial preparation

For endometrial preparation, artificial cycle protocol was used, downregulation was done with injection decapeptyl 0.1/0.05 mg for 5 days premenstrually and the patients were called on day 2 of menses. Downregulation was confirmed on the scan by absence of any cyst, presence of thin shedding endometrium, day 2 LH <5 IU and estradiol <50 pg/ml. From day 2/3 oral estradiol valerate 4 mg BD was given for 5 days followed by 4 mg TDS, patient was then called for scan on day 9 and estradiol hemihydrate 2 mg TDS till day 13 was added if the endometrial growth was less than 7.5 mm. On day 13/14 endometrial uterine artery RI was assessed using colour doppler and progesterone supplementation was then started from day 13/14 after supplementing estradiol for a minimum period of 10 days.

Serum estradiol measurement

Serum estradiol levels were measured on the day of starting progesterone supplementation using electrochemi-luminescence immunoassay. Serum progesterone levels were also analyzed using the same technique.

Embryo transfer

On the day of embryo transfer endometrial thickness was assessed. Cryopreserved embryos were thawed and single/sequential transfer was done. Day 3 embyo transfer was done on day 17 and day 5 blastocyst transfer was done on day 19. Transfer was postponed if the serum progesterone levels were more than 1 or endometrial thickness was less than 8 mm. Patients having difficult embryo transfer were excluded from the study.

Cycle outcome

Serum β-hCG was tested after 14 days of embryo transfer. Clinical pregnancy was defined by the presence of gestational sac on ultrasound and β-hCG levels >100 mIU/ml. Implantation rates were defined by the number of gestational sac observed on ultrasound divided by the number of embryos transferred.

Statistical analysis

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean±SD (minimum-maximum) and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance. Chi-square/Fisher exact test had been used to find the significance of study parameters on categorical scale between two or more groups, non-parametric setting for qualitative data analysis. Fisher exact test used when cell samples were very small, p value <0.1 was considered significant.

RESULTS

A total of 64 FET cycles were analyzed in this study. Based on the serum estradiol levels patients were divided into group A (0-25th centile), group B (25th-75th centile) and group C (>75th centile). Mean serum estradiol was 154.46±7.37, 408.95±23.39 and 849.02±33.75 in group A, B and C respectively. The clinical characteristics of these 3 groups are depicted in Table 1. Mean age was 32±1.72, 32±0.9 and 33.69±6.13 in group A, B and C respectively. Mean age between these groups were comparable and no significant difference was seen (p>0.1). Mean serum progesterone levels on the day of starting progesterone were not significantly different in group A, B and C values being 0.27±0.04, 0.25±0.03 and 025±0.03 respectively (p>0.1). There was no significant difference seen between the number of days estradiol treatment was given with the mean days being

12.06±0.14, 12.22±0.11 and 12.38±0.18 in group A, B and C respectively (p>0.1). Mean endometrial thickness on the day of transfer in group A, B and C was 11.60±1.74, 11.94±1.98 and 11.38±1.93 respectively and it was not statistically significant (p>0.1). Sequential transfer was done in 87.5%, 81.3% and 87.5% respectively in groups A, B and C (p>0.1).

Table 1: The clinical characteristics of these 3 groups.

Variables	Serum estradiol			Total (n=64)	P value
	1-25th percentile (n=16)	25-75th percentile (n=32)	More than 75th percentile (n=16)		
Age (years) (mean±SD)	32.00±1.72	32.00±0.90	33.69±6.13	32.42±5.79	0.12
Days E2 (mean±SD)	12.06±0.14	12.22±0.11	12.38±0.18	12.22±0.08	0.379
P4 (mean±SD)	0.27±0.04	0.25±0.03	0.25±0.03	0.26±0.02	0.912
E2 (mean±SD)	154.46±7.37	408.95±23.39	849.02±33.75	455.34±34.6	<0.001
ET (mean±SD)	11.60±1.74	11.94±1.98	11.38±1.93	11.71±1.90	0.606
RI (mean±SD)	0.79±0.02	1.04±0.23	0.83±0.02	0.92±0.11	0.600
First BHCG (mean±SD)	2086.38±640.2	1882.25±408.52	1531.51±427.81	1845.59±277.46	0.778
Sequential frequency (%)	14 (87.5)	26 (81.3)	14 (87.5)	54 (84.4)	0.789
Clinical pregnancy rate frequency (%)	11 (68.8)	25 (78.1)	12 (75)	48 (75)	0.812
Implantation rate (mean±SD)	36.98±9.06	32.03±4.48	29.69±5.69	32.68±3.44	0.748

Uterine artery RI value was also seen on the day of starting progesterone and the mean RI was 0.79±0.02, 1.04±0.23 and 0.83±0.02 in group A, B and C respectively. This difference was not different statistically (p>0.1).

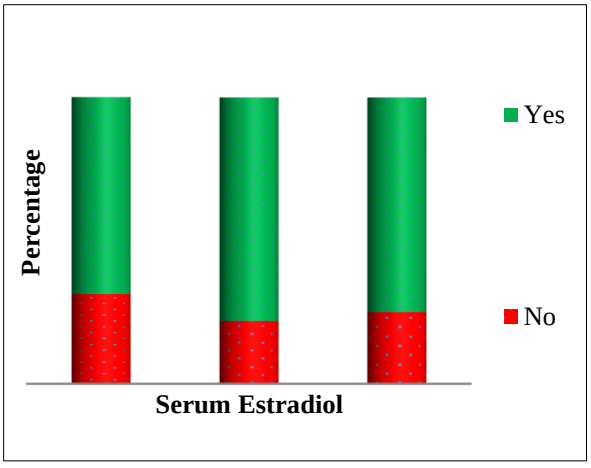

Figure 1: Clinical pregnancy rates in group A, B and C.

First β-hCG in group A, B and C was 2086.38±640.2, 1882.25±408.52, 1531.51±427.81 respectively (p>0.1). After evaluating the baseline characteristics of the patients a comparative study was done between clinical pregnancy and implantation rates. Clinical pregnancy rates were 68.8%, 78.1%, 75% in group A, B and C respectively as sown in (Figure 1) (p>0.1). Implantation rates were maximum in group A (36.98±9.06) and minimum in group C (29.69±5.69). Implantation rate in group B was 32.03±4.48, however the difference was not statistically significant (p>0.1). The implantation rates in group A, B and C is represented in (Figure 2).

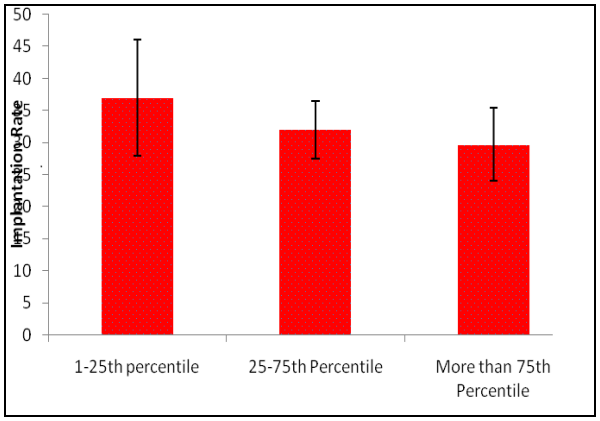

Figure 2: Implantation rates in group A, B and C.

DISCUSSION

It is well established that successful outcome in a FET cycle depends on proper synchronization between the developing endometrium and the implanting embryo which is determined by the window of implantation. Studies in the past have reported the role of estradiol in pinopode expression which is a marker to locate

implantation window.[10] However the impact of the serum levels of estradiol on outcome of FET cycle was not understood. In a natural cycle estradiol rose progressively and LH surge occured at levels more than 200 pg/ml and there was a LH peak at levels 300-400 pg/ml followed by an abrupt decline.[11] In our study levels of serum estradiol was measured prior to starting progesterone supplementation and was found to be in the range of 100.5 pg/ml to 1015.13 pg/ml showing that many patients had supraphysiological estradiol levels that were not seen in a natural cycle, interestingly some patients also showed lower than physiological levels of serum estradiol even after supplementation and resulted in clinical pregnancy. Elevated serum estradiol may have a detrimental effect on the endometrium, clinically elevated levels in an IVF cycle have also been linked to poor pregnancy rates.[9,12] In a mouse model elevated estradiol levels was associated with altered endometrial expression of genes needed for implantation.[13] Also Remohi et al demonstrated normal implantation to occur at levels <50 ng/ml which was considered to be the limit for the arrest of ovaries when a gonadotropin releasing hormone analogue was used.[14] Routine estradiol monitoring before FET was a common practice in most of the IVF clinics thus adding to the cost and unnecessary intervention. This study aimed to analyze the correlation between the serum estradiol levels and pregnancy outcome if any. Work had been done in the past and some studies had shown no correlation between serum estradiol levels and ART outcome, while some have shown a negative outcome with elevated serum estradiol levels.[15-17] Our conclusion was in line with the majority of the studies published in the past.

In the study conducted by Wen et al a total of 193 cycles were studied, 110 cycles in day 3 transfer group and 83 cycles in day 5 transfer group, both these groups were divided into 2 subgroups based on serum estradiol levels on the day of embryo transfer that is low E2 (≤ 150 pg/ml) and high E2 (>150 pg/ml). This study indicated similar biochemical pregnancy, clinical pregnancy and implantation rates in patients with low and high E2 groups. However in this study serum estradiol was measured on the day of transfer instead of on the day of starting progesterone as done in our study.[18] Our study supported the results of Remohi et al that found similar implantation rates in all the four groups based on E2 levels in artificial oocyte donation FET cycles.[15] Patients in this study were desensitized with leuprolide acetate after which oral estradiol was started at 2 mg/day and serum E2 levels were measured on the day of initiation of progesterone supplementation. On the basis of E2 levels four groups were made levels <100 pg/ml, 100-199 pg/ml, 200-299 pg/ml, 300-399 pg/ml and ≥400 pg/ml. All the four groups showed similar pregnancy and implantation rates. Niu et al conducted a retrospective cohort study in which 274 artificial frozen-thawed embryo transfer cycles were analyzed, groups were made based on different serum estradiol levels on the day of progesterone initiation (percentile 0-25th, 25th-75th and 75th to 100th) and it was concluded that serum estradiol levels did not predict successful pregnancy rates in artificial FET cycles.[16] The difference from our study was that lower dose of oral estradiol was used for endometrial preparation hence the highest E2 levels in the 75th-100th range were lower on average that was 299 pg/ml. Our findings differed from the study conducted by Fritz et al in which 110 autologous artificial FET cycles from 95 patients were analyzed. This study was different from ours as E2 supplementation was done using transdermal and intramuscular route and serum E2 levels were measured from initiation of FET cycle to levels prior to progesterone supplementation initiation. Another important difference was that comparisons were made using average E2 levels and peak E2 throughout the cycle. It was seen that average estradiol levels were lower in cycles resulting in ongoing pregnancy/live births as compared to those who did not. It was also seen that that ongoing pregnancy/live birth rates decreased from 54% when peak serum E2 was <234 pg/ml to 9% for peak E2 levels above 692 pg/ml.[17]

CONCLUSION

Serum estradiol levels before progesterone supplementation in FET cycles do not predict the outcome of FET cycle therefore making routine monitoring of serum estradiol in FET cycle of questionable value.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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