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ASSISTED REPRODUCTION TECHNOLOGIES

Elevated serum estradiol levels in artificial autologous frozen embryo transfer cycles negatively impact ongoing pregnancy and live birth rates

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Abstract
Purpose The aim of this study is to evaluate the correlation between serum estradiol (E_2) levels during artificial autologous frozen embryo transfer (FET) cycles and ongoing pregnancy/live birth rates (OP/LB).
Methods A historical cohort study was conducted in an academic setting in order to correlate peak and average estradiol levels with ongoing pregnancy/live birth rates for all autologous artificial frozen embryo transfer cycles performed from 1/2011 to 12/2014.
Results Average and peak E_2 levels from 110 autologous artificial FET cycles from 95 patients were analyzed. Average E_2 levels were significantly lower in cycles resulting in OP/LB compared to those that did not (234.1 ± 16.6 pg/ml vs. 315 ± 24.8 pg/ml, respectively, $p = 0.04$). Although peak E_2 levels were not significantly different between cycles resulting in OP/LB compared with those that did not (366.9 ± 27.7 pg/ml vs. 459.1 ± 32.3 pg/ml, respectively, $p = 0.19$), correlation analysis revealed a statistically significant ($p = 0.02$) downward trend in OP/LB rates with increasing peak E_2 levels.
Conclusions This study suggests that elevated E_2 levels in artificial autologous FET cycles are associated with lower OP/LB rates. Estradiol levels should be monitored during artificial FET cycles.

Keywords Estradiol · In vitro fertilization · Frozen embryo transfer cycles · Live birth rates

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Introduction

Frozen non-donor embryo transfer cycles account for approximately 24.5% of total IVF cycles reported to the CDC in 2013 [1]. The percentage of frozen embryo transfer (FET) cycles is dramatically increasing, in part due to a trend to transfer fewer embryos, improved cryopreservation techniques, and elective oocyte cryopreservation [2]. Additionally, recent evidence suggests that pregnancy rates following in vitro fertilization (IVF) could be improved with elective FET cycles in place of fresh embryo transfers [3–5]. Frozen embryo transfer cycles, either natural or artificial, require synchronization of the endometrium with development of the embryo. Natural FET cycles rely on growth of a dominant follicle with subsequent endometrial maturation by endogenous estradiol (E_2) and progesterone. Artificial FET cycles commonly rely on suppression of the hypothalamic-pituitary-ovarian axis with administration of exogenous E_2 and progesterone for endometrial/embryo synchronization.

Successful implantation and subsequent healthy pregnancy are dependent on invasion of embryo-derived trophoblast cells through the maternal decidua and myometrium, with eventual remodeling of the myometrial spiral arteries [6]. This process is regulated by a complex interaction between maternal and fetal tissues, mediated by numerous cytokines, growth factors, and peptides [7]. Estradiol is critical to endometrial and placental development [8, 9]; however, excess E_2 in the early stages of pregnancy can have adverse effects on placentation. Elevated E_2 has been shown to adversely affect endometrial receptivity [10] and elevated levels in fresh cycles prior to transfer are correlated with decreased implantation rates [11, 12]. Additionally, elevated E_2 levels during IVF cycles have been linked to an increased incidence of adverse pregnancy outcomes including pre-eclampsia and intrauterine growth restriction [13, 14].

Estradiol levels are a modifiable variable in artificial FET cycles. The aim of this study is to evaluate the impact of peak and average E_2 concentrations prior to progesterone administration in autologous artificial FET cycles on ongoing pregnancy/live birth rates (OP/LB).

Materials and methods

A total of 110 artificial autologous FET cycles from 95 patients with a subsequent embryo transfer were conducted from 1/2011 to 12/2014 at Montefiore's Institute for Reproductive Medicine and Health. Inclusion criteria included patients undergoing an artificial autologous FET cycle primarily with transdermal or intramuscular E_2 supplementation with or without the addition of vaginal E_2. Exclusion criteria included natural FET cycles, donor FET cycles, and initiated artificial cycles without a subsequent embryo transfer. This study was approved by the Albert Einstein College of Medicine Institutional review board (IRB number 2016-6057). Data were extracted including patient's age, duration of FET cycle, maximum historical serum follicle stimulating hormone (FSH) level, route of insemination, gravidity, parity, body mass index (BMI), race, route of E_2 administration, use of vaginal E_2 supplementation, maximal endometrial stripe (EMS) during the FET cycle, route of progesterone administration, serum E_2 levels from initiation of the FET cycle to levels immediately prior to progesterone supplementation, peak E_2 levels, average E_2 levels, day of embryo transfer, number of embryos transferred, and whether the transfer resulted in a biochemical pregnancy, clinical pregnancy, ongoing pregnancy/live birth, or spontaneous abortion. Biochemical pregnancy was defined as positive β human chorionic gonadotropin (βhCG) level without evidence of a gestational sac on ultrasound. Clinical pregnancy was defined as evidence of a gestational sac on ultrasound. Ongoing pregnancy was defined as intrauterine pregnancy with a heart beat at 20 weeks or later. Peak serum E_2 levels were considered the highest serum E_2 level (pg/ml) obtained from initiation of the artificial FET cycle to the level immediately prior to progesterone supplementation, whereas average serum E_2 levels (pg/ml) were the average of all serum E_2 levels recorded from the initiation of the artificial FET cycle to the last level prior to progesterone supplementation.

Stimulation protocol

Frozen embryo transfer cycles were started either following hormonal suppression with oral contraception, GnRH agonist (leuprolide acetate) suppression, combination of leuprolide acetate, and hormonal suppression, or no suppression. Following confirmation of suppressed E_2 levels (defined as serum E_2 level below 50 pg/ml) and a negative βhCG test, supplemental E_2 was initiated primarily transdermally (100mcg/patch) every other day or intramuscularly (2 mg) twice per week. Trough E_2 levels were measured twice weekly for at least 2 weeks and E_2 dosage was adjusted accordingly to achieve an E_2 level of 200–500 pg/ml following 8–10 days of E_2 supplementation. Progesterone supplementation was added once adequate E_2 levels were achieved, and an adequate EMS was appreciated by ultrasound (≥ 7 mm). Progesterone was either administered in oil intramuscularly (50 mg) daily or vaginally via Endometrin (Ferring Pharmaceuticals, Parsippany, NJ) at 100 mg three times a day or Crinone (Allergan, Irvine, CA). Estradiol was measured in pg/ml using Immulite 2000 (Siemens, Munich, Germany). Embryo transfer was performed under ultrasound guidance on the 5th day of progesterone administration for a day 3 embryo transfer and on the 7th day of progesterone administration for a day 5 embryo transfer.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Student's *t* test or Mann Whitney U test (if data were not normally distributed) were used to compare the demographic and cell cycle characteristics that are characterized with continuous data. Chi square or Fisher exact tests were used for categorical data. Logistic regression on achieving an OP/LB was implemented using the generalized estimating equations [15] that was performed to account for possible correlation between contributions of more than 1 cycle from a single patient, with average and peak serum E_2 levels as primary exposure variables in the model. Pearson's correlation was used to assess the association between peak serum E_2 levels categorized in percentiles and OP/LB rates. Additionally, a one-sided Fisher's exact test was used to compare the OP/LB rates between the lowest and highest 10th percentiles peak serum E2 levels. In order to determine the predictive ability of average and peak serum E_2 levels on achieving a clinical pregnancy, a receiver operating characteristic (ROC) curve was created to calculate the cutoff, sensitivity, specificity, and area under the curve (AUC). All statistical analyses were performed using STATA/IC 12.0 software. $P < 0.05$ was considered statistically significant.

Results

A total of 110 artificial FET cycles from 95 patients were analyzed from 1/2011 to 12/2014. Overall pregnancy rate was 56.3%, clinical pregnancy rate was 43.6%, and OP/LB rate was 32.7%. Average number of E_2 measurements in all cycles following exogenous E_2 administration and prior to progesterone supplementation was 4.4 ± 0.1 Demographic and cycle characteristics of women are shown in Table 1.

Table 1 Baseline characteristics in cycles resulting with and without ongoing pregnancy/live birth following an artificial FET cycle

Characteristics	Positive OP/LB (n = 36)	No OP/LB (n = 74)	P value
Age at oocyte cryopreservation (years)	32.1 ± 0.6	34.3 ± 0.5	0.01
Max follicle stimulating hormone (IU/L)	6.4 ± (5.4–7.8)	5.8 (4.9–8.7)	0.79
Gravidity	1 (1–2)	1 ± (0–2)	0.66
Parity	1 (0–1)	0 (0–1)	0.11
Body mass index (kg/m^2)	26.2 ± 0.9	26.6 ± 0.7	0.72
Race (%)			0.64
Caucasian	38.9	28.4	
African American	22.2	20.3	
Hispanic	11.2	12.2	
Other	27.7	39.1	
FET cycle length (days)[a]	23.9 ± 0.7	23.2 ± 0.4	0.29
Max endometrial stripe during FET cycle (mm)	9.6 ± 0.3	9.5 ± 0.3	0.85
Route of progesterone administration (%)			0.49
Intramuscular	69.4	75.7	
Vaginal	30.6	24.3	
Route of E2 administration (%)			0.33
Transdermal	44.4	41.9	
Intramuscular	52.8	56.8	
Other[b]	2.8	1.4	
Vaginal E_2 supplementation (%)			0.54
Yes	8.3	13.5	
No	91.7	86.5	
FET cycle GnRH agonist use (%)			0.90
GnRH agonist use	58.3	59.5	
No GnRH agonist use	41.7	40.5	
Embryos transferred (number)	1.8 ± 0.1	1.8 ± 0.09	0.98
Day of embryo transfer (%)			0.008
5	94.4	72.9	
3	5.6	27.1	
Baseline E_2 at start of FET cycle (pg/ml)	35.8 ± 2.6	32.8 ± 2.0	0.39

Values are presented as mean ± standard error of the mean if normally distributed and median with interquartile range if non-normally distributed

FET frozen embryo transfer, *E_2* estradiol, *GnRH* gonadotropin-releasing hormone agonist

[a] Denotes days from start of estradiol supplementation to embryo transfer

[b] 1 cycle in cohort without live birth/ongoing pregnancy received estrace vaginally only. One patient in the live birth/ongoing pregnancy cohort received estrace PO only

Age at embryo cryopreservation was significantly lower in cycles resulting in OP/LB compared to cycles not resulting in OP/LB (32.1 ± 0.6 vs. 34.3 ± 0.5 years, respectively, $p = 0.01$). Additionally, patients who had an OP/LB had more often a day 5 embryo transfer (compared to day 3 embryo transfer) then patients without an OP/LB (94.4% vs. 73.0%, respectively, $p = <0.01$).

Average serum E_2 levels were lower in cycles with positive OP/LB outcome compared to cycles with no OP/LB (234.1 ± 16.6 pg/ml vs. 315 ± 24.8 pg/ml, respectively, $p = 0.04$). As stated above, average age at cryopreservation was lower and percentage of day 5 embryo transfer were higher in cycles that ended up with OP/LB; however, these variables were not associated with either average or peak E_2 levels. Hence, these variables were not considered as potential confounders, and were excluded from the regression model when average E_2 and peak E_2 levels were analyzed to predict live birth rates. Logistic regression implemented with generalized estimating equations showed that average E_2 levels predicted lack of OP/LB rate ($p = 0.04$, 95% CI: 0.0001–0.007). Since higher serum average E_2 levels were associated with lower likelihood of achieving a clinical pregnancy, we wanted to determine an optimal average serum E_2 cutoff level to predict OP/LB with good accuracy. For this purpose, an ROC curve was created (Fig. 1) and demonstrated that average serum E2 level of ≥ 330 pg/mL was associated with no OP/LB

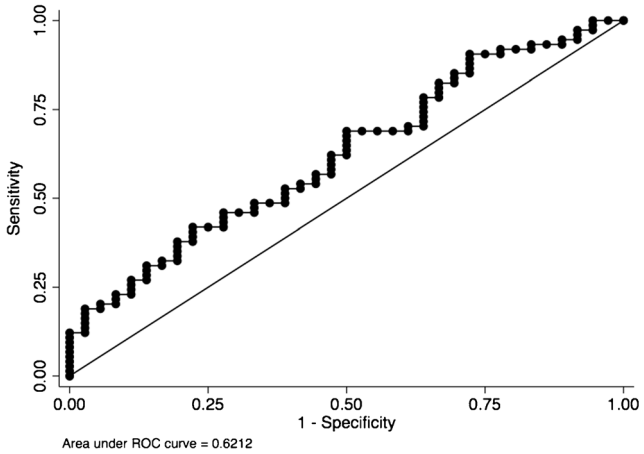

Fig. 1 Receiver operating characteristics curve. Average serum E2 level of ≥ 330 pg/mL is associated with no ongoing pregnancy/live birth (OP/LB) with a low sensitivity of 37.8% but a specificity of 80.6% and an AUC of 0.62

with a low sensitivity of 37.8%, but a specificity of 80.6%, and an AUC of 0.62.

There was not a significant difference between peak E_2 levels in OP/LB group compared to those without OP/LB (366.9 ± 27.7 pg/ml vs. 459.1 ± 32.3 pg/ml, respectively, $p = 0.19$). The patients were categorized into three groups according to their peak serum E2 levels: lowest 10th percentile, 11th to 90th percentile, and the highest 10th percentile. Correlation analysis revealed a statistically significant ($p = 0.02$) downward trend in OP/LB rates with increasing peak E_2 levels (Fig. 2). Cycles with the highest 10th percentile peak E_2 levels (692–1713 pg/ml) had a OP/LB rate of 9.1% compared to 32.9% in the 11th to 90th percentile (215–689 pg/ml) and 54.6% in the lowest 10th percentile peak E_2 levels (135–214 pg/ml). Having a serum estradiol level in the lower 10th percentile was more likely to achieve an OP/LB when compared to having a serum estradiol level in the upper 10th percentile (54.6 vs. 9.1%, respectively, OR: 12, $p = 0.03$).

Discussion

In this study, we have shown that increasing average serum E_2 levels during autologous FET cycles are associated with decreasing OP/LB rates. We have also shown that OP/LB rates decrease from 54% for peak serum E_2 levels below 234 pg/ml to 9% for peak serum E_2 levels above 692 pg/ml.

Placental implantation is a complex process requiring communication between maternal and fetal tissues [16]. For implantation, placentation, and a healthy subsequent pregnancy outcome, trophoblast cells derived from the placenta must invade through the endometrium, inner third of the myometrium, and eventually remodel the spiral arteries [6]. In the follicular phase, E_2 is necessary for proper endometrial maturation through induction of endometrial progesterone receptors

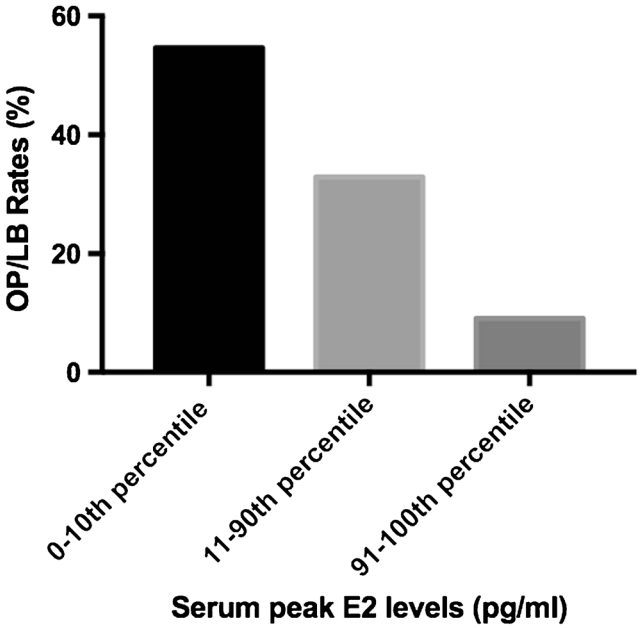

Fig. 2 Correlation between ongoing pregnancy/live birth (OP/LB) rates and peak serum estradiol (E2) levels. Ongoing pregnancy/live birth rates are negatively correlated with increasing peak serum estradiol levels ($p = 0.02$)

necessary for implantation [17]. Despite the necessity of E_2 in the follicular phase for implantation, excess E_2 during the follicular phase may have a detrimental role. Estradiol has been shown in vitro to cause cell death and inhibit trophoblast invasion in both first-trimester human cytotrophoblast cell line and first-trimester placental explants [18]. In a mouse model, E_2 must be held in relatively narrow range for the endometrium to be in a receptive state, and elevated E_2 in preparation for a receptive state is associated with altered endometrial expression of genes necessary for implantation [19]. In a baboon model, elevated E_2 levels in the first 60 days of pregnancy adversely affected extravillous trophoblast invasion and uterine artery function [20]. Clinically, elevated E_2 levels following controlled ovarian hyperstimulation during IVF cycles have been associated with poorer pregnancy rates [11, 12] and adverse placenta-related pregnancy outcomes [13, 14].

Exogenous E_2 administered in artificial FET cycles prior to progesterone supplementation is analogous to the endogenous E_2 during the follicular phase in natural cycles. During natural cycles, E_2 progressively rises, triggering an LH surge at levels greater than 200 pg/ml for at least 50 h [21], and peaks at approximately 300–400 pg/ml around the time of ovulation followed by an abrupt and steep decline [22]. In our study, higher average E_2 levels were found in cycles not resulting in a OP/LB compared to those resulting in an OP/LB despite E_2 levels being in the range found during the follicular phase of natural cycles. This may be explained by the timing of E_2 measurements. E_2 levels were measured on the day the patient was scheduled to administer exogenous E_2, and therefore represented a trough level. Given measurement of trough levels,

it is likely that the E_2 levels the endometrium was exposed to during the artificial FET cycles were higher than those measured. In addition, in natural cycles there is an immediate drop in E_2 following the LH surge, which does not occur during artificial FET cycles. Although peak levels did not significantly differ between cycles with an OP/LB compared to those without, there was a significant difference when extreme levels were analyzed. In the cycles with the highest 10th percentile peak E_2 levels, there was a sixfold decrease in OP/LB compared to cycles with the lowest 10th percentile peak E_2 levels. The levels obtained in the top 10thpercentile of peak E_2 levels ranged between 692 and 1713 pg/ml, representing supraphysiologic levels that are not normally seen prior to the rise of progesterone in natural cycles. This suggests that elevated E_2 levels prior to progesterone stabilization of the endometrium may have detrimental effects on endometrial/blastocyst synchrony. Interestingly, the lowest 10th percentile of E_2 levels ranged between 135 and 214 pg/ml and resulted in the highest OP/LB rates despite being lower than physiologic levels seen during a natural follicular phase. As described above, these levels likely do not represent the true peak E_2 levels as trough levels were obtained. Additionally, low levels of E_2 (< 100 pg/ml) in artificial FET donor oocyte cycles have not been associated with adverse pregnancy rates [23].

Our findings are in contrast to a retrospective study by Niu et al. evaluating the predictive value of E_2 in artificial FET cycles, revealing that elevated E_2 was not predictive of pregnancy rates [24]. Differences in our findings may be related to the timing of E_2 measurements. In the study by Niu et al. [24], prior to progesterone administration, E_2 levels were measured only on the day ultrasound was performed and day of progesterone initiation, whereas in our study E_2 was measured on average 4.4 times per cycle following the onset of E_2 administration and prior to the onset of progesterone supplementation. In the above study, E_2 levels were separated into three percentiles (0–25th%, 25th–75th%, and 75th–100th%). The highest E_2 levels on the day of progesterone administration in the 75th to 100th% range were lower on average (299 pg/ml) than levels we obtained in our cycles. Another major and important difference was the route of E_2 administration. Cycles in the present study were administered predominantly with transdermal and intramuscular E_2 whereas, in the study by Niu et al. the route of E_2 administration was oral. Oral E_2 is extensively metabolized by the intestine and liver to estrone, an estrogen with weaker estrogenic activity and weaker binding affinity for estrogen receptors compared to E_2 [17]. In contrast, transdermal and intramuscular administration of E_2 bypass first-pass metabolism and result in less conversion to estrone [17]. It is possible that the endometrium is more sensitive to E_2 administered via the intramuscular or transdermal routes compared to E_2 administered orally due to less conversion to estrone.

Our findings are also in contrast to findings of Remohi et al. that found no difference in implantation rates in artificial oocyte donation FET cycles with E_2 levels measured on the day of progesterone administration. They grouped cycles into E_2 levels < 100 pg/ml, 100–199 pg/ml, 200–299 pg/ml, 300–399 pg/ml, and ≥ 400 pg/ml [23]. In this study, patients were desensitized with leuprolide acetate followed by oral estradiol initiated at 2 mg/day. This study also used oral E_2, which may account for the discrepant findings. Additionally, the patient population in this study were donor oocyte recipients, whereas, patients in our study were autologous FET recipients.

Despite physiological differences in artificial and natural FET cycles, meta-analyses reveal no significant differences in pregnancy rates [2, 25]. Advantages of artificial FET cycles benefit anovulatory patients and include ability to control the timing of FET cycle start and embryo transfer. Our study suggests that when performing an artificial FET cycle, E_2 levels should be monitored and E_2 dosage should be adjusted accordingly. Results of this study demonstrate that, ideally, trough peak E_2 levels should not reach above approximately 234 pg/ml. In cycles in which peak E_2 levels are approaching this level, dosage may be lowered, or alternatively, if levels exceed this threshold, cycle can be canceled and restarted.

In conclusion, elevated E_2 levels in artificial autologous FET cycles utilizing intramuscular or transdermal E_2 could negatively impact OP/LB rates, possibly due to adverse effect on the endometrium from excess unopposed E_2 exposure. This is a retrospective study in a single facility, and therefore, larger prospective studies are needed to confirm our results and further elucidate optimal monitoring regimens and E_2 levels in artificial autologous FET cycles.

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