The Association between Serum Estradiol and Progesterone on the Same Day of FET and the Pregnancy Outcome; a cross sectional study

Shortened running title:

Effect of serum E2 &P. on FET outcome

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Abstract

Research question:

Precise timed synchronization between endometrium and the embryo is essential for high implantation and pregnancy rate, it is worthy to mention that endometrial thickness is not the only factor, E2 and P levels are also regularly monitored for endometrial receptivity. So, we decided to go for this study, to investigate the impact of serum E2 and P levels on the same day of embryo transfer on pregnancy outcomes for FET cycles.

Design:

This was a retrospective cross sectional study for 402 FET cycles which conducted between April 2018 and May 2019. All participants started endometrial preparation for FET with 6 mg/day oral estradiol for 13 days. When endometrium reached 8 mm or greater, patients were initiated on both micronized vaginal and oral P treatment. On FET day, serum level of E2 and P were assessed. Then, transfer of PGT euploid embryos was performed. 12 days later pregnancy test was assessed, and then 4 weeks after FET date ultrasound was scheduled to check the viability and the clinical pregnancy.

Results:

The mean E2 value was 931.41 ± 438.65 pg/ml, while mean P value was 8.47 ± 9.4 ng/ml. 240 out of 402 cases got pregnant (59.7%) while the clinical pregnancy rate was 53.9% with no correlation between serum (E2, P & E/P ratio) and the outcome.

Conclusion:

Our results revealed that the association between E2 and P on FET day and the pregnancy outcome is still not proven and those markers can't serve as predictors for the outcome.

Key Words: Estradiol, Progesterone, Frozen Embryo Transfer

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Introduction

Great efforts have been made over the last two decades to improve clinical and embryologic strategies with the aim of improving outcomes of assisted reproductive technologies. The endometrium is accepted as a final destination allowing blastocysts to attach under sufficient amounts of biologically relevant receptivity molecules. Understanding endometrial receptivity or more accurately, detecting the window of implantation, has become crucial in ART practice in order to go one step further (*Salamonsen, et al., 2013 & Garrido-Gómez*, *et al., 2013*). The outcomes of frozen embryo transfer (FET) have substantially improved, due to the improvements in the cryopreservation process with robust development in vitrification protocols which have undeniably improved the outcome (*Rienzi. et al., 2017*)

We can notice that the number of women undergoing frozen embryo transfer has increased for many reasons including both elective and medically indicated oocyte and embryo preservation.

As an important part of IVF/ICSI techniques, frozen-thawed embryo transfer (FET) has multiple advantages: 1) Preventing complications of IVF/ICSI such as ovarian hyper-stimulation syndrome; 2) Increasing the cumulative pregnancy rate; 3) Acquiring a better endometrium synchronism (*Rienzi, et al., 2017, Verwoerd, et al, 2008, Guzeloglu-Kayisli et al. 2007 & Arslan , et al., 2005*)

Precise timed synchronization between endometrial development and the implanting embryo is essential for obtaining high embryo implantation rate and pregnancy rate. Endometrial preparation plays an important role in FET cycle. *(Rienzi, et al, 2017 & Wen, et al., 2018).*

There are various protocols for endometrial preparation in FET cycles; however no endometrial preparation protocol is consistently superior to the others (*Burks*, et al, 2015 *& Yarali, et al., 2016*). Nevertheless, many centers prefer artificial endometrium preparation cycles with hormonal replacement therapy (HRT) because it allows the day of embryo transfer (FET) to be scheduled. It also controls days of exposure to exogenous progesterone (P) very precisely, which is essential for controlling the window of implantation to favor synchrony between the embryo and endometrium (*Blesa, et al., 2014*). This treatment consists in giving estrogens for 12–14 days. Once endometrium

thickness reaches about 7.5mm and over, exogenous P is introduced to prepare the endometrium for embryo implantation (*El-Toukhy, et al., 2008*).

So far, most studies about FET cycles have focused on embryological factors and the thickness of endometrium, while little attention has been paid to the serum steroid hormone levels on the day of embryo transfer.

Besides endometrial thickness, estradiol (E2) and P levels are also regularly monitored for endometrial receptivity. However, whether serum E2 and P levels on the day of embryo transfer can serve as an indicator for clinical pregnancy is doubtful. The effect of serum E2 and P levels on the day of embryo transfer is a matter of controversy in the literature and needs further evaluation (*Salumets, et al., 2006*).

The effect of serum E2 levels on the day of embryo transfer is poorly defined in the literature and needs further evaluation (*Labarta, et al., 2017*) A debate also exists regarding the optimal duration and dose of P supplementation in relation to pregnancy rates and early pregnancy loss, in addition to the optimal serum P levels on the day of ET among women undergoing FET cycles (*Alsbjerg, et al., 2013 & Brady, et al., 2014*).

Aim of the study

The main purpose of this study was to investigate the impact of serum E2 and P levels on the same day of embryo transfer on pregnancy outcomes for FET in hormone replacement cycles (HRT) cycles.

Materials and methods

Study design

This was a cross-sectional study (registered at ***clinical trials.gov with ID NCT04114500)***, for 402 FET cycles which conducted in Al- Baraka Fertility Hospital, Manama, Bahrain, between April 2018 and May 2019, and it was approved by our ethical committee.

Study population

- ***Inclusion criteria*** were women who underwent FET, were age below 40 yrs., body mass index (BMI) below 30 kg/m^2, patients who underwent FET treatment using the endometrial preparation was initiated with oral estradiol valerate, the endometrial thickness was no less than 8 mm on the day when P was administrated; with normal endometrial ultrasound imaging and euploid pre-genetically tested embryos;

- ***Exclusion criteria*** were chromosomal and genetic disorders, age> 40 years, BMI > 30 , abnormal ultrasonogram of uterine cavity (acquired or congenital) and abnormal embryos not suitable for transfer.

Study protocol

Endometrial preparation in frozen embryo transfer (FET) briefly, patients received treatment with 2 mg/8h E2 (Estrofem, Novo Nordisk) for 12–14 days, E2 treatment started after we get sure that down regulation happened either by noticing thin endometrium via ultrasound or confirmed by estimation of basal serum E2 level less than 50 pg (usually started on the second day of the menstruation); Endometrial thickness was evaluated with transvaginal sonography. When endometrial thickness reached 8 mm or greater and was trilaminar in appearance, patients were initiated on both vaginal micronized P (Crinone gel, Merck) and oral P (Duphastone, Abott) treatment at 200 mg/8h. P4 was given as supplementation because normal ovarian steroid production and the ovarian follicular-to-luteal transition were suppressed with estradiol. A depot GnRH agonist (Decapeptyl CR 3.75, Ferring) was administered in the midluteal phase of the preceding cycle) because it allows the day of FET to be scheduled at clinician's discretion. It also controls days of exposure to exogenous progesterone (P) very precisely, which is essential for controlling the window of implantation to favor synchrony between the embryo and endometrium. On the early morning of day 5 of P treatment, the same day of FET, a blood sample was obtained and immediately analyzed. Hormone determinations of E2 and P were performed. And it was done for all patients at the same lab. (Our own hormonal lab). Then, embryo transfer of the pre-genetically tested euploid

blastocyst embryos was performed under ultrasound guidance. 12 days later pregnancy test was assessed, (which is the primary outcome) and then 4 weeks after FET date ultrasound was scheduled to check the viability and the clinical pregnancy.(Secondary outcome)

Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 15.0. Quantitative data were expressed as mean± standard deviation (SD), median and IQR. Qualitative data were expressed as frequency and percentage.

Mean (average): the central value of a discrete set of numbers, specifically the sum of values divided by the number of values.

Standard deviation (SD): is the measure of dispersion of a set of values. A low SD indicates that the values tend to be close to the mean of the set, while a high SD indicate that the values are spread out over a wider range.

Median: is the value separating the higher half from the lower half of data. The basic advantage of median comparing to mean is that the median is not skewed so much by small proportion of extremely large or small values.

IQR: it is the measure of statistical dispersion, being equal to the difference between 75^{th} and 25^{th} percentile.

The following tests were done:

Mann-Whitney Test: was used when comparing between two means (for abnormally distributed data).

Chi-square test: was used when comparing between non-parametric data.

Kolmogorov-Smirnov: For testing of normality distribution of data.

Probability (P-value)

- P-value < 0.05 was considered significant.

- P-value < 0.001 was considered as highly significant.
- P-value > 0.05 was considered insignificant.

Serum P and estradiol levels the same day of FET were measured. Then the analysis to assess the relationship between serum E2 & P level and pregnancy outcomes was performed. For categorical analyses, Progesterone levels were split in terciles: T1: <15 ng/ml; T2: 15–30 ng/ml; T3: > 30 ng/ml. Progesterone was also grouped according to the median and according to groups performed from terciles.

Results:

All patients were homogenous group regarding the age, BMI, infertility duration, AMH, E2, serum P. or E2/P ratio. After measurement of serum P and estradiol levels the same day of FET, and then analysis of these values to assess the relationship between serum E2 & P level and pregnancy outcomes was performed, we found that Mean E2 value was 931.41 ± 438.65 pg/ml, among the studied patients while mean P value was 8.47 ± 9.4 ng/ml. ***(table 1)***. And the results revealed that, out of 402 cases; 240 cases had positive pregnancy test (59.7%) while the clinical pregnancy rate was 53.9% (217 cases out of 402) ***(table 2).*** with no correlation between serum (E2, P & E/P ratio) and the pregnancy rate; As we found that Mean E2 value was 914.53 ± 487.07 pg/ml, among the patients with positive pregnancy test while it was 942.8 ± 403.38 pg/ml in patients with negative pregnancy test, and the mean value of Progesterone was 7.9 ± 8.85 ng/ml, among the patients with positive pregnancy test while it was 8.85 ± 9.75 ng/ml in patients with negative test. ***(table 3),*** the results revealed also that no correlation between serum (E2, P & E/P ratio) and the clinical pregnancy rate, As we found that Mean E2 value was 903.4 ± 443.5 pg/ml, among the patients with positive clinical pregnancy rate, while it was 923.3 ± 399.2 pg/ml in patients with negative clinical pregnancy rate, and the mean value of Progesterone was 7.5 ± 7.9 ng/ml, among

the patients with positive clinical pregnancy rate, while it was 8.4 ± 8.6 ng/ml in negative group ***(table 4).***

Discussion:

Principal Findings:

The results of the present study showed that serum P and E2 level on the day of FET has no predictive value for success after an artificial endometrial preparation cycle. Having adjusted for all the potential confounders, including the number of euploid transferred embryos, the relationship between serum E2 and P on the day of FET and the likelihood of ongoing pregnancy is still not proven.

Results of the study in the context of other observations:

Regarding estradiol, to our knowledge, there were some studies reported about the impact of serum E2 on pregnancy rate in FET cycles and in fresh embryo transfer cycles. A recent study analyzed the relation between E2 (late follicular phase) levels and pregnancy outcomes in HRT cycles. The results showed that late follicular phase serum E2 levels were not able to predict pregnancy outcomes in HRT cycles (*Bocca, et al., 2015*). In addition, another study on 1287 cycles demonstrated that serum E2 levels had no impact on the outcomes of FET cycles when the endometrial thickness was between 7 mm to 15 mm (*Salumets, et al., 2006*). In line with our data, several studies suggested that serum E2 levels were an unimportant index to FET outcomes and there existed a wide range of E2 levels to receive optimal endometrial receptivity (*Niu,et al,2008, Remohi,et al,1993 & Simon, et al, 1999*). What is noteworthy is that all these findings failed to examine the E2 levels on the expected time of embryo transfer.

On the other hand, as regard to the progesterone; our results are in contrary with a previous study measuring P levels on the day of ET of non-genetically tested embryos showing different results ***Labarta et al, 2017*** , and also disagree with other study measuring P levels one day before FET of genetically tested euploid embryos. ***Gaggiotti-Marre, et al., 2018***

Data about an optimal range of serum progesterone on the day of ET are scarce. In FET cycles, very few retrospective studies have analyzed this issue, and obtained contradicting results when using IMP *(Brady et al., 2014; Kofinas et al., 2015).* When using intravaginal P however, Yovich et al*., 2015,* found an optimal serum P concentration interval, while values outside this interval significantly related to lower pregnancy rates, and that study showed that low P levels on mid-luteal phase (2–3 days after ET) may result in low pregnancy rates, while other studies described a deleterious effect of high p values on LBR. However, serum P might not reflect neither the actual absorption nor the level of endometrial support (*Shapiro, et al, 2014*).

Estradiol and progesterone levels are critical modulators of immune reactions during pregnancy and play a key role in inducing peripheral tolerance (*La Rocca, et al., 2014*). It could be speculated that a certain serum P values should be attained to allow for adequate immunological environment to reduce pregnancy loss, although lower serum P levels are sufficient to allow implantation to occur. In the natural cycle, P is secreted during the luteal phase in order to prepare the uterus for implantation. Through two receptors, PR-A and PR-B, P controls and ensures correct endometrial epithelial proliferation, stromal differentiation, local immune response and angiogenesis, altogether allowing embryo implantation (*Bhurke, et al., 2016*). P decreases active uterine contractions to ensure a correct embryo attachment (*Kuijsters, et al., 2017*) and has been associated with pinopode

development, with a positive correlation between pinopode abundance and implantation success (*Nikas, et al., 2002*). Once implantation occurs, other unidentified factors may be important for the maintenance of the early stages of pregnancy, which could account for the observed deleterious effect of lower P among patients with higher miscarriages.

Strengths and limitations:

The current study has two major differences as compared with the study by Labarta et al; first of all, we focused only on FETs of genetically-tested embryos. Embryo aneuploidy is one of the main aspects related to IVF failure, causing implantation failure, miscarriages and affected pregnancies (*Rubio, et al, 2017*). Preimplantation genetic testing for aneuploidies (PGT-A) may allow the de-selection of aneuploidy embryos for transfer, and improve final outcomes (*Dahdouh, et al., 2015*), practically eliminating one of the strongest confounders (embryo euploidy status), increasing the external validity of our findings. Secondly, we didn't use donated oocytes but we used their own oocytes. Our study is also different from Gaggiotti-Marre et al, 2018, as we measured P levels on the same day of (not the day before) embryo transfer, which is supposed to reflect actually the current serum level of both hormones that theoretically seems to be more realistic as a predictor for the outcome. Moreover we evaluate serum E2 and E2/P as well.

In our study, all patients received the same dose of P and for the same duration before ET. Our use of euploid embryos, in turn, allows for the more direct assessment of role of P4 levels on implantation without the fear of genetically abnormal embryos confounding the overall analysis.

A major limitation of the current study is its retrospective design, which precludes drawing conclusions regarding how to improve pregnancy outcomes in FET

patients using serum estradiol and progesterone levels as predictors; especially we are using combined oral and vaginal progesterone - both routes of administration - which is according to our own protocol in the hospital, and we believe that the serum level might not reflect the effect of vaginal progesterone on the endometrium, also in our study, we didn't evaluate live birth rates (LBR). Besides, still it is unclear the clinical value of serum P measurement on the day of ET, given that at this point no intervention is possible.

We attached table (5) that will summarize and demonstrate some published studies in relation to our current study.

Conclusion and clinical implications:

The implantation process is the most vital and the least understood part of reproduction. It's a matter of seeds and soil, the cross-talk between the endometrium and the developing embryo is mediated by many substances. So, trying to understand this enigma we decided to go for this study. Our results revealed that the association between serum estradiol and progesterone levels on the same day of FET and the pregnancy outcome is still not proven and those markers can't serve as predictors for the outcome. (Specially while using combined vaginal and oral P)

Recommendations : in the future we are planning to go for a prospective study using only vaginal progesterone, with evaluation of secondary outcomes e.g. live birth rates (LBR), and spontaneous abortions/biochemical pregnancies.

DISCLOSURE

- ***Nothing to disclose***

- ***There's no financial/personal interest or belief that could affect our objectivity***

Contribution to authorship:

Dr. Kamal Rageh (M.D.) & Dr. A. Barakat (FRCOG), are the IVF consultants who managed those cases of infertility and they were the treating physicians, hand in hand they went through the whole process of this work, planning, carrying out, analyzing and writing up of this work. ***Miss Nada Barakat (M.Sc.***) is the laboratory in charge, who completes their work, through assessment of the serum levels of estradiol and progesterone hormone, and embryogenesis as well. All authors contributed equally to the design, planning, conduct and manuscript preparation for this article.

Details of ethical committee approval:

Board Name: Independent Research Ethical Committee (IREC)

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Tables:

Table (1): The description of the data in studied patients

Variables		**Studied patients (N = 402)**
Age (years)	**Mean ±SD**	31.3 ± 4.3
	Min - Max	26 – 35
BMI (kg/m^2)	**Mean ±SD**	26.4 ± 5.2
	Min - Max	22 – 31
Infertility duration (years)	**Mean ±SD**	5.2 ± 1.9
	Min – Max	3.5 – 7
AMH	**Mean ±SD**	1.8 ± 0.9
	Min - Max	0.8 – 2.5
E2 (pg/mL)	**Mean ±SD**	931.41 ± 438.65
	Min - Max	19.4 – 1995
P (ng/ml)	**Mean ±SD**	8.47 ± 9.4
	Min - Max	0.29 – 47.89
E2 / P ratio	**Mean ±SD**	268.5 ± 446.5
	Min - Max	1.8 – 3906.1

AMH: *Anti Mullerian Hormone,* ***BMI****: Body Mass Index,* ***E2:*** *Estradiol,* ***P:*** *Progesterone*

Table (2): The description of pregnancy test and clinical pregnancy rate among studied patients

Variables (N = 402)		Positive	Negative
Pregnancy test	**N**	240	162
	%	59.7%	40.3%
Clinical Pregnancy rate	**N**	217	23
	%	53.9%	5.7%

This table revealed that the pregnancy test was positive in 59.7% while the clinical pregnancy rate was 53.9%.

Table (3): the correlation between serum (E2, P & E/P ratio) and pregnancy test in studied patients

Variables		Pregnancy test				Test	P-value
		Positive (N = 240)		Negative (N = 162)			
Serum E2 (pg/mL)	**Mean ±SD**	914.53 ± 487.07		942.8 ± 403.38		MW = 18017	0.213
	Median - IQR	924.4 – 636.7		899.8 – 769.5			
	Min - Max	130.2 – 1869.4		19.4 - 1995			
Serum P(ng/ml)	**Mean ±SD**	7.9 ± 8.85		8.85 ± 9.75		MW = 18389	0.358
	Median - IQR	6 – 6.53		5.5 – 5.16			
	Min - Max	0.33 – 47.9		0.29 – 45.2			
Serum P(ng/ml)	**< 15**	214	89.2%	144	88.9%	$X^2 = 0.165$	0.920
	15 – 30	10	4.2%	8	4.9%		
	> 30	16	6.7%	10	6.2%		
E2 / P ratio	**Mean ±SD**	307.1 ± 596.45		242.41 ± 305.21		MW = 19226	0.851
	Median - IQR	147.7 – 230.5		153.6 – 177.2			
	Min - Max	1.8 – 3906.1		9.18 – 2125.3			

This table describes the correlation between serum (E2, P & E/P ratio) and pregnancy test in studied patients. It's clear that there's no association between E2, P & E/P ratio and the pregnancy test.

E2: *Estradiol,* ***P:*** *Progesterone,* ***MW:*** *Mann-Whitney Test,* $X^{2:}$: chi square test

Table (4): the correlation between serum (E2, P & E/P ratio) and clinical pregnancy rate

Variables		Clinical Pregnancy rate		Test	P-value
		Positive (N = 217)	Negative (N = 23)		
Serum E2 (pg/ mL)	Mean ±SD	903.4 ± 443.5	923.3 ± 399.2	MW = 17011	0.786
	Median – IQR	912.3 – 623.4	883.3 – 743.8		
	Min - Max	19.4 – 1869.4	130.2 - 1995		
Serum P(ng/ml)	Mean ±SD	7.5 ± 7.9	8.4 ± 8.6	MW = 19379	0.635
	Median - IQR	5.5 – 6.1	5.8 – 5.3		
	Min - Max	0.33 – 45.2	0.29 – 47.9		
Serum P(ng/ml)	< 15	133 61.3%	16 69.6%	$X^2 = 0.604$	0.739
	15 – 30	60 27.6%	5 21.7%		
	> 30	24 11.1%	2 0.1%		
E2 / P ratio	Mean ±SD	307.1 ± 596.45	242.41 ± 305.21	MW = 18225	0.731
	Median - IQR	127.8 – 218.2	134.8 – 167.1		
	Min - Max	9.18 – 3906.1	1.8 – 2125.3		

It's clear that there's no association between E2, P & E/P ratio and the clinical pregnancy rate.

E2: *Estradiol,* ***P:*** *Progesterone,* ***MW:*** *Mann-Whitney Test,* $X^{2:}$: chi square test

Table (5):

The following table will summarize and demonstrate some published studies in relation to our study (arranged in a chronological manner)

Study	Year	Sample size	Maneuver	Conclusion
Jason D. Kofinas, et al	**2015**	**213**	Patients underwent single euploid embryo frozen transfer cycles from 2010 to 2013 at a single large academic center. Patients using donor oocytes and patients with changes in progesterone dose during the cycles in question were excluded. All cycles were programed and intramuscular P4 was used exclusively. Only patients administering the same daily dose of P4 throughout the cycle were included (N=213 patients). Main outcomes were ongoing pregnancy/live birth rates (OPR/LBR), clinical	Two groups based on day 19 P4 levels were compared (group A, P4<20 ng/ml; group B, P4>20 ng/ml). OPR/LBRs were65 vs.49 %,group A vs .B, p value=0.02, RR=1.33(1.1–1.7).Missed abortion and biochemical rates were higher in group B as opposed to group A, 27vs.12%,p=0.01,RR=0.45(0.24– 0.86). When P4 was stratified into five groups based on nano-gram per milliliter of progesterone on day 19(10–15,15–20,20– 30, 30–40, and >40), there was a trend downward in OPR/LBR (70, 62, 52, 50, and 33 %,

			pregnancy rates (CPR), and spontaneous abortions/biochemical pregnancies.	respectively). There was also an increase in missed abortion/biochemical rates (7, 15, 27, 32, and 20 %, respectively). Multiple logistic regression showed an increase in OPR/LBR when accounting for age, day2 FSH, weight, number of embryos biopsied, and number of euploid embryos. Conclusion P4 levels >20ng/ml on the day of transfer (during frozen single euploid embryo transfer cycles) were associated with decreased OPR/LBR..
E.Labarta, et al	**2017**	**244**	Prospective cohort study with 244 patients who underwent their first/second oocyte donation cycle, aged <50, BMI < 30kg/m2, triple layer endometrium >6.5mm and 1–2 good quality transferred blastocysts. A private infertility center. Serum P determination and3Dultrasound of uterine cavity were performed on the day of ET. Endometrial volume measurements were taken using a virtual organ computer-aided analysis (VOCAL™) system. The primary endpoint was OPR beyond pregnancy week12.	About 211 of the 244 recruited patients fulfilled all the inclusion/exclusion criteria. Mean serum P on the day of embryo transfer was 12.7±5.4ng/ml (Centiles 25, 9.2; 50, 11.8; 75,15.8). OPRs according to serum P quartiles were: Q1: 32.7%; Q2: 49.1%; Q3: 58.5%; Q4: 50.9%. The OPR of Q1 was significantly lower than Q2–Q4: 32.7% versus 52.8%; P = 0.016; RR (95% CI): 0.62 (0.41–0.94). The mean endometrial volume was 3.4 ± 1.9ml. Serum P on the day of ET did not correlate with endometrial volume. A logistic regression analysis, adjusted for all the potential confounders, showed that OPR significantly lowered between women with serum P < 9.2ng/ml versus ≥9.2ng/ml (OR: 0.297; 95%CI: 0.113–0.779); P = 0.013. The ROC curve showed a significant predictive value of serum P levels on the day of ET for OPR, with an AUC (95%CI)=0.59 (0.51–0.67).
S. Alur-Gupta ***et al,***	**2017**	389	Medical records of all programmed FETs were reviewed. A protocol of checking serum estradiol and progesterone one day prior to FET was instituted at Penn Fertility Care in June 2015. There were no other changes in the performance of FET at this center during the study period. Progesterone dosage was increased from 50mg/mL to 75mg/mL if serum progesterone was below 15ng/mL, and estradiol dosage was increased by 2mg oral estradiol and/ or the addition of vaginal estradiol if serum estradiol was below 150pg/ mL. Multivariable logistic regression was used to compare the likelihood of CIG after institution of the flexible dose protocol based on pre-FET blood serum levels compared to the likelihood before implementation of the protocol Adjustment for potential confounders included age, BMI, diagnosis, number of embryos transferred and pre-implantation genetic testing.	Overall, cycles in which pre-FET labs were measured and used to adjust hormone supplementation were more likely to result in CIG, despite a lower pregnancy rate in those who needed hormonal adjustment. This suggests that patients requiring adjustment may represent a poor prognosis group, such as obese individuals, and when identified may have benefited from improved hormonal preparation prior to FET.
Wen He., et al	**2018**	**193**	A total 193 HRT cycles was retrospectively identified. Cycles were divided into two groups: high E2 group (>150 pg/ ml) and low E2 groups (≤ 150 pg/ml). Stimulation and embryological characteristics were compared between the two groups	The concentration of serum E2 on the day of embryo transfer cannot serve as an indicator to predict the outcomes of artificial FET cycles.
S. Gaggiotti-Marr, et al	**2018**	**244**	Endometrial preparation was achieved with estradiol valerate and vaginal micronized progesterone. Serum P and estradiol levels the day prior to embryo transfer were measured. A multivariable analysis to assess the relationship between serum P level and pregnancy outcomes	Patients included in the lower P quartile had a significantly higher miscarriage rate and significantly lower live birth rate (LBR) compared to the higher ones. A low serum P level (10.64ng/ml) one day before FET is associated with a

			was performed, adjusted for confounding variables. Mean P value was 11.3±5.1ng/ml. Progesterone levels were split in quartiles: Q1: 8.06ng/ml; Q2: 8.07–10.64ng/ml; Q3: 10.65–13.13ng/ml; Q4: > 13.13ng/ml.	lower pregnancy and LBR following FET of euploid embryos.
Our study	**2019**	402	402 infertile women gave their written consent to be included in this study. All were recruited from the outpatient clinic of Al-Baraka fertility hospital - with age below 40 yrs, body mass index (BMI) below 30 kg/m2, whose uteri were morphologically normal - Endometrial preparation in frozen embryo transfer (FET) briefly, patients received treatment with 2 mg/ 8h oral E2 for 12–14 days, Endometrial thickness was evaluated by TVS. When it reached 8 mm or greater, patients were initiated on both vaginal micronized P and oral P treatment. A depot GnRH agonist was administered in the midluteal phase of the preceding cycle. On the early morning of the same day of FET, serum level of E2 and P were assessed. Then, embryo transfer of the pre-genetically tested euploid embryos was performed. 12 days later pregnancy test was assessed, and then 4 weeks after FET date ultrasound was scheduled to check the viability and the clinical pregnancy.	Our results revealed that the association between serum estradiol and progesterone levels on the same day of FET and the pregnancy outcome is still not proven and those markers can't serve as predictors for the outcome.

443

444