Original Article

Does Pretrigger Echogenic Endometrium in Assisted Reproductive Technology Cycles Reflect Raised Serum Progesterone Level?

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Abstract

Background: Serum progesterone is the main hormone of the luteal phase. The hyperechoic pattern of the endometrium in the luteal phase is believed to be induced by raised serum progesterone. Serum progesterone is found to be raised cases of controlled ovarian stimulation (COS) cycle on the day of ovulation trigger. **Aim:** This study aims to find the association between echogenicity of endometrium and raised serum progesterone **Objective:** The objective of this study is to determine whether raised pretrigger serum progesterone influences the echogenicity of the endometrium **Materials and Methods:** In this prospective observational study, we evaluated 221 patients who underwent COS. Echogenic patterns of the endometrium on transvaginal sonography were described as hypoechoic/trilaminar endometrium (Type A), isoechoic (Type B), and hyperechoic (Type C). The endometrial pattern and serum progesterone levels were evaluated on the day of ovulation trigger and value of ≥1 ng/ml was considered as elevated. **Results:** A total of 168 patients out of 221 patients (76.01%) had elevated serum progesterone levels on the day of ovulation trigger. Type A endometrium was found in a total of 174 patients, of these 132 patients (75.86%) had raised serum progesterone. Type B endometrium was found in 35 patients, of these 27 patients (77.14%) had raised serum progesterone. Type C endometrium was seen in 12 patients, out of these 9 patients (75.00%) had raised serum progesterone level. There was no statistically significant difference in the echogenic patterns of endometrium in patients with raised progesterone (≥1 ng/ml). On intergroup comparison, the difference in the progesterone levels between type A and type C was statistically significant (P = 0.02), and on receiver operating characteristic curve analysis, echogenic endometrium was found to predict progesterone level of 1.57 ng/ml with a sensitivity of 58.3% and specificity of 58.4% only. **Conclusion:** Echogenicity of the endometrium does not reliably predict raised serum progesterone on the day of ovulation trigger.

Keywords: *Assisted reproductive technology, echogenicity, endometrium, in vitro fertilization, progesterone, transvaginal sonography*

Introduction

Identification of optimal conditions in controlled ovarian stimulation (COS) and embryo transfer (ET) is of substantial clinical interest to increase the pregnancy rates in assisted reproductive technology (ART) cycles. Advancements in fertilization methods and embryo culture techniques have led to only marginal improvement in the pregnancy rates. Endometrial receptivity has been the focus of interest recently, and its disorders are likely to represent an important cause of the suboptimal embryo implantation rates.[1]

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In an ART cycle, direct assessment of the endometrial receptivity by endometrial biopsy is impossible in the ET cycles. As an alternative to endometrial biopsies, high-resolution transvaginal sonography (TVS) makes it possible to monitor noninvasively the endometrium through TVS assessment of the pattern of the endometrium wherein the endometrial echogenicity is assessed.[2,3] Patterns of endometrium were graded as Type A-hypoechoic/trilaminar (hypoechoic endometrium with prominent central and outer echogenic lines), Type B-isoechoic (same reflectivity of the endometrium as the surrounding myometrium and a poorly defined central echogenic line), and Type C-hyperechoic (increased reflectivity compared to myometrium and the central and the outer echogenic line not differentiated clearly.[5] It is well established that in the proliferative phase, the endometrium has a triple line/hypoechoic endometrium (Type A) and this appearance changes in the secretory phase, becoming hyperechoic (Type C). Serum Progesterone is the main hormone of the luteal phase and the hyperechoic pattern of the endometrium in the luteal phase is believed to be due to changes in the endometrium induced by raised serum progesterone. Recently, serum progesterone is found to be elevated during the late follicular phase in as many as 20—38%[4] of ART cycles and is reported to decrease the pregnancy rates by interfering with implantation.[6] We were interested to determine whether the hyperechoic (Type C) endometrium as detected by TVS on the day of ovulation trigger in some patients, be a reliable ultrasound marker of raised serum progesterone during the late follicular phase. The effect of raised serum progesterone on the endometrial echogenicity in the late follicular phase is controversial. There are few studies stating that follicular phase echogenicity of the endometrium is due to raised serum progesterone but few others refute this proposition.[7,8] We analyzed 221 ART cycles to determine if raised serum progesterone (≥1 ng/ml) in the late follicular phase was associated with increased echogenicity of the endometrium.

Materials and Methods

We conducted a prospective cohort study of patients who underwent COS for *in vitro* fertilization in the Department of Reproductive Medicine and Surgery at a tertiary care university teaching hospital, from January 2013 to December 2016. Institutional Ethical Committee clearance was obtained (Ref-IEC/12/MAR/94/09). A total of 221 patients were included in the study after obtaining written and informed consent. Patients in whom serum progesterone levels were not estimated, those with a history of pelvic tuberculosis, Asherman's syndrome, adenomyosis, and with retroverted fixed uterus, were excluded from the study. Patients underwent COS with flexible GnRH antagonist protocol. COS was started from day 2 or day 3 of menstrual cycle (spontaneous or withdrawal to combined estrogen-progesterone pill). Recombinant follicle stimulating hormone (rFSH) with or without human menopausal gonadotropin was started, dose individualized as per departmental protocol. When at least two follicles were 18 mm in diameter ovulation trigger was given with recombinant human chorionic gonadotropin 250 mcg. On the day of ovulation, trigger echogenicity of the endometrium was assessed by noting the endometrial pattern by TVS using a transvaginal probe 9 MHZ Ultrasoniox-SonixOP ultrasound system and the pattern were graded as hypoechoic/trilaminar (hypoechoic endometrium with central and outer echogenic lines-Type A), isoechoic (same reflectivity of the endometrium as the surrounding myometrium and poorly defined central echogenic line-Type B), and hyperechoic (increased reflectivity compared to myometrium and the central and the outer echogenic line not differentiated clearly-Type C).[2] Serum Estradiol and Progesterone levels were estimated (using ADVIA Centaur XP system by chemiluminescence method with analytical sensitivity for serum estradiol of 7.0 pg/ml and for serum progesterone 0.21 ng/ml) before administration of ovulation trigger. Serum progesterone levels of ≥1 ng/ml on the day of ovulation trigger was considered to be elevated in our study. Oocyte retrieval was performed approximately 35–36 h after ovulation trigger. ICSI was performed in all the cases. Fresh ET was done on the 3rd or the 5th day after oocyte retrieval or underwent freeze-all and subsequent frozen ET, as per department protocol. Serum β-human chorionic gonadotropin (hCG) concentrations were estimated on the 14th day of ET and a value of >5 mIU was considered positive for pregnancy.

Statistical analysis

The collected data were analyzed with Statistical Package for Social Sciences for Windows, Version 16.0 (SPSS Inc., Chicago, IL, USA). To describe about the data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean and standard deviation were used for continuous variables. For the multivariate analysis, the one way ANOVA was used. Pearson's correlation was used to assess the relationship between the variables. Chi-square test was used to find the significance in categorical data. In all of the above statistical tools, the $P < 0.05$ was considered as statistically significant.

Results

A total of 221 women undergoing COS for ART were evaluated. Indications for COS are tabulated in Table 1a.

Male factor which accounted for 43.43% followed by tubal factor which accounted for 24.85% were the most common indications. Demographic and COS details are summarized in Table 1b. There was no statistically significant difference in the distribution of patient, as per age and body mass index between the three groups. The COH details such as duration of stimulation, total dose of gonadotropins, and total dose of rFSH and HMG was not statistically different between Type A, Type B, and Type C endometrium. Pretrigger serum estradiol, endometrial thickness, and the number of oocytes obtained in all the three groups also did not show any statistical difference between all the three groups.

Out of 221, 127 patients had fresh ET, 50 patients conceived (39.3%). Pregnancy rates in patients with Type A, Type B, and Type C endometrium on the day of hCG trigger were 42.1%, 30%, and 25%, respectively. There was no statistical difference between the pregnancy rates between all the three groups.

The pattern of endometrium was not found to be significantly associated with elevated pretrigger serum progesterone as shown in Table 2a-c. However, in the intergroup analysis, the pretrigger serum progesterone levels were significantly higher in patients with compared to Type A – 2.41 ± 2.7 ng/ml versus 1.60 ± 0.8 ng/ml, P = 0.02 [Table 2c]. Receiver operating characteristic curve was plotted for the values of progesterone in patients with Type C endometrium, to know the reliability of endometrium as a predictor of elevated progesterone level. On analysis, the echogenic endometrium (type C) is found to reliably predict elevated progesterone level at 1.57 ng/ml, with a sensitivity of 58.3% and specificity of 58.4% [Table 2d].

Table 1a: Indications for Assisted Reproductive Technology

Indications for ART	Total patients (%)
Tubal factor	55 (24.85)
Male factor	96 (43.44)
Unexplained	34 (15.4)
DOR	12 (5.4)
Endometriosis	14 (6.3)
PCOS	6 (2.7)
Hypogonadotropic Hypogonadism	3 (1.4)
Retro +ve	1 (0.5)
Total	221 (100.0)

DOR=Decreased ovarian reserve, PCOS=Polycystic ovarian syndrome, ART=Assisted Reproductive Technology

Discussion

The impact of premature serum progesterone elevation at the end of the follicular phase in COS cycle for *in vitro* fertilization is extensively studied. Most of the studies have reported lower pregnancy rates in patients with high progesterone concentration on the day of ovulation trigger. Premature progesterone elevation does not alter the oocyte quality has been proved by many studies[9] while there are accumulated data suggesting its negative impact on the endometrium.[10] Elevated progesterone levels induce premature endometrial maturation (secretory changes), and as a consequence, there is an earlier opening of the implantation window that leads to lack of synchronization of the crosstalk between embryo and endometrium.[11] The elevated serum progesterone on the day of ovulation trigger is known to cause premature maturation changes in the endometrium. However, does this raised serum progesterone in the follicular phase result in echogenic endometrium as seen during the luteal phase?

In our study, we have found that preovulatory progesterone raise did not significantly increase the endometrial echogenicity, as is known to occur with luteal phase progesterone. A similar study was conducted by Corbacioglu *et al*. where the authors did not find any significant association between various patterns of endometrium and serum progesterone concentrations. The mean concentration of serum progesterone in their study was 1.5 ± 1.38 ng/ml in trilaminar endometrium, 1.81 ± 1.8 ng/ml in isoechogenic

Table 1b: Demographic and controlled ovarian stimulation details

Variables	Type A (mean)	Type B (mean)	Type C (mean)	*P*
Age (years)	30.70±4.22	31.29±3.56	30.58±3.63	0.729
BMI (kg/m^2)	26.47±4.25	27.79±4.09	28.27±3.84	0.108
FSH dose (mIU/ml)	2933.82±926.70	2811.00±1158.08	2939.58±833.63	0.786
HMG dose (mIU/ml)	892.30±772.77	767.86±761.18	912.50±832.61	0.676
Total dose of gonadotropin (mIU/ml)	3824.86±1388.70	3566.00±1455.31	3852.08±1409.89	0.600
Duration stimulation days	11.60±1.61	11.97±3.04	12.42±1.83	0.240
Pretrigger estradiol (ng/ml)	3117.90±2621.14	3562.54±2400.67	3011.11±1229.62	0.621
M2 oocytes	10.79±6.10	10.77±7.77	8.58±5.32	0.680
Endometrial thickness (mm)	10.21±1.94	10.38±2.35	11.22±1.20	0.507

BMI=Body mass index, FSH=Follicle stimulating hormone, HMG=Human menopausal gonadotropin

Table 2a: Pattern of endometrium and serum progesterone levels

Pattern of endometrium (*n*=221)	Serum progesterone (ng/ml)	
	<1 (%)	≥1 (%)
Type A (174)	42 (24.14)	132 (75.86)
Type B (35)	8 (22.86)	27 (77.14)
Type C (12)	3 (25.00)	9 (75.00)
Total (221)	53 (23.98)	168 (76.02)

There is no statistical significant association found between the types of endometrium and progesterone levels, *P*=0.98

Table 2b: Pattern of endometrium and mean serum progesterone

Endometrial pattern	Serum progesterone (mean±SD)
Type A (174)	1.60±0.8
Type B (35)	1.78±1.1
Type C (12)	2.41±2.7
Total (221)	1.68±1.0

SD=Standard deviation

endometrium and 1.12 ± 0.59 ng/ml in hyperechogenic endometrium (*P* = 0.25).[8] In the present study, the mean serum levels were 1.60 ± 0.85 in Type A, 1.78 ± 1.15 ng/ml in Type B, and 2.41 ± 2.37 ng/ml in Type C. In our study, although the difference in the mean progesterone value between Type A and Type C endometrium was significant, the echogenic endometrium could not reliably predict raised progesterone between 1 and 1.56 ng/ml range. Only at serum progesterone level of 1.57 ng/ml, the type C endometrium has the highest predictability of elevated progesterone, but the sensitivity and specificity are very low – 58.3% and 58.4%, respectively. Thus, in our study, type C endometrium could not reliably predict elevated pretrigger serum progesterone. In a study by Julian A. Gingold *et al*., hyperechoic endometrium on the of trigger day, showed significantly high progesterone value (1.21 ± 0.5 ng/ml) than the other patterns with a lower implantation rate but again with a limited statistical power <50%.[7]

Fanchin *et al*. studied the endometrial echogenicity values on the day of hCG administration, oocyte retrieval and ET in two groups of patient those with serum progesterone ≤0.9 ng/ml and with serum progesterone values >0.9 ng/ml. They found that on the day of hCG administration, the degree of endometrial echogenicity was similar in both groups (41% vs. 40%), but after hCG administration, it increased significantly faster in the high progesterone group than in the low progesterone group (70% vs. 63% at oocyte retrieval and 90% vs. 79% at ET, respectively).[12]

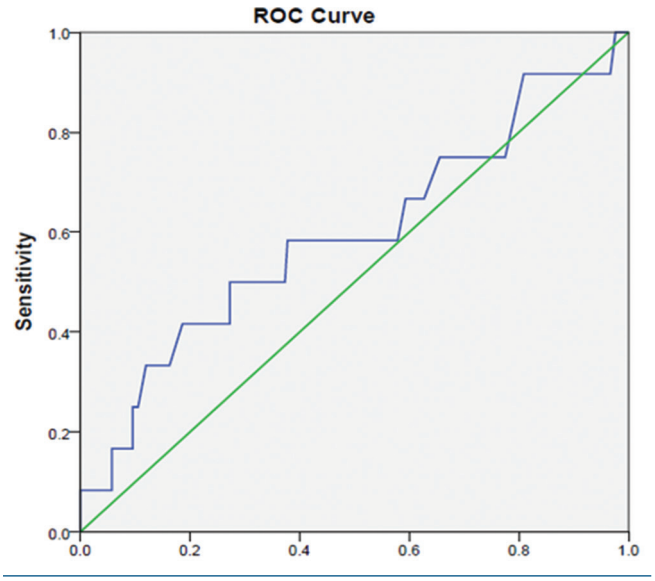
On studying the transformation induced by progesterone during the normal spontaneous menstrual cycle, stromal edema is observed relatively late (days 21–22), whereas

Table 2c: Comparison of serum progesterone levels between different pattern of endometrium

Pattern of endometrium		Mean difference	SE	Significant
Type A	Type C	−0.80606*	0.30941	0.026
Type B	Type A	0.17437	0.19205	0.636
Type C	Type B	0.63169	0.34679	0.165

*The difference in the progesterone levels between Type A and Type C was statistically significant (the *P*=0.026). SE=Standard error

Table 2d: Pretrigger serum progesterone in patients with Type C endometrium



Cut off	1.57 ng/ml
Sensitivity	58.30%
Specificity	58.40%

endometrial echogenicity often starts to increase earlier, during the first days of exposure to progesterone. Therefore, it is conceivable that other histologic events, such as glandular coiling (day 16 onward) and/or secretion (day 17 onward), contribute to increase the endometrial echogenicity.[13] The absence of hyperechogenicity due to raised progesterone in the follicular phase may be explained by the fact that the magnitude of the histologic transformation triggered by progesterone on the endometrium may be still too incipient at this phase of COS to be discerned clearly by ultrasonography.

Ubaldi *et al*. performed endometrial biopsy on the day of oocyte retrieval and compared the difference in the secretory changes between patients with normal progesterone (P) ≤0.9 ng/ml Group 1 and in those with raised progesterone (P) of ≥1.1 ng/ml (Group 2). In group II, the endometrial maturation was advanced by 0.7 days (*P* < 0.01) more than the normal *P* group.

Although advancement of endometrial maturation was significantly different between groups I and II, it was less than that might be expected from the number of days of premature progesterone rise and cumulative exposure to this. They speculated that it might be due to insufficient progesterone receptor priming of the endometrium at the time of premature progesterone elevation.[14]

The lack of correlation between circulating progesterone levels and follicular phase echogenicity, observed in previous investigations and confirmed by the present study raises the question of other factors which would alter the echogenicity of the endometrium during the follicular phase. Corbacioglu *et al*.[8] found that older patients and patients in whom the total gonadotropin dose was higher were more in the hyperechoic group. They also suggested that the other factors such as serum androgens may be responsible for the echogenicity. In our study, we tried to correlate the effect of age and total dose of gonadotropin levels on the echogenicity of the endometrium, but no significant association with any of the above factors were noted [Table 1b]. However, one of the major limitations of our study is the small number of patients in the echogenic endometrium (Type C) group.

Conclusion

Elevated pretrigger progesterone does not result in a significant change in the endometrial echogenicity. Further large prospective controlled studies are required to identify the factors responsible for endometrial echogenicity in the follicular phase.

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Conflicts of interest

There are no conflicts of interest.

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