Periodicity in the levels of serum plasminogen activator inhibitor-1 is a robust prognostic factor for embryo implantation and clinical pregnancy in ongoing IVF cycles

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ABSTRACT

CONTEXT: Plasminogen activator inhibitor-1 (PAI-1) has been inversely correlated to proteolytic extracellular-matrix degradation exerted by urokinase-type (u-PA) and tissue-type plasminogen activators (t-PA). Any pathological disturbance in PAI-1 levels may lead to several pregnancy complications. **AIMS:** To assess the influence of periodicity in serum PAI-1 levels on embryo implantation and clinical pregnancy outcome in IVF cycles **SETTINGS AND DESIGN:** Prospective study of 120 IVF cycles at private infertility centre. **MATERIAL AND METHODS:** Endometrial response (ER) assessment by measuring Endometrial thickness (cm) and echopattern (grade). Serum PAI-1 (ng/ml) measurement by ELISA method on day of hCG, day of ET and days 7 and 14 of ET. Main outcome measure was clinical pregnancy. **STATISTICAL ANALYSIS:** Student "*t*" test, ANOVA, Post-test for linear trend, Pearson Correlation. **RESULTS:** PAI-1 levels declined from dhCG to dET (318.8 ± 36.1 to 176.1 ± 28.4) whereas they increased steadily from dET to d7 to d14ET (176.1 ± 28.4 to 285.2 ± 30.4 to 353.5 ± 150.4; $P = 0.0004$) in pregnant group ($n = 31$). Conversely, dhCG to dET levels increased in both nonpregnant ($n = 75$; 173.8 ± 18.3 to 280.8 ± 26.1) and biochemical pregnancy BCP ($n = 14$; 172.7 ± 31.1 to 216 ± 30.1) groups. The rising pattern from dET to d7 to d14ET was not observed in non-pregnant and BCP groups. ER thickness and grade shared significant correlation with serum PAI-1 on dET (Pearson *r*: ER = 0.28, Grade = 0.29) and d7ET (Pearson *r*: ER = 0.40, Grade = 0.23). **CONCLUSIONS:** Periodicity in serum PAI-1 levels offers a robust prognostic factor for predicting clinical pregnancy outcome. The dhCG to dET PAI-1 transition is a decisive factor for either transferring embryos in same/ongoing cycle or cryopreserving them and postponing ET to subsequent natural cycle.

KEY WORDS: Implantation, IVF, PAI-1; PA system, pregnancy outcome

INTRODUCTION

Human embryo implantation is interstitial, in which the embryonic trophoblastic cells pass through the luminal epithelium to invade the endometrial stroma and become embedded into the uterine wall.[1] Establishment of blood supply with the maternal endometrium through angiogenesis is indispensable to this physiological process. Invasion of embryonic cytotrophoblasts to the proper depth and angiogenesis at the implantation site involves proteolytic invasion of the endometrial extra cellular matrix (ECM).

Plasminogen activator inhibitor-1 (PAI-1), a member of the serine family of inhibitors, is recognized to be inversely correlated to proteolytic ECM degradation exerted by both urokinase type (u-PA) and tissue type plasminogen activators (t-PA).[2-4] In fact, enhanced expression of PAI-1 by the decidual cells has been reported to play a decisive role in regulating proteolysis,[5] remodeling of matrix proteins, and migration of endothelial cells.[6-9] Any pathological disturbance in PAI-1 levels may lead to several pregnancy complications.[10] Whereas insufficient endovascular invasion has been associated with hypertension,

pre-eclampsia and inadequate fetal growth; unrestricted trophoblastic invasion leads placenta accreta and a variety of pre-malignant conditions such as hydatidiform moles and choriocarcinoma.[11] Therefore, an active, targeted, yet tightly regulated proteolysis giving rise to highly localized degradation seems to be a prerequisite for successful implantation.

Previous research has implicated PAI-1 in the implantation process either through *in-vivo* and *in-vitro* studies in animal models[12,13] or *in-vitro* tumor invasion studies[5,14-18] presuming that implantation is a process very similar to tumor invasion[18] and metastasis.[14,16,17] However, human implantation is quite unique[19] as is best illustrated by the fact that ectopic pregnancy is not an uncommon event in humans, though it is very rare in other species. Moreover, the specie-specific variations that exist in the implantation process preclude extrapolation with other mammals.[20] Also, though similar in being invasive, there is a molecular impropriety between tumourogenesis and human implantation. Thus, in order to decode the intricacies of the idiosyncratic human implantation process, especially with respect to IVF cycles, a more in-depth investigation of the mechanism in humans is warranted.

Cell culture of human endometrium-derived endothelial cells has reportedly exhibited an enhanced proteolytic as well as angiogenic capability.[21] However, endometrial tissue analysis done during one IVF cycle does not allow embryos to be transferred in the same/ongoing cycle. Moreover, data so generated cannot be applied to the next cycle as the endometrium undergoes intercyclic changes. Thus, a unifying scheme for the human implantation mechanism vis-a-vis proteolysis still remains obscure in women though it apparently is one of the most vital steps in the management of infertility.

Despite reports indicating that besides being present in granulosa cells,[22] trophoblasts, cytotrophoblasts, and trophectodermal cells,[23] circulating levels of PAI-1 in serum are about five times higher than in plasma;[24] surprisingly, serum as a medium demonstrative of successful human embryo implantation in IVF cycles has remained unexplored. Moreover, although some reports[25,26] have indicated that high PA activity in the proliferative phase and low PA activity in the secretory phase may be required to prepare the endometrium for implantation; no study has yet emerged on evaluation of PAI-1 levels at periodic intervals in an IVF cycle. Since tightly regulated proteolysis is essential at various stages of implantation, a tacitly implicit rhythmicity in the PAI-1 levels must necessarily exist at varied intervals not only to allow implantation but also to maintain pregnancy till term; an aspect not hitherto examined.

Another primary requisite for successful implantation besides invasive proteolysis and changes in vascular permeability is the availability of an adequately prepared endometrium. Earlier studies have unilaterally attempted to assess endometrial receptivity by evaluating implantation and pregnancy rates alone without measuring either endometrial thickness or grade (echopattern). Considering that endometrium undergoes cyclic changes in order to prepare for nidation of the embryo, it becomes imperative to also evaluate endometrial response (ER) in terms of its thickness and grade at periodic intervals in an IVF cycle and assess its effect on pregnancy outcome; an approach not previously attended.

Therefore, the aim of this study was to assess the influence of periodicity in serum PAI-1 levels and differences in ER thickness and grade on human embryo implantation and pregnancy outcome in IVF cycles, in order to gain further insight into this rather enigmatic process.

MATERIALS AND METHODS

This prospective study comprised 146 cycles of non-PCOS, normo-ovulatory women (menstrual cycle length range 25–32 days, mean age 32.22 ± 4.25 years, BMI 23.97 ± 4.53, Waist to hip (W/H) ratio 0.88 ± 0.06) undergoing their first conventional IVF cycle. Out of the 146 cycles, 17 cycles with severe male factor requiring ICSI, and 9 cycles where no blastocysts were available for transfer, were excluded from the study. A standard long protocol involving pituitary desensitization with GnRH agonist (500 μg/day of Lupride, Sun Pharma, India) and COH using recombinant FSH (Foligraph, Bharat Serum Pvt. Ltd. India, 225 IU/day) was followed. Follicular monitoring was carried out using transvaginal ultrasound scan from day 8 of menstrual cycle. Final oocyte maturation and trigger for ovulation was induced with 5,000 IU of hCG (Fertigyn 5000 IU, Bharat Serum Pvt. Ltd. India), when there was at least one leading follicle reaching a mean diameter of 18 mm and two to four other follicles reaching mean diameter of 16 mm. Transvaginal ultrasound-guided oocyte retrieval was done approximately 34–36 h after hCG administration under patient sedation.

Two to three embryos were replaced in the uterine cavity on day 5 (blastocyst stage) after retrieval. Blastocysts were graded according to Gardner's system of classification.[27]

Injection micronized progesterone (IM Susten 100 mg daily, Sun Pharma India) were administered daily to support the luteal phase starting from evening of d OPU. On d14 ET, a serum β-hCG concentration > 100 mIU/ml was considered as a positive indicator of pregnancy.

Informed consent was sought from all patients for participation in the study. The study protocol was approved by the local Hospital Ethical Committee.

Serum separation and hormonal measurements

Women underwent blood sampling by venipuncture on day of h CG administration (d hCG), day of embryo transfer (d ET), day 7 after embryo transfer (d7 ET) and day 14 after embryo transfer (d14 ET). Sera were separated and frozen in aliquots at –80° C for subsequent centralized analysis.

PAI-1 level in serum was estimated by ELISA method using diagnostic kits obtained from American Diagnostica Inc. (No. 85400). The protocol was followed as per manufacturer's instructions. The theoretical sensitivity or lowest detection limit was 2.2 ng/ml for PAI-1.

Estradiol (E2) level in serum was measured by Radio Immuno Assay (RIA) kits obtained from Diagnostic Systems Laboratories, Texas, USA (DSL-4400). Estimations were done as per manufacturer's protocol. The values were expressed as pg/ml with theoretical sensitivity or lowest detection limit 4.7 pg/ml.

Measurement of endometrial response

The endometrial thickness and grade was measured on d hCG, d ET and d7 ET using transvaginal sonography (TVS: Aloka, 550, Japan). All scans were performed by a single operator.

Endometrial thickness (in cm) was measured on frozen gray-scale images. Mean endometrial thickness was derived from average measurements of the two planes (longitudinal and transverse) of anterio-posterior distance between the echogenic endometrial–myometrial interfaces at the level of the uterine fundus. The thickness range from 0.7 to 1.5 cm was considered optimal.

The multilayerd or nonmultilayered endometrial pattern was graded as described by Sher *et al*.:[28] Grade 1 = single line, Grade 2 = double line, Grade 3 = triple line or Grade 4 = dense diffused endometrium, depending on intensity of hyper-echoic image of endometrium visualized as ultrasonologically discernible layers.

Main outcome measures

Clinical pregnancy (CP): Confirmed by the presence of at least one gestational sac with positive cardiac activity on transvaginal ultrasonography at around 6 weeks of amenorrhea.

Biochemical pregnancy (BCP): Defined as decreasing serum β-hCG levels before visualization of a gestational sac on ultrasonography.

Endometrial response (ER) in terms of thickness (cm) and echopattern (grade) of endometrium as visualized on transvaginal ultrasound scan on d h CG, d ET and d7 ET.

Definition of study groups

Cycles were sorted into pregnant, nonpregnant and biochemical pregnancy groups depending on pregnancy outcome.

Statistical analysis

Data was statistically analysed for relevance using the Graph Pad Prism 5.0 statistical package. Non-parametric Student's *t* test was used to assess difference between means. Comparisons between continuous variables from more than two groups were performed using one way analysis of variance (ANOVA) when data distribution was normal. A post-test for linear trend was used to statistically establish a rising pattern. Pearson's test was used to determine correlation coefficients to assess relationship between continuous variables. All values are expressed as Mean ± SD unless otherwise specified. In all cases, P value < 0.05 was considered statistically significant.

RESULTS

Of the 120 cycles studied, 14 cycles resulted in biochemical pregnancies whereas clinical pregnancy rate (CPR) and embryo implantation rate (total no. of gestational sacs × 100/ total no. of embryos transferred) was 25.83% and 14.34%, respectively. Table 1 shows that such characteristics as age, number of days of stimulation, indications for treatment, number of eggs retrieved and number of embryos transferred of the patients did not significantly differ between the pregnant, nonpregnant and biochemical pregnancy groups.

Table 2 depicts intercomparison of serum PAI-1 levels between pregnant, nonpregnant and BCP groups on the strategic days of IVF cycle. Though these results appeared to be inconclusive as most of the intergroup comparisons exhibited a nonsignificant difference; a close observation at the intra-group levels of serum PAI-1 on different days [Figure 1a] depicted a particular trend within the pregnant group where the levels recorded a drop to almost half on d ET from dhCG with subsequent steady rise from dET to d7 to d14 of ET (post test for linear trend: Slope = 88.67, r^2 = 0.26, P = 0.0004). On the other hand, within the nonpregnant group, an altogether different pattern was discerned where serum PAI-1 levels showed a steady but subtle rise from dhCG to dET to d7ET but dropped suddenly on d14ET. Though the overall one way ANOVA test for serum PAI-1 levels from dhCG to d14ET within this group was found to be significant (P = 0.04, r^2 = 0.048), one way ANOVA and post test for linear trend from dET to d14 did not significantly differ (Slope= -11.39, r^2 = 0.002, P = 0.63).

Similarly, PAI-1 levels in the biochemical pregnancy group showed a steady rise from dhCG to dET but recorded a dramatic rise on d7ET followed by a gradual decline on d14ET [Figure 1a]. The overall one way ANOVA test for difference in PAI-1 levels from d hCG to d14ET as well as the one way ANOVA and post test for linear trend from dET to d14ET was found to be nonsignificant in the BCP group (Slope = 86.75, $r^2 = 0.053$, $P = 0.29$).

Intercomparison of E2 levels between pregnant and nonpregnant groups showed a small but significant difference on dhCG (1342 ± 697.1 vs. 1091 ± 606.4 pg/ml, $P = 0.04$) as well as on dET (518.0 ± 350.1 vs. 325.6 ± 248.8 pg/ml, $P = 0.04$) but a highly significant difference on d7 (211.3 ± 169.1 vs. 82.42 ± 33.6 pg/ml, $P = 0.0004$) and d14 (381.8 ± 231.6 vs. 59.03 ± 20.1 pg/ml, $P < 0.0001$) of ET.

Although, serum E2 levels between the pregnant and BCP groups did not show any significant differences on dhCG (1342 ± 697.1 vs. 1276 ± 719.8 pg/ml, $P = 0.72$) and dET (518.0 ± 350.1 vs. 282.7 ± 124.9 pg/ml, $P = 0.14$), their levels depicted a statistically relevant difference between these two groups on d7ET (211.3 ± 169.1 vs. 90.38 ± 31.8 pg/ml, $P = 0.04$) and d14ET (381.8 ± 231.6 vs. 157.4 ± 93.41 pg/ml, $P = 0.03$).

Ultrasonographic measurement of endometrial thickness (1.0 ± 0.19 vs. 0.97 ± 0.22, $P = 0.53$) and grade (2.96 ± 0.18 vs. 2.95 ± 0.19, $P = 0.70$) showed no significant difference between the pregnant and non-pregnant groups on dhCG but differed significantly on dET (ER: 1.22 ± 0.26 vs. 1.12 ± 0.21, $P = 0.03$ and grade: 3.97 ± 0.16 vs. 3.86 ± 0.26, $P = 0.03$). However, though ER

Table 1: Patient characteristics in the three study groups

Parameters	Pregnant (31)	Non pregnant (75)	*P* value (ns)	BCP (14)	*P* value (ns)
Indications for treatment					
Tubal factor no. (%)	14 (45)	40 (53)	0.45	6 (43)	0. 89
Ovulatory dysfunction	6 (19)	13 (17)	0. 81	2 (14)	0.69
Endometriosis	5 (16)	12 (16)	0.99	3 (21)	0.68
Unexplained factor	6 (19)	10 (13)	0.44	3 (21)	0. 88
Mean age (years)	31.92±3.84	31.86±4.35	0. 72	33.25±5.17	0.4613
No. of days of stimulation	11.1±1.1	11.3±1.4	0.52	11.15±1.2	0.49
No. (Mean) of eggs retrieved	284 (9.16)	616 (8.21)	0.09	127 (9.07)	0.92
No. (Mean) of d5 embryos transferred	63 (2.03)	159 (2.12)	0.38	29 (2.07)	0.78

Statistically significant difference between groups was obtained by the nonparametric student *t*-test. Values are mean±SD; Column (c) represents statistical difference between columns (a) and (b) whereas column (e) represents difference between columns (a) and (d). *P*>0.05=nonsignificant (ns); D5=transfer of expanded blastocyst stage embryos. BCP: Biochemical pregnancy

Table 2: Inter-comparison of serum PAI-1 levels on different days of an IVF cycle

Day of IVF treatment	Serum PAI-1 (ng/ml)				
	Pregnant (31)	Non pregnant (75)	*P* value	BCP (14)	*P* value
dh CG	318.8±36.12	173.8±18.28	0.0003***	172.7±31.11	0.0192*
dET	176.1±28.37	280.8±26.08	0.0399*	216.0±30.06	0.4267 ns
D7 ET	285.2±30.43	321.2±36.59	0.6076 ns	450.1±112.4	0.0947 ns
D14 ET	353.5±38.84	258.0±30.27	0.0814 ns	389.5±118.8	0.0814 ns

PAI-1 levels were measured in serum on day of hCG administration (dhCG), day of embryo transfer (d ET), day 7 after embryo transfer (d7 ET) and day 14 after embryo transfer (d14 ET). Statistically significant difference between groups was obtained by the non-parametric student t test. Values are mean±SD; Column (c) represents statistical difference between columns (a) and (b) whereas column (e) represents difference between columns (a) and (d). *P*>0.05=nonsignificant (ns); *P*<0.05=significant*; *P*<0.0005=extremely significant***. PAI-1: Plasminogen Activator Inhibitor-1, IVF: *In-vitro* fertilization, BCP: Biochemical pregnancy, dhCG: day of hCG injection

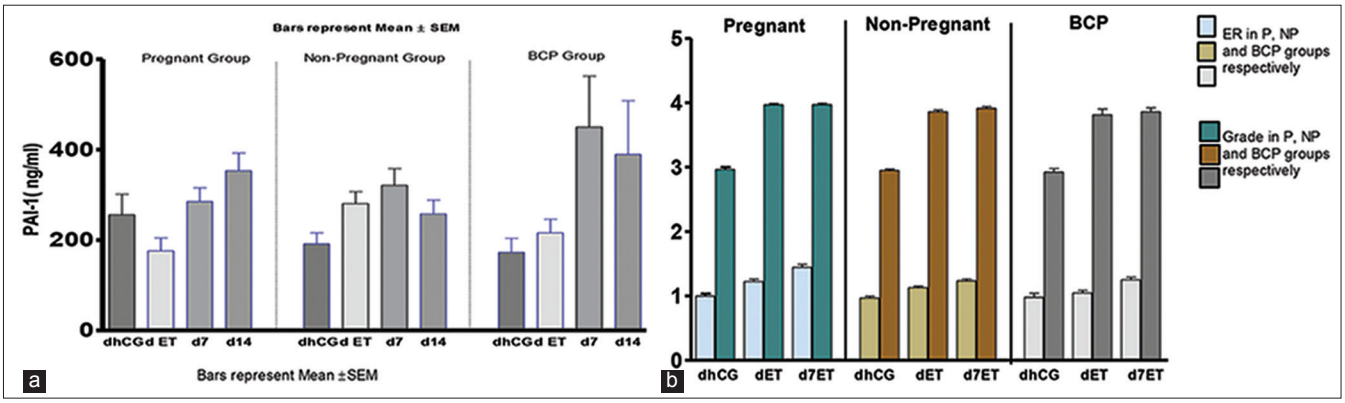

Figure 1: (a) Trend of serum PAI-1 levels on different days in pregnant, non-pregnant and BCP groups, (b) ER and grade on various days in pregnant, non-pregnant and BCP groups

denoted highly significant statistical difference between the above groups (1.44 ± 0.26 vs. 1.23 ± 0.21, *P* = 0.0001); the grade (3.97 ± 0.12 vs. 3.91 ± 0.20, *P* = 0.17) did not show any significant difference between them on d7ET.

Inter comparison between the pregnant and BCP groups showed that though ER (1.0 ± 0.19 vs. 0.98 ± 0.22, *P* = 0.77) and Grade (2.96 ± 0.18 vs. 2.92 ± 0.19, *P* = 0.54) did not differ significantly on dhCG; both ER and grade recorded significant differences between these groups on dET (ER: 1.22 ± 0.26 vs. 1.05 ± 0.22, *P* = 0.019 and grade: 3.97 ± 0.16 vs. 3.81 ± 0.35, *P* = 0.038) as well as on d7ET (ER: 1.44 ± 0.26 vs. 1.25 ± 0.16, *P* = 0.01 and Grade: 3.97 ± 0.12 vs. 3.86 ± 0.23, *P* = 0.039), respectively.

It is noteworthy that within each of the three study groups, a rising trend in endometrial thickness as well as grade was observed from dhCG to d7ET [Figure 1b].

Table 3 denotes correlationship of serum PAI-1 levels with endometrial response on various days of study. Although, both ER and grade did not exhibit any significant correlation with serum PAI-1 levels on dhCG, these parameters shared a significant correlation on dET as well as d7ET.

DISCUSSION

The uterus is considered to have an unfavorable environment for blastocyst implantation except for a stringent "implantation window" phase during which it undergoes ovarian hormone-induced anatomical and molecular changes. The present study has attempted to delve upon the enigmatic human implantation process and endeavored to answer how implantation is precisely regulated and brought about in this narrow time frame.

Our results indicate that serum PAI-1 level increases with a linear rhythmicity from day of embryo transfer (dET) to d14 of ET in pregnant group. Understandably, lower inhibitor level observed at the time of embryo transfer (ET) is presumably necessary for active proteolysis of extracellular matrix components in order to prepare a "pocket" or "cavity" for the incoming embryo to implant. Thereafter, when the supposed 'implantation window' closes, persistance in proteolytic activity may cause hindrance in the development of implanted embryo. Therefore, the observed rise in PAI-1 levels at d7 and d14 may logically account for the protection of embryo as well as prevention against any possible pathological invasion of embryo within uterus. The continuous rise in PAI-1 levels recorded during this study till sixth week of gestation (data not presented here), is in tune with earlier reports of increase in serum PAI-1 levels with gestational age.[10,29]

The linear trend of rhythmicity in PAI-1 levels however appears to be disrupted in the nonpregnant as well as biochemical pregnancy (BCP) groups. Higher PAI-1 level on dET in nonconception cycles arguably may impede the required proteolysis of endometrial ECM membrane, sufficient for creating a pocket for the ensuing embryo. Further, a marginal rise in the inhibitor levels on day 7 probably seals the fate of the embryo by hampering implantation altogether since the "window of implantation" supposedly closes by this time. A sudden drop in its levels on day 14 may augment some proteolytic activity thereby causing a profound damage to the embryo rendering it incapable to implant. This perception is in consonance with earlier report that regulation by PAI-1 protects the pre-implantation embryo from proteolytic degradation.[30]

Although, PAI-1 level on dET is elevated in BCP group as compared to pregnant group, the extent of elevation is found to be comparatively lesser than in non-pregnant group. This might explain the lodging of embryo within the uterine cavity in the BCP group due to allowance of just sufficient proteolysis needed to do so. However, a drop in PAI-1 levels on day 14 may indirectly cause obstruction in proper embryonic development owing to suddenly enhanced proteolysis. Further monitoring of serum PAI-1 levels beyond day 14 in this study (data not depicted) displayed a random fluctuation in its value which may probably account for the aborted embryonic development leading to discontinuation of pregnancy in this group.

The entire discussion above regarding implantation window and its "closure" by d7ET makes sense and assumes significance considering that all cycles in this study involved transfer of d5 expanded stage blastocysts. The extended culture to d5 after egg retrieval not only imparts required duration for preparation of endometrium and coincides more or less with the physiological timing of embryo implantation, but by d7ET, also covers the stringent implantation window.

Table 3: Correlation of Serum PAI-1 (ng/ml) with endometrial response (ER) (in terms of thickness) and Grade of endometrium on different days

Correlation	d hCG		d ET		d7 ET	
	ER	Grade	ER	Grade	ER	Grade
Pearson r (95% CI)	0.18 (−0.09 to 0.42)	0.07 (−0.2 to 0.33)	0.29 (0.05 to 0.48)	0.29 (0.06 to 0.49)	0.41 (0.2 to 0.58)	0.23 (0.004 to 0.43)
P value	0.1919 ns	0.5969 ns	0.0166*	0.0138*	0.0003***	0.0463*

ER and grade=endometrial response in thickness (cm) and grade of endometrium, dhCG=day of hCG administration, d ET=day of embryo transfer, d7 ET=day 7 after embryo transfer
Correlation was obtained using Pearson r correlation coefficient, CI=Confidence Interval *P*>0.05=nonsignificant (ns); *P*<0.05=significant*; *P*<0.0005=extremely significant***

The higher serum PAI-1 level on dhCG in the pregnant group seems to be in conformity with earlier studies in mouse ovary where levels of PAI-1 were reported to be slightly induced around the time of ovulation post treatment with hCG.[31,32] Interestingly, PAI-1 levels on dhCG in nonpregnant as well as in BCP group were found to be significantly lower as compared to pregnant counterpart despite providing comparable number of days of stimulation and same dose of hCG administered in all three study groups. This minor observation though apparently seems innocuous; the dhCG to dET PAI-1 transition may in fact serve as a major pointer towards taking a clinical decision for either transferring embryos in the same cycle or cryopreserving them and postponing ET to subsequent natural cycle.

In this study, significantly higher E2 levels observed on all days of IVF cycle in the pregnant group as compared to the other two study groups corroborates the relevant role of E2 in stipulating endometrial receptivity.[33-35] Also, there have been contrary reports on adverse effects of exposure to supra-physiologic levels of E2 associated with controlled ovarian hyperstimulation (COH) during IVF-ET cycles on endometrial receptivity and implantation rates.[36-47] Our result cannot claim to substantiate either of the two conjectures unless we compare E2 levels in natural vs. IVF cycles.

Pertinently, it may be mentioned that though none of the earlier studies attempted to evaluate endometrial thickness and echo-pattern (grade) employing transvaginal sonography for the measure of endometrial response (ER), Basir *et al*.[48] did measure endometrial thickness on day of hCG administration in women undergoing their first *in-vitro* fertilization cycles wherein they observed significant lowering in pregnancy rates in the suboptimal (≤8 mm) range as compared to optimal (>8 mm) thickness group. In contrast, this study depicts no significant differences in endometrial thickness and grade on dhCG between pregnant and nonpregnant or biochemical pregnancy groups. However, both these parameters are found to differ significantly on d ET and d7ET thus demonstrating for the first time a variation in ER between pregnant, nonpregnant and BCP groups. Interestingly, though no correlation of PAI-1 levels with ER was observed on dhCG, a significant, albeit weak, correlation of PAI-1 levels with ER on dET and d7ET observed in all three study groups indicates that serum PAI-1 may be involved in preparing the endometrial bed and influencing endometrial receptivity. This is further corroborated by the fact that irrespective of the intergroup differences; the intra-group ER thickness and grades show an increasing trend from dhCG to d7ET within each of the three groups. The result is clinically relevant considering that all patients undergoing IVF are provided luteal phase support in lieu of reports that IVF patients suffer from luteal phase defect and that the uterus is made receptive through transformation of a thin, dense endometrium into a thick, highly edematous secretory endometrium.[49] Thus our findings emphasize that serum PAI-1 levels are a governing factor not only for implantation and endometrial response but also for discrimination between pregnancy, non-conception and biochemical pregnancy.

An apparent limitation of this study may seem to the fact that this study does not measure PAI-1 levels in endometrial tissue. However, our basic aim was to investigate a noninvasive marker for human embryo implantation which may hold relevance in the same/ongoing treatment cycle. The drawback of endometrial biopsy studies is that the results obtained *in-vitro* cannot be applied to an ongoing cycle. Hence our limitation actually transforms into a rather strong point of our study. However, it would be interesting to estimate PAI-1 levels in natural cycles involving transfer of frozen-thawed embryos. This extension of our original prospective study is now underway at our clinic and we should be able to come out with promising results soon.

CONCLUSION

An incredibly remarkable synchrony between the developing embryo and the differentiating endometrium is the hallmark of successful implantation in both natural and assisted reproductive cycles. The present study offers a new paradigm in the form of 'PAI-1 Algorithm' for successful implantation and pregnancy outcome. The linear rhythmic rise in serum PAI-1 levels accompanied with the concomitant periodicity in endometrial response measured on ultrasound clearly discriminates between pregnant, non-pregnant and biochemical pregnancy. The dhCG to dET PAI-1 transition is a decisive factor for either transferring embryos in same cycle or cryopreserving them and postponing ET to subsequent natural favourable cycle. Thus periodicity in serum PAI-1 levels offers a robust prognostic factor for predicting clinical pregnancy outcome in IVF cycles.

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