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Original Research Article

Enhanced pregnancy outcomes with intrauterine platelet-rich plasma infusion in women following failed personalized embryo transfer guided by endometrial receptivity assay

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ABSTRACT

Background: Altered endometrial receptivity is an important cause of implantation failure. The embryo-endometrial asynchrony due to ovarian stimulation in IVF cycles contributes to low success rates. Frozen embryo transfer (FET) has become popular a method to overcome this asynchrony. Endometrial receptivity array (ERA) is a molecular test that differentiates a receptive from a non-receptive endometrium. Studies suggest that a personalised embryo transfer (pET) guided by ERA in FET cycles, improves pregnancy rates in recurrent implantation failure (RIF) patients. Despite pET not all patients achieve implantation. Intrauterine infusion of platelet-rich plasma (IUPRP) is emerging as an innovative technique to enhance fertility outcomes in patients with impaired endometrial receptivity. Platelet rich plasma (PRP) contains an abundance of growth factors and cytokines thought to enhance endometrial growth and receptivity. Aim of the study was to evaluate the role of IUPRP in improving pregnancy rate in patients with failed pET.
Methods: 53 patients with two previous pET failures, having an endometrial thickness ≥7 mm were included in this observational cohort study. FET was planned in a hormone replacement cycle. 0.4 ml PRP prepared by double centrifugation technique was infused into the uterus on the day of starting progesterone.
Results: A pregnancy rate of 64.1% and Live birth rate of 49.06% was achieved at FET after IUPRP infusion. Miscarriage rate was 17.6%. These results are similar to pregnancy and live birth rates achieved in our non-RIF IVF patients.
Conclusions: Our study suggests an improved reproductive outcome with infusion of IUPRP in patients with who failed to implant after pET.

Keywords: Platelet rich plasma, ERA, Recurrent implantation failure, Endometrial receptivity, FET

INTRODUCTION

The endometrium is a dynamic structure that undergoes complex changes at a structural and functional level during the transitory window of implantation (WOI), that facilitate embryo implantation.[1] These changes are consequent to the action of progesterone on an estrogen primed endometrium, resulting in secretory transformation of the endometrium, vascular remodeling, decidualization of stromal fibroblasts and immune modulation.[2] Endocrine, paracrine, and autocrine factors are thought to be involved in the orchestration of these events. Interestingly, the endometrium blocks embryo implantation outside this narrow WOI.[3] Key factors to successful implantation are the presence of a healthy embryo, a receptive endometrium that allows embryo-endometrial dialogue and maternal immune protection. The endometrial factor has been implicated in approximately 2/3rd of women who have implantation failure.[4] Ovarian stimulations for IVF leads to early endometrial maturation resulting in the embryo being transferred into a non-receptive endometrium. Frozen

embryo transfer's (FET) using a hormone replacement cycle are being used increasingly to overcome embryo-endometrial asynchrony, yet repeated implantation failure remains a significant challenge. Recurrent implantation failure (RIF) remains an ill-defined entity as there is no consensus on the number of failed attempts to be used for diagnosis.

Accurate markers of endometrial receptivity (ER) are elusive as the highly intricate changes occurring during the WOI are not completely understood. Currently, ultrasound measurement of endometrial thickness (EMT) remains the most commonly used modality to assess endometrial receptivity. Failure of euploid blastocyst implantation in an endometrium of normal thickness underscores the importance of developing a better marker for endometrial receptivity.[5,6] Transcriptomic analysis of the endometrium using microarray technology has given an insight into gene expression during the WOI. An endometrial receptivity array (ERA) based on 238 genes that are differentially expressed in the receptive phase, has been developed to overcome implantation failure resulting from embryo - endometrial asynchrony in FET cycles.[7] An improvement in pregnancy rates has been reported in RIF patients compared to controls, when a personalized embryo transfer (pET) guided by ERA was used.[8,9] However, the efficacy of ERA to overcome implantation failure has been questioned.[6,10]

Many empirical treatments eg intrauterine (IU) infusion of granulocyte colony-stimulating factor (G-CSF), IU–HCG, endometrial scratch have emerged to overcome implantation failure, but have met with limited success. Intrauterine infusion of platelet rich plasma (IUPRP) has recently been added to this plethora of treatments for enhancing endometrial receptivity. Platelets contain numerous growth factors and cytokines in their alpha granules that are activated on release, facilitating tissue healing and repair. PRP prepared from the patient's own blood, contains a high concentration of these factors and has been used successfully for tissue regeneration in dermatology and orthopaedics. A case report by Chang et al demonstrated that IUPRP can effectively improve endometrial thickness and pregnancy outcome in women with refractory endometrium.[11] Numerous studies and meta-analysis followed, demonstrating an improvement in both endometrial thickness and endometrial receptivity with the use of IUPRP.[12-17] The exact mechanism of action of PRP however, remains uncertain.

The aim of the study was to evaluate the role of IUPRP infusion in improving pregnancy rate in women with implantation failure after two personalised frozen embryo transfers (pET) guided by ERA.

METHODS

A prospective observational cohort study was conducted on fifty-three women aged 25-50 years with a body mass index (BMI) ≤35, endometrial thickness ≥7 mm and two previous failed frozen embryo transfers with good quality blastocysts, guided by ERA (pET cycles). Total number of embryos transferred previously were three, one blastocyst in the first transfer and two blastocysts in the second transfer. They had received empirical treatments with LMWH, s/c G-CSF, IU-GCSF infusion, prednisolone, Intralipids or endometrial scratch in their earlier transfers. Patients were planned for a frozen embryo transfer cycle with embryos that had been generated within the last three months from autologous or donor eggs.

Exclusion criteria

Women with congenital uterine anomalies, active genital tract infection, previous history of endometrial atypia, cancer survivors, genetic, chromosomal and haematological abnormalities and co-morbid conditions were excluded.

All women underwent a hysteroscopy, screening for antiphospholipid antibodies, thyroid antibodies, karyotype for both partners and an endometrial biopsy (EB) to exclude genital tuberculosis and chronic endometritis.

The study was conducted at Mother and Child Hospital and Ferticity Fertility Clinics, New Delhi, India, between March 2021 and March 2023.

Endometrial preparation for ERA

It was done in a hormone replacement (HRT) cycle. Estradiol valerate 6mg/day orally was started from cycle day 2 after a baseline ultrasound. Serum progesterone (P) was measured once an endometrial thickness (EMT) of ≥7 mm was achieved. If p value was <0.9 ng/ml, 50 mg of injectable progesterone (aqueous based) was administered daily by sub-cutaneous route. An endometrial biopsy was taken with a pipelle after five full days of progesterone administration (P+5) and sent for analysis. ERA results were classified as receptive (P+5) or non-receptive, non-receptive results were either pre- or post-receptive. The number of hours ±3, after progesterone administration suggested for personalized ET was advised.

PRP preparation

PRP was prepared by a double centrifugation technique to separate the pellet of platelets from whole blood. 20 ml of blood was drawn from the patient and mixed with 10 ml of ACD-A solution. After 5-10 minutes at room temperature first centrifugation was done at 200 g for 10 minutes, the plasma layer and buffy coat containing platelets was transferred to a fresh conical tube and centrifuged at 500 g for 8 minutes.

The resulting pellet of platelets was mixed with 1 ml of supernatant. Platelet count was done before and after preparation. A platelet concentration of more than 5 times the baseline was achieved in all patients.

IUPRP procedure

A volume of 0.4 ml of the PRP concentrate was instilled into the endometrial cavity with an embryo transfer catheter under ultrasound guidance, on the day of starting progesterone supplementation. This volume was chosen to avoid backflow. PRP was delivered slowly over 2-3 minutes to give it time to spread in the endometrium.

Personalized embryo transfer

Vitrified blastocysts (Gardner grade 4BB) were thawed and transferred in an HRT cycle mimicking the ERA cycle, under ultrasound guidance using a Cook's echotip catheter (K-Jets-7019-ET). In patients with a receptive endometrium at P+5 pET was done after 5 full days of P. In an altered implantation window, P administration was adjusted based on the personalized WOI identified by ERA. If the serum P4 level on day of FET was below 15 ng/ml, P supplementation was increased using either vaginal pessary 400 mg twice a day, Duphaston 20 mg twice a day or increasing the dose of injectable P to 100 mg daily, as per the clinic protocol.

Pregnancy confirmation

It was done 14 days after embryo transfer. Serum ß-HCG level of more than 25 mIU/ml was used for pregnancy confirmation. Clinical pregnancy was diagnosed by ultrasound visualization of one or more gestational sacs. Miscarriage was defined as clinical pregnancy loss before 12 weeks of gestation and live birth was defined as the delivery of a live baby after 24 weeks of gestation.

Statistical analysis

Categorical variables were presented as counts and percentages, while quantitative data were presented as mean±SD. Data normality was assessed using the Shapiro-Wilk test.

Appropriate statistical tests were applied (paired t-test, Mann-Whitney test, independent t-test, Chi-square test, and Fisher's exact test) based on data type and distribution. A p value of <0.05 was considered statistically significant.

RESULTS

Mean age of the 53 patients was 34.6 years (range 27-46), mean BMI was 26.06±2.6 (range 17.5-31.3) and mean AMH was 2.4±1.31 kg/m² (range 0.6-6.5 ng/ml). 71.7% (28/53) of the patients had primary infertility (PI) and 28.3% (15/53) had secondary infertility (SI). ERA was receptive at P+5 in 39/53 women (73.5%), pre-receptive in 10/53 (18.86%) and post-receptive in 4/53 (7.5%) patients. Three of the 53 (5.6%) patients had a donor-egg IVF (Table 1).

The pregnancy rate (PR) and live birth rate (LVBR) post IUPRP infusion were 64.15% (34/53), and 49.06% (26/53) respectively. Miscarriage rate was 17.65% (6/53). One patient had an ectopic pregnancy and there was one still birth (Table 2).

Table 1: Demographic characteristics of patients.

Clinical variables	N (%)	Mean±SD	Median (25^{th}-75^{th} percentile)
Primary/secondary infertility			
Primary infertility	38 (71.70)	-	-
Secondary infertility	15 (28.30)		
Endometrial receptivity array (ERA)			
Receptive (P+5)	39 (73.5)		
Pre-receptive	10 (18.8)		
Post-receptive	4 (7.5)		
Age (years)	-	34.62±3.8	35 (32-37)
Body mass index (kg/m²)	-	26.06±2.76	26 (24.8-28.1)
AMH (ng/ml)	-	2.4±1.31	2.3 (1.46-2.9)

Table 2: Obstetric outcomes of 53 patients.

Obstetric outcomes	N=53 (%)
Clinical pregnancy rate	34/53 (64.15)
Live birth rate/ongoing PR	26/53 (49.06)
Abortion rate	6/53 (17.65)
Ectopic	1/53 (2.94)
Still birth	1/53 (2.9)

Table 3: Comparison of endometrial thickness in patients pre and post IUPRP infusion.

Endometrial thickness (mm)	Pre PRP (N=53)	Post PRP (N=53)	P value
Mean±SD	7.32±0.39	7.81±0.46	<0.0001*

*Paired t test

This PR and LVBR results were similar to our non-RIF ART patients. A significant increase in mean endometrial thickness from 7.32 mm before PRP to 7.81 mm after PRP (p<0.0001) was observed, suggesting an enhancement in endometrial growth with IUPRP (Table 3).

Univariate logistic regression analysis for factors (age, AMH, BMI, pre and post EMT) affecting PR did not show a significant effect, indicating that within this group, these demographic and clinical factors were not independently predictive of pregnancy success (Table 4).

Table 4: Univariate logistic regression to find out significant factors affecting positive pregnancy rate in RIF.

Variables	Beta coeff-icient	Stand-ard error	P value	Odds ratio
Age (years)	-0.004	0.076	0.95	0.99 (0.85-1.16)
AMH (ng/ml)	0.053	0.223	0.81	1.06 (0.68-1.63)
Body mass index (kg/m²)	0.045	0.104	0.67	1.05 (0.85-1.3)
Pre PRP ET (mm)	-0.655	0.743	0.38	0.52 (0.12-2.23)
Post PRP ET (mm)	-0.371	0.630	0.56	0.69 (0.20-2.37)

DISCUSSION

Implantation failure continues to be a major obstacle to the success of IVF. The pathophysiology is likely multifactorial, contributing to the complexity of effective management. Though both embryonic and endometrial factors contribute to implantation failure, endometrial receptivity plays a crucial role in the success of embryo implantation. The pivotal role of endometrial receptivity is highlighted by studies showing implantation failure, even after the transfer of euploid embryos.[18,19] The study by Almohammadi et al reported that at least 20% of their patient failed to achieve sustained implantation (the appearance of fetal heart beat) even after three euploid transfers and that the possibility of implantation reduced with each failed attempt.[19] Furthermore, a decreased pregnancy and neonatal outcome is seen in intending parents compared to surrogates when the embryonic factor is eliminated using donor eggs.[20] At present there is no universal consensus on the benefit of PGT-A in management of RIF.[21] Women suffering repeated unexplained implantation failures are often advised surrogacy to fulfil their desire for a child.

Unfortunately, accurate markers of endometrial receptivity remain elusive. Currently, ultrasound evaluation of endometrial thickness is used as a surrogate marker for endometrial receptivity in the clinical setting. An endometrial thickness of <7 mm in FET cycles is associated with decreased pregnancy rates.[22] Endometrial thickness however is not a reliable marker, as pregnancies have been reported even with thin endometrium (≤6 mm).[23] To avoid the impact of endometrial thickness on implantation we included only patients with an endometrium of ≥7 mm, in our study. 'Omics' has improved our understanding of the molecular events involved in ER and the embryo-endometrial cross-talk and identified potential biomarkers of endometrial receptivity.[24] The ERA test based on molecular dating of the endometrium identifies the receptive window and offers an opportunity to overcome embryo-endometrial asynchrony encountered in IVF, thereby improving reproductive outcome. In this study we included women where endometrial receptivity had been ascertained by ERA yet they failed two embryo transfers. They had also received various empirical therapies to improve endometrial receptivity. There is an ongoing debate regarding the efficacy of pET in improving PR's in patients with repeated implantation failure.[10] The empirical therapies being used widely by reproductive physicians have also not met with much success.[25]

Platelet rich plasma has recently gained a lot of attention in various disciplines of medicine because of its regenerative properties. The success achieved with this therapy in dermatology and orthobiologics, has led to its use in women with endometrial factor and poor ovarian reserve.[12-17] Though the precise mechanism of action is still to be elucidated the proliferative, immunomodulatory and anti-inflammatory effects of the growth factors and cytokines in PRP are thought to be responsible for the positive effects on the endometrial environment.[26,27] An improvement in endometrial vascular perfusion is seen due to an increase in vascular endothelial growth factor (VEGF), that enhances growth and function. A deficient expression of fibroblast growth factor-1 (FGI-1) which is also involved in angiogenesis and improves endometrial trophoblastic interaction, has been found in patients with RIF.[28] An insight into the changes induced by IUPRP at a molecular level has revealed that in the proliferative phase of the menstrual cycle PRP promotes gene expression related to cell growth and pro-inflammatory response, while in the secretory phase it improves immune tolerance by inhibiting phosphoinositide 3-kinase signalling.[29] Hence timing of IUPRP is important, administered early in the cycle it may increase endometrial thickness without a positive effect on receptivity. We performed IUPRP on the day of starting progesterone for embryo transfer to maximize its effect on immune modulation and enhance ER. To minimize endometrial trauma, the procedure was carried out under ultrasound guidance. PRP can be administered hysteroscopically as well however it is an invasive procedure requiring anaesthesia and its superiority over intrauterine infusion with a catheter has not been confirmed.[30] The beneficial effect of PRP has been observed in studies using varied protocols but the overall quality of evidence is low.[31]

In our study that included 53 women who had 2 previous failed blastocyst transfers guided by ERA, a pregnancy and live birth rate of 64.15%, and 49.06% respectively, was achieved. The only additional intervention in the study cycle was the infusion of IUPRP. A significant increase in endometrial thickness was also observed after IUPRP (7.32 mm-7.81 mm PRP ($p<0.0001$) which is consistent with observations made in other studies. An improvement in PR after IUPRP has been reported in patients who failed a previous euploid transfer.[17]

We could not perform PGT-A in our patients because of cost considerations. The embryo quality for all patients was Gardner grade 4BB.

Limitations

Limitation of the study was the lack of randomization and use of embryos selected only by morphology. We acknowledge that transfer of euploid embryos would have improved accuracy of findings however financial constraints did not allow for PGT-A.

CONCLUSION

Recurrent or repeated implantation failure in IVF leads to a considerable emotional, physical and financial burden. The multifactorial nature of the condition precludes effective management. Endometrial receptivity is a critical factor for embryo implantation. The factors that initiate and orchestrate an effective embryo-endometrial dialogue have not been completely elucidated. Accurate markers of endometrial receptivity remain elusive. Ultrasound measurement of endometrial thickness has been used for many years as a prognostic marker of ER. Endometrial thickness <7 mm is considered sub-optimal for implantation in FET. Advances in molecular diagnostics lead to the development of ERA, a test that ascertains the WOI based on its transcriptomic signature. A personalised time is calculated for embryo transfer based on the numbers of hours of progesterone administration required after estrogen priming, to achieve the requisite secretory changes. The use of ERA has not been able to completely overcome implantation failure due to the endometrial factor. Numerous empirical therapies are being used to improve ER but have not met with much success.

Platelet rich plasma is abundant in growth factors and cytokines that play an important role in tissue regeneration and growth. The infusion of IUPRP has shown to be effective in enhancing reproductive outcome in women failing IVF due to thin refractory endometrium. Our study reports on the benefit of IUPRP in women who had two implantation failures despite a personalised embryo transfer guided by ERA and an endometrial thickness of ≥7 mm. A PR of 64% and a LVBR of 49% were achieved. IUPRP infusion shows promise as a therapy for women with recurrent implantation failure.

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