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Original Research Article

A Hospital-Based Study Assessing the Effect of Intrauterine Instillation of Autologous Platelet Rich Plasma and G-CSF on Endometrial Thickness and Pregnancy Rate in Frozen Embryo Transfer for Women with Thin Endometrium: A Randomized Clinical Study

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Abstract

Aim: The aim of the present study was to evaluate the effect of intrauterine instillation of autologous platelet rich plasma and G-CSF on endometrial thickness and pregnancy rate in frozen embryo transfer for women with thin endometrium.

Methods: The present retrospective cohort study was conducted in the department of reproductive medicine, IGIMS, Patna, Bihar, India. All the patients of age group 22 – 40 years coming to OPD for ART during the period of 9 months from March 2022 to November 2022 was selected after randomization into two groups for this study after fulfilling the inclusion & exclusion criteria. Sample sizes of 100 subjects were included in the final analysis.

Results: The mean age, BMI, S AMH in both the groups were not statistically significant. The most frequent factor for thin endometrium is AKT-or patients on anti-kochs treatment followed by uterine anomalies. The distribution of various other factors have been discussed here like RIF, RPL, endometriosis and ashermanns syndrome which are commonly associated with thin endometrium. Both the groups have similar distribution of factors. The mean of the endometrial thickness before and after are compared in both the groups with injection GCSF showing higher increase in endometrial thickness than intrauterine PRP after 48 hours of administration. It was found that for chemical and clinical pregnancy rates, the p values were 0.77 and 0.37 respectively and hence statistically not significant.

Conclusion: Through the analysis done in this study it becomes evidently clear that injection GCSF is superior in its action as compared to intrauterine Platelet Rich Plasma in increasing the endometrial thickness in patients diagnosed as thin endometrium for infertility.

Keywords: Clinical pregnancy rates, Chemical pregnancy rates, Endometrial receptivity, Injection GCSF, Intrauterine PRP, Thin endometrium

Introduction

Infertility affects 13% of couples worldwide [1] and despite extensive workup in many couples; etiology in around 10–25% of the couples remains unexplained which has brought implantation and endometrial receptivity into the light. [2] Endometrial tissues contain receptors for cytokines, growth factors, and lipids that are vital for endometrial proliferation as well as its receptivity for conceptus. Endometrial thickness (ET) promotes successful implantation. [3] High impedance blood flow in radial arteries of the uterine vasculature results in poor epithelial growth, reduced expression of vascular endothelial growth factor (VEGF) leading to implantation failure.

According to the European Society of Human Reproduction and Embryology (EHRE) consortium, recurrent implantation failure (RIF) is defined as the absence of gestational sac on ultrasound at 5 weeks or more after frozen embryo transfer (FET) following 3 FET with high-quality embryos or after the transfer of 10 or more embryos in multiple transfers. [4,5] Recurrent implantation failure is a

major challenge in reproductive medicine and despite several advances; still, no universal consensus exists. Many strategies such as estrogen, low-dose aspirin, heparin, vaginal sildenafil, pentoxifylline, and granulocyte-colony stimulating factor (G-CSF) intrauterine perfusion have been extensively used to increase the ET if not optimal. [6,7]

However, these methods were not found to be very impressive in all cases especially with a thin refractory endometrium. Platelet-rich plasma (PRP) may be effective in promoting endometrial growth, increasing ET and improving endometrial vascularity, and improving pregnancy outcomes in repeated implantation failure due to thin endometrium. Platelet-rich plasma is fraction of plasma prepared from autologous blood which is highly enriched with platelets and contain a variety of cytokines and growth factors like VEGF, transforming growth factor (TGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) that support cellular migration, proliferation, and growth. [8]

The measurement of endometrial volume has been proposed as a predictor of implantation in the recent years. However, in practice, most clinicians empirically prefer endometrial thickness (ET) >7mm. The available evidence does not support any specific thickness, and pregnancies with similar success have been described in endometrium from 5 to 15mm. [9] In a meta-analysis by Kasius et al., published in 2014, evaluating the optimal ET required for implantation, the probability of clinical pregnancy for an ET≤7mm was significantly lower compared with cases with ET>7mm [23.3 versus 48.1%]. Positive and negative predictive values for the outcome of clinical pregnancy were 77 and 48%, respectively. [10] Presence of granulocyte colony stimulating factor (GCSF) receptors in placental tissues, trophoblastic cells and endometrial cells indicate the importance of this cytokine in implanation. [11-13] The use of GCSF in assisted reproductive technology (ART) has been reported by many studies to improve the inadequacy of the endometrium. [14,15]

The aim of the present study was to evaluate the effect of intrauterine instillation of autologous platelet rich plasma and G-CSF on endometrial thickness and pregnancy rate in frozen embryo transfer for women with thin endometrium.

Materials and Methods

The present retrospective cohort study was conducted in the department of reproductive medicine, IGIMS, Patna, Bihar, India. All the patients of age group 22 – 40 years coming to OPD for ART during the period of 9 months from March 2022 to November 2022 was selected after randomization into two groups for this study after fulfilling the inclusion & exclusion criteria. Sample sizes of 100 subjects were included in the final analysis.

Inclusion Criteria:

1. Women aged 22-40 years

2. Women diagnosed with thin endometrium in previous/present cycle

3. Implantation failure in previous cycles due to thin endometrium

4. Normal shape & size of uterine cavity (No obvious intrauterine adhesion/No submucous uterine myoma/ no endometrial polyps)

Exclusion Criteria:

1. Women with history of hematological disorders (e.g.Leukopenia,thrombasthenia)

2. Active debilitating disorders like acute PID or other infections

Consent

The study was done after getting approval from institutional ethical committee. Informed consent was taken from each subject.

Collection of Data

The relevant parameter was recorded in a pre-designed performa which included detail history taking, clinical examination along with checking of infertility records

Procedure

For all women, a basic transvaginal sonography was done on 2nd day of FET cycle and estradiol hemihydrate 2 mg three times daily was initiated. On 12th day of cycle, transvaginal sonography was done again. When the endometrial thickness was <7mm, the same physician measured it repeatedly for two times to confirm thin endometrium and the average value of the two different measurements were recorded. Then the women with endometrial thickness <7mm was randomized in two groups,(G-CSF group B & PRP group A) according to table of random numbers with computer software. In PRP group, 0.5-1 ml of PRP was infused intrauterine with IUI (intrauterine insemination) catheter. In G-CSF group, G-CSF (300mcg/1ml) was instilled slowly into the uterine cavity using IUI catheter. Endometrial thickness was assessed after 48 hrs. If endometrial thickness was <7 mm, either a second infusion of G-CSF/PRP was given or cycle was cancelled and if endometrial thickness was > 7mm, progesterone was started & embryo transfer was done on Day 3 /day 5 accordingly. Appropriate luteal phase support after embryo transfer was given and two weeks later, pregnancy was confirmed by serum beta hcg level.

Both the groups had 50 women each hence the distribution was 50% in each group. This study shows a distribution of 50% of patients treated with injection GCSF and 50% of the patients treated with intrauterine Platelet Rich Plasma for previously diagnosed thin endometrium.

Statistical analysis

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables. Data was also represented using appropriate diagrams like bar diagram and, pie-diagram. All Quantitative variables were checked for normal distribution within each category of explanatory variable by using visual inspection of histograms and normality Q-Q plots. Shapiro-wilk test was also conducted to assess normal distribution and p-value of >0.05 was considered as normal distribution.

Results

Table 1: Comparison of mean of secondary variables between the study groups

Parameter	Study group		p value
	Platelet rich plasma (PRP) (N=50)(Mean±SD)	Granulocyte colony stimulating factor(GCSF) (N=50) (Mean±SD)	
Age	31.09±5.68 years	32.78±4.86	0.540
BMI	23.73±4.06 kg/m^2	24.16±4.36	0.752
SR AMH (ng/ml)	2.14 (1.46, 3.205)	1.84 (1.06, 2.27)	0.102
FET cycles	17 +33	16 +34	0.034

The mean age in both the groups A and group B was 31.09±5.68 years and 32.78±4.8 years respectively and the p-value is 0.579 which was not statistically significant. Hence age does not act as a compounding factor in both the groups. The mean BMI in both the groups A and group B was 23.73±4.06 and 24.16±4.36 respectively and the p-value is 0.752 which is not statistically significant hence it can be safely said that both the groups was comparable BMI. Hence BMI does not act as a compounding factor in the comparison in both the groups Serum Anti-Mullerian hormone is measured in ng/ml and is a good indicator of ovarian reserve. The mean values for both groups A and B was 2.14 and 1.84 respectively. The p - value was 0.102 and hence was statistically not significant. Hence the variable in both groups were comparable.

Table 2: Comparison of factor for thin endometrium between the study groups

Factor for thin endo	Study group	
	Platelet rich plasma (PRP) (N=50	Granulocyte colony stimulating factor (GCSF (N=50)
AKT	9 (18%)	5 (10%)
Anovulation	5 (10%)	1 (2%)
Ashermanns syndrome	4 (8%)	3 (6%)
Endometriosis	2 (4%)	0 (0%)
Hyperprolactinemia	2 (4%)	0 (0%)
Poor ovarian reserve	4 (8%)	10 (20%)
Post op myomectomy	4 (8%)	7 (14%)
RIF	4 (8%)	6 (12%)
RPL	6 (12%)	4 (8%)

The distribution of factors of infertility and possibly thin endometrium in both the groups of patients. The most frequent factor for thin endometrium is AKT-or patients on anti-kochs treatment followed by uterine anomalies. The distribution of various other factors have been discussed here like RIF, RPL And endometriosis and ashermanns syndrome which are commonly associated with thin endometrium. Both the groups have similar distribution of factors. The factors involved are patients who were treated for tuberculosis (ART), ovulatory cycles, Ashermanns syndrome, endometriosis, recurrent pregnancy loss etc. Both the groups suffered from the similar distribution of these variables.

Table 3: Descriptive analysis of endometrium before PRP (mm), endometrium after 48 hrs of PRP (mm) and injection GCSF before and after 48 hrs in study population

Parameter	Mean±SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Endometrium before PRP (mm)	6.54±0.66	6.84	5.20	7.2	6.24	7.37
Endometrium before Inj GCSF	6.78±0.42	6.94	5.40	7.00	6.56	6.90
P value- 0.1741						
Endometrium after 48 hours of PRP (mm)	8.06±1.14	7.80	7.00	12.70	7.53	8.35
Endometrium after inj GCSF 48 hours	9.5±0.72	8.80	7.00	11.60	8.31	9.9
P value -<0.0001						
Endometrium diff PRP before ET	1.810±0.842	2	0.5	3.8	1.37	2.23
Endometrium diff GCSF after ET	2.68±0.548	2.5	0.7	3	1.55	2.1
P value- <0.0001						

The mean of the endometrial thickness before and after are compared in both the groups with injection GCSF showing higher increase in endometrial thickness than intrauterine PRP after 48 hours of administration. This could be demonstrable through the p value of the differences in endometrium over 48 hours which was 0.0001 and was statistically significant.

Table 4: Comparison of chemical pregnancy between the study group

Chemical pregnancy	Study group		Chi square	p-value
	Platelet rich plasma (PRP)	Granulocyte colony stimulating factor (GCSF)		
0	29 (58%)	24 (48%)		
1	21 (42%)	26 (52%)	0.084	0.777
Clinical pregnancy				
0	36 (72%)	28 (56%)	0.765	
1	14 (28%)	22 (44%)		0.3734

It was found that the chemical and clinical pregnancy rates the p values were 0.77 and 0.37 respectively and hence statistically not significant.

Discussion

Implantation is a process dependent on three main factors good quality embryos, receptive endometrium with good endometrial thickness and the endometrium-embryonal cross dialogue. Hence implantation of a mature embryo into receptive endometrium is key to build a successful pregnancy. [16] Despite many advances in the past decade for betterment of the thin unresponsive endometrium, implantation failure still continues to befuddle the IVF doctor. Endometrial thickness in turn is an important component of endometrial receptivity. [17] Endometrium below 7mm in thickness is widely considered sub- optimal for transfer and associated with reduced pregnancy rates. Successful implantation requires a complex molecular process including endometrial integrins, extracellular matrix molecules, adhesion molecules, growth factors and ion channels. [16] Intrauterine perfusion of platelet rich plasma was described by Chang et al for patients of thin endometrium. [18] The effectiveness of endometrial improvement has also been described by Zadehmodares et al. [19] The mechanism for increase of thickness of the endometrium by PRP, is that PRP has many cytokines and growth factors including transforming growth factor (TGF-β), platelet derived growth factor (PDGF), interleukin - 8 (IL-8) and many factors that promotes cellular migration, proliferation and differentiation processes. Nowadays, PRP has been widely used in different clinical scenarios such as orthopedics, ophthalmology and wound healing. [20]

The mean age, BMI, AMH, and FET cycels in both the groups were not statistically significant. The distribution of factors of infertility and possibly thin endometrium in both the groups of patient. The most frequent factor for thin endometrium is AKT-or patients on anti-kochs treatment followed by uterine anomalies. In study injection GCSF given intrauterine has proven to be more efficacious compared to intrauterine PRP for improvement of endometrial thickness in patients diagnosed with thin endometrium. However the chemical and clinical pregnancy rates do not differ much.

The distribution of various other factors have been discussed here like RIF, RPL, endometriosis and ashermanns syndrome which are commonly associated with thin endometrium. Both the groups have similar distribution of factors. The mean of the endometrial thickness before and after are compared in both the groups with injection GCSF showing higher increase in endometrial thickness than intrauterine PRP after 48 hours of administration. It

was found that the chemical and clinical pregnancy rates the p values were 0.77 and 0.37 respectively and hence statistically not significant. In another study it was reported that patients with a good response to ovarian stimulation cycles had shown high levels of G-CSF in blood and follicular fluid compared to patients who had low ovulation stimulation response. Also her pregnancy rate in the first group was 33.5% whereas there was no pregnancy in the other group. Also GCSF plays a part in the implantation window due to its presence in the endometrium during implantation. [21] Due to the action of macrophages one may debate that GM-CSF may have better prognosis for endometrial thickness. [22] Local GCSF significantly decreases CD16 And CD56 and it also increases LIF (Leukemia inhibiting factor), as a result of which pregnancy rates may improve.

Eftekhar et al [23] conducted random control trail (RCT), including 83 women with a poor endometrial response to standard hormone replacement therapy (HRT) <7 mm. Endometrial thickness increased significantly to 8.67 ± 0.64 in PRP group than in controls (p = 0.001). This increase was higher in women who conceived in the PRP group (p ++value: 0.031). The implantation rate and per-cycle clinical pregnancy rate were significantly higher in the PRP group (p = 0.002 and 0.044, respectively). A study by Coksuer et al[24] to evaluate the effect of intrauterine PRP treatment on frozen-thawed embryo transfer (FET) cycles in patients suffering from unexplained infertility patients with a history of RIF. Endometrial thickness, clinical pregnancy rate, and live birth rate were also significantly higher in the PRP group than the control group.

Conclusion

Through the analysis done in this study it becomes evidently clear that injection GCSF is superior in its action as compared to intrauterine Platelet Rich Plasma in increasing the endometrial thickness in patients diagnosed as thin endometrium for infertility. Although the other variables were comparable, the chemical and clinical pregnancy rates when compared showed a slightly higher effect of injection GCSF over intrauterine PRP as the clinical and chemical pregnancy rates, though not statistically significant, were slightly higher than that of group given intrauterine PRP.

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