Intrauterine Follicular Fluid Injection: A Potential Novel Approach To Improve The Endometrium

Original Article

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ABSTRACT

Introduction: Recently intrauterine infusion of various drugs and autologous components has been tried to improve assisted reproductive technology (ART) cycles outcome. Follicular fluid (FF) is a complex fluid rich in growth factors and cytokines. These mediators may enhance implantation potentials. This randomized controlled trial (RCT) aimed at evaluating the effect of intrauterine infusion of FF in ART cycles.

Patients and Methods: 60 infertile patients indicated for ICSI were randomized into two groups; study group in which intrauterine injection of follicular fluid in the endometrial cavity was done at the time of oocyte retrieval, and a control group. During oocyte retrieval clear FF was centrifuged for 2 minutes and 1 ml was injected into the uterine cavity using intrauterine insemination (IUI) catheter.

Results: Demographic characteristics, number of retrieved oocytes and embryos available for transfer were comparable among both groups. There was no statistical difference between both groups regarding endometrial thickness and clinical pregnancy rate. In patients with previous ICSI failure the study group showed a trend toward a better clinical pregnancy rate compared to control (62.25% versus 44.4% respectively).

Conclusion: IU injection of FF at the time of oocyte retrieval does not improve clinical pregnancy rate. However, this new modality may have a role in patients with previous ICSI failure.

Key Words: Endometrium, Intrauterine follicular fluid, platelet rich plasma, recurrent implantation failure

Received: 05 September 2024, **Accepted:** 17 September 2024

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ISSN: 2090-7265, November 2024, Vol. 14, No. 4

INTRODUCTION

Implantation is one of the most critical steps in reproduction. One-third of implantation failures can be attributed to embryonic reasons, while unsatisfactory endometrial receptivity and poor embryo-endometrium communication account for the remaining two-thirds[1].

In an attempt to improve implantation rates, intrauterine infusion of various drugs has been tried. It aims at direct delivery of the drug into the uterine cavity and acts via transmucosal absorption of the active ingredient. Options for intrauterine infusion therapy include drugs as human chorionic gonadotropin (HCG) and granulocyte colony-stimulating factor (G-CSF) or processed blood samples as peripheral blood mononuclear cells (PBMCs), and autologous platelet-rich plasma (PRP). These procedures gained worldwide popularity because of their simplicity and the encouraging results[2].

Angiogenesis is a critical step in the formation of a vascularized receptive endometrium. Vascular endothelial growth factor (VEGF), which is expressed in the human endometrium, is an important angiogenic mediator. Platelet rich plasma (PRP) is rich in VEGF. Moreover, PRP contains several growth factors such as, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), and other cytokines that stimulate proliferation and growth. All these mediators have a role mplantation[3].

Follicular fluid (FF) is a complex dynamic biological fluid rich in growth factors (e.g. VEGF, TGF and insulin-like growth factor) and cytokines. These mediators may influence implantation via paracrine and autocrine effects[4]. Mehta *et al*. found a connection between the FF characteristics and the quality of the oocyte and concluded that FF insulin-like growth factor 1 (IGF-1) is not only a potential biochemical marker of embryo quality but also influences implantation rates[5]. It is reported that a portion of the FF reaches the fallopian tube with the oocyte following spontaneous ovulation. FF may also promote implantation via its immunosuppressive activity[6].

DOI:10.21608/EBWHJ.2024.318625.1358

In IVF/ICSI cycles following oocyte retrieval the FF is discarded and the endometrium may lose all these mediators that might positively enhance implantation. So, we conducted this randomized controlled trial (RCT) to evaluate the value of intrauterine infusion of FF in ICSI cycles.

PATIENTS AND METHODS

A randomized control study (RCT) was conducted at El-Shatby University Maternity Hospital and a private Fertility Centers (Alexandria, Egypt) to evaluate the effectiveness of endometrial infusion with follicular fluid on endometrial thickness, chemical and clinical pregnancy rates.

Sample Size Justification[7]

A minimal total sample size of (60) females undergoing ICSI (30 per group) is needed to assess an assumed difference of (2 mm) in endometrial thickness between a group of patients undergoing intrauterine injection of follicular fluid in the endometrium cavity at the time of oocyte retrieval and another control group to evaluate the effectiveness of this procedure assuming that standard deviation in both groups is (2.6, 2 respectively) using a two-sided independent t-test, a significance level of 0.05 and 90% power.

60 infertile patients indicated for ICSI were recruited and were randomized using computer generated **tables** into two groups:

Group I (study group): 30 women underwent intrauterine injection of follicular fluid in the endometrial cavity at the time of oocyte retrieval.

Group II (control group): 30 women as a control group.

Inclusion criteria were patients suffering from primary infertility and indicated for ICSI, aged 20-40 years old, with BMI < 35kg/m2 and having normal uterine cavity diagnosed by 3 D ultrasound. Poor responders, cases indicate for freeze-all and cancelled embryo transfer cases were excluded from the study. Moreover, females having azoospermic partners were also excluded.

All patients signed an informed written consent before being enrolled in the study.

Patients were subjected to complete history talking and thorough examination. Basal transvaginal ultrasound (TVUS) was done in addition to basal hormonal profile that included anti-mullerian hormone (AMH), thyroid stimulating hormone (TSH) and prolactin (PRL).

Controlled Ovarian Stimulation (COS)

Cases were stimulated using either antagonist or luteal long agonist protocols.

A-Antagonist Protocol

FSH Fostimon® (IBSA-Switzerland) SC ± HMG Meriofert® (IBSA-Switzerland) SC daily was administrated from cycle day 2. The starting dose ranged between 150:300 IU according to the ovarian reserve. Then the dose was adjusted according to patient's response. The GnRH antagonist (Cetrotide®, Serono, Geneva, Switzerland) was given when the leading follicle was from 12 to 14 mm, at a daily dose of 0.25 mg subcutaneously. When the leading follicles reach 18 mm in diameter, HCG (choriomon® IBSA-Switzerland) was administration. After 35-36 hours oocyte retrieval was done.

B-Luteal Long Agonist Protocol

The GnRH agonist (Decapeptyl; Ferring, Switzerland) was started on day 21 of the preceding cycle at a dose of 0.1 mg/day, subcutaneously. On the second day of menstruation, FSH Fostimon® (IBSA-Switzerland) SC ± HMG Meriofert® (IBSA-Switzerland) SC daily was added. The starting dose ranged between 150:300 IU according to the ovarian reserve and dose was adjusted according to patient response. When most of the follicles reach 18mm in diameter, HCG (choriomon® IBSA-Switzerland) was administration mm. After 35-36 hours oocyte retrieval was done.

Follicular Fluid Preparation and Injection

During oocyte pickup, 6-8 ml of clear follicular fluid was collected in a conical tube then centrifuged at speed of 2000 RBM for 2 minutes. After conclusion of the oocyte pick up procedure, a vaginal speculum was inserted to expose the cervix and using intrauterine insemination (IUI) catheter 1 ml supernatant was injected into the uterine cavity.

Endometrial Assessment

Using TVUS the endometrial thickness and pattern were assessed on the day of HCG, oocyte retrieval, and embryo transfer.

Embryo Transfer

All cases were transferred on day 5 by the same clinician using Labotect® (Labotect, Germany) embryo transfer catheter.

Measuring the Level of Beta HCG

The level of HCG was measured for diagnosis of pregnancy fourteen days after ET (embryo transfer).

Detection of Clinical Pregnancy Rate

Transvaginal ultrasonography was done 2 weeks after positive pregnancy test to detect the gestational sac/sacs and the number of fetal pole (poles) in the uterus.

Statistical Analysis of the Data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level.

The Used Tests Were

1- Chi-square test

For categorical variables, to compare between different groups

2- Fisher's Exact

Correction for chi-square when more than 20% of the cells have expected count less than 5.

3- Mann Whitney test

For abnormally distributed quantitative variables, to compare between two studied groups.

RESULTS

Demographic characteristics, hormonal profile and controlled ovarian stimulation:

There was no statistically significant difference between both groups regarding the demographic characters and the basal hormonal profile (Table 1). The long agonist protocol was used in73.3% of group I (study group) patients and 60% of group II (control group) patients. The antagonist protocol was used in the remaining patients, the difference was statistically not significant (Table 2).

As shown in table 2 number of retrieved oocytes and number of mature oocytes were comparable among both groups. All cases in both groups were transfer on day 5 and the number of transferred embryos ranged from 1-3 on both groups with no statistically significant difference between both groups.

Endometrium Thickness

There was no statistical difference in the endometrial thickness between group I and II on the day of HCG (9.9 and 9.6 mm respectively) nor on the day of embryo transfer (8.5 mm and 8.4 mm respectively) (Table 3).

Pregnancy Rates

As shown in (Table 4) there was no statistically significant difference between both group regarding chemical and clinical pregnancy rates. The clinical pregnancy rate was 76.7% in group I and 66.7% in group II.

Table 1: Demographic characters and hormonal profile

	Group I (n=30)	Group II (n=30)	Test of significance
Age in years:			
Min. – Max.	23 – 40	20 – 39	Mann-Whitney test
Median (IQR)	33.0 (26.2 -34.0)	29.0 (26.0 -33.0)	0.275
Body Mass Index (BMI):			
Min. – Max.	23.4 – 33.7	23 – 34.4	Mann-Whitney test
Median (IQR)	27.0 (25.7 -29.0)	27.4 (26.1 -29.7)	0.428
AMH:			
Min. – Max.	1.3 – 5.95	1.4 – 8.2	Mann-Whitney test
Median (IQR)	1.9 (1.2 to 2.6)	2.2 (1.5 to 4.4)	0.412
PRL:			
Min. – Max.	3.12 – 34	5.3 – 20.1	Mann-Whitney test
Median (IQR)	16.4 (14.2 -18.8)	14.2 (11.6 - 16.9)	0.065
TSH:			
Min. – Max.	0.3 – 3.8	0.255 – 3.4	Mann-Whitney test
Median (IQR)	2.0 (1.4 - 2.5)	1.8 (1.1 - 2.4)	0.237

Table 2: Comparison between the two studied groups regarding the controlled ovarian stimulation

	Group I (n=30)	Group II (n=30)	Test of significance
Protocol:			
Agonist	22 (73.3%)	18 (60.0%)	Chi-Square test
Antagonist	8 (26.7%)	12 (40.0%)	0.411
No. of oocyte retrieval			
Min. – Max.	6 – 27	7 – 19	Mann-Whitney test
Median (IQR)	12.0 (8.0 to 14.0)	10.0 (8.0 to 14.8)	0.847
M2: (Maturity of oocyte)			
Min. – Max.	4 – 21	5 – 17	Mann-Whitney test
Median (IQR)	9.0 (7.2 - 12.0)	8.0 (7.0 - 12.0)	0.624
No. of embryos			
Min. – Max.	1 – 3	1 – 3	Mann-Whitney test
Median (IQR)	2.0 (2.0 - 2.0)	2.0 (2.0 - 2.0)	0.7

Table 3: Comparison between the two studied groups according to endometrial thickness

	Group I (n=30)	Group II (n=30)	Test of significance
Endometrium thickness in day of HCG:			
Min. – Max.	8.5 – 12	8.4 – 12	Mann-Whitney test
Median (IQR)	9.9 (9.1 - 10.9)	9.6 (9.0 - 10.3)	0.338
Endometrium thickness in day of Oocyte retrieval:			
Min. – Max.	8 – 11.8	8.0 – 11.5	Mann-Whitney test
Median (IQR)	9.5 (8.9 - 10.4)	9.2 (8.7 - 9.9)	0.366
Endometrium thickness in day of Embryo transfer:			
Min. – Max.	7.5 – 11.0	7.4 – 10.5	Mann-Whitney test
Median (IQR)	8.5 (8.0 - 9.5)	8.4 (8.0 - 8.9)	0.445
Compaction:			
Min. – Max.	0.5 – 2	0.5 – 2	Mann-Whitney test
Median (IQR)	1.4 (1.0 - 1.5)	1.4 (1.0 - 1.5)	0.850

Table 4: Chemical and clinical pregnancy rate

	Group I (n=30)	Group II (n=30)	Test of significance
Chemical Pregnancy:			
			Chi-Square test
Positive	23 (76.7%)	20 (66.7%)	0.567
Clinical Pregnancy:			
			Chi-Square test
Positive	23 (76.7%)	20 (66.7%)	0.567

DISCUSSION

The follicular fluid provides the microenvironment in which oocytes develop, mature, and ovulate[8]. It has been investigated as a possible predictor of oocyte and embryo quality[5,9]. FF is rich in cytokines and growth factors (e.g., endothelial growth factors, VEGF, and LIF). These factors can activate numerous pathways, which have a key role in the process of implantation. Moreover, it is proven that FF in vitro increases the replication and decidualization of the endometrial cells. This represents the theoretical basis behind using it in flushing the endometrium in attempt to improve implantation rates in ART cycles[10,11].

Moharrami *et al* investigated the effect of FF on various endometrial receptivity genes. They found that exposure of endometrial stromal cells to FF resulted in a significant elevation in the expression of HOXA10, HOXA11, LIF, ITGB3 and ITGAV compared to controls. The group concluded that FF may modulate endometrial receptivity and improve the implantation rate in ART cycles through the up-regulation of endometrial receptivity genes[12].

Previous studies showed the relationships between growth factors, proteins,[13] reactive oxygen species,[14] and metabolites[15] in the follicular fluid and oocyte quality, fertilization rate, embryonic developmental potential, and pregnancy outcome[4].

In this RCT 60 patients were randomized into 2 groups; intrauterine follicular fluid injection group and control group. There was no statistically significant difference between both groups regarding the endometrial thickness nor the pregnancy rates. However, the was a trend towards a higher clinical pregnancy rate in study group compared to the control group (76.7% versus 66.7% respectively. This may be explained by the small sample size.

A subgroup analysis of cases with previous ICSI failure showed a trend towards better pregnancy rates in the study group as compared to the control group (62.25% versus 44.4% respectively), suggesting a possible positive impact of FF injection in this subgroup of patients. This nonsignificant improvement may be explained by the small number of patients in this subgroup.

To the best of our knowledge, no study was conducted before to investigate the role of the follicular fluid in improving endometrial thickness. However, various studies have shown possible improvement of endometrial thickness with different therapeutic modalities such as the use of prolonged estradiol valerate, aspirin, sildenafil citrate, L-arginine, and pentoxifylline, as well as intrauterine infusion of G-CSF and PRP[16,17,18].

Platelet-rich-plasma (PRP) is a platelet-rich whole blood extract, in which red and white blood cells are removed. It is considered an inexpensive means of delivering high concentrations of growth factors since activated platelets release, high concentrations of vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF)[19,20]. A meta-analysis by Maleki-Hajiagha *et al*, included seven studies (625 participants), evaluated the effect of the intrauterine infusion of PRP on women undergoing IVF/ICSI. They found that all reproductive outcomes were significantly improved in PRP-treated cases[21].

Tehraninejad *et al*. used intrauterine infusion of G-CSF five days before embryo transfer in 15 patients with a history of thin endometrium. The endometrial thickness increased from 3.59 ±0.25 mm to 7.12 ± 0.84 mm, suggesting that G-CSF may increase endometrial thickness in patients suffering from thin endometrium[17].

In the present study IU injection of FF did not have a significant effect on the endometrial thickness. Moreover, there was no significant difference between the endometrial thickness of pregnant and non-pregnant patients. This finding is in agreement with Momeni *et al* metanalysis that included 4,922 cycles from 14 studies. The metanalysis was

not able to draw a convincing conclusion on the relationship between endometrial thickness and the pregnancy rate in IVF[22]. Kasius *et al*, meta-analysis that included 22 studies concluded that, there is no justification for using endometrial thickness as a tool for cycle cancellation, freeze-all, or refraining from further IVF treatment[23].

In the present study, regarding the association between the pregnancy rate and endometrium thickness, we found no statistically significant difference between pregnancy rate and endometrium thickness on the day of HCG, day of oocyte retrieval, or day of embryo transfer at (p=0.307, p=0.379, p=0.445) respectively. However, pregnant cases had a significantly higher compaction rate (1.5 verse 0.7, ^{p},0.001). Haas *et al*. also reported that the greater the degree of endometrial compaction, the higher the ongoing pregnancy rate[24].

The limitation of the current study is the small sample size, which is attributed to the new modality and it was not easy to find patients who consent for trying this intervention. Moreover, the study was conducted on general ICSI population. Replicating the trial in patients with thin endometrium or patients with recurrent implantation failure may be more beneficial.

CONFLICT OF INTERESTS

There are no conflicts of interest

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