scientific reports

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# Intrauterine infusion of autologous platelet rich plasma can be an efficient treatment for patients with unexplained recurrent implantation failure

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**The studies posits that there is not sufficient evidence to support the use of intrauterine platelet-rich plasma (PRP) infusion in patients with recurrent implantation failure (RIF). This study aims to investigate the effects of infusion of PRP on patients with unexplained-RIF in fresh and frozen embryo transfer (ET) cycles. A total of 80 participants were included in this study. The participants were randomly assigned to one of two groups with and without PRP infusion. Each of the PRP and control groups were also divided into fresh and frozen ET subgroups. ET outcomes were compared between groups. Clinical pregnancy rate was significantly higher in Frozen ET in PRP group than other subgroups ($p < 0.0001$). Miscarriage rate were significantly lower in PRP group than control group. Pregnancy complications and preterm labor were significantly higher in PRP group than control group ($p < 0.0001$). Live birth and healthy baby rate were significantly higher in PRP group than control group ($p < 0.0001$). The intrauterine infusion of 0.8-1 ml of PRP 48 h before blastocyst ET at fresh and frozen cycles can be an efficient treatment option for u-RIF patients. Also, results indicated that the clinical pregnancy rate was equal to the live birth rate at fresh ET cycles, whereas the live birth rate was lower than the clinical pregnancy rate at frozen ET cycles. Therefore, considering the superiority of fresh cycles over freeze cycles, the infusion of PRP into the uterus of patients with RIF is recommended to be done at fresh ET cycles.**

***Trial registration*: NCT, NCT03996837. Registered 25/06/2019. Retrospectively registered, http://www.clinicaltrial.gov/ NCT03996837.**

**Keywords** Platelet-rich plasma, Recurrent implantation failure, Unexplained infertility, Fresh embryo transfer, Frozen embryo transfer, Pregnancy outcome

**Abbreviations**

| | |
|---|---|
| ARTs | Assisted reproductive technologies |
| BMI | Body mass index |
| ESHRE | European Society of Human Reproduction and Embryology |
| ET | Embryo transfer |
| GBO | Good obstetric outcome |
| GCSF | Granulocyte-colony stimulating factor |
| GDM | Gestational diabetes mellitus |
| GH | Gestational hypertension |
| HCG | Human chorionic gonadotropin |
| HPO | Hypothalamus pituitary ovary |
| IM | Intramuscular |
| PBMCs | Peripheral blood mononuclear cells |

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| | |
|---|---|
| PCOS | Polycystic ovary syndrome |
| PRP | Platelet-rich plasma |
| RCTs | Randomized controlled trials |
| RIF | Recurrent implantation failure |
| SD | Standard deviation |
| u-RIF | Unexplained RIF |

## Background

Despite the remarkable advances that have been made in assisted reproductive technologies (ARTs), recurrent implantation failure (RIF) continues to be a major issue. The failure to achieve a clinical pregnancy after transferring four high-quality embryos during at least three cycles of fresh or frozen embryo transfer (ET) is the most widely accepted definition for RIF[1]. The estimated incidence of RIF is 10%, with about 50% of the cases having unexplained causes[2]. Unexplained RIF (u-RIF) is a clinical problem as well as a major economic and psychological issue that makes considerable demands on healthcare resources. Nevertheless, there has been no considerable progress in its effective management. It is hence necessary to come up with effective treatments to improve pregnancy outcomes in patients with RIF[3,4]. The etiology of RIF includes a decline in endometrial receptivity, fetal defects, and immunological factors[1]. Implantation failure can be primarily attributed to poor endometrial receptivity and to the interplay between embryo and endometrium. Successful implantation requires a receptive endometrium, a functional embryo, and coordinated embryo-endometrial cross talk[4].

Although various interventions have been proposed to improve implantation, there is no consensus on the optimal approach for dealing with u-RIF[5]. Drug therapy through intrauterine infusion has been considered due to its advantages including its safety and less invasiveness. In this approach, the drug does not directly enter the circulatory system; instead, it is absorbed from the uterine mucosa. Intrauterine infusion can improve pregnancy outcomes in patients with RIF by improving endometrial receptivity, increasing the chances of embryo implantation, and modulating maternal immune function. Human chorionic gonadotropin (HCG), granulocyte-colony stimulating factor (GCSF), peripheral blood mononuclear cells (PBMCs), and Platelet-Rich Plasma (PRP) are the commonly administered intrauterine infusions for treating RIF. Kong et al. performed a network meta-analysis to investigate pregnancy outcomes in RIF patients treated with intrauterine infusion of different drugs and showed that PRP intrauterine infusion was the most effective approach to improve clinical pregnancy and live birth[6].

Cell-based therapies such as PRP due to their ability to regenerate tissue through cell differentiation have become increasingly popular over the past decade. Autologous PRP is prepared from fresh whole blood drawn from a peripheral vein. It can be processed to have supra-physiological platelet levels three to five times higher than the normal one. PRP contains alpha-granules that store cytokines and growth factors. PRP growth factors regulate cell migration, differentiation, and proliferation via autocrine and paracrine mechanisms[7]. Studies have shown that PRP can potentially increase endometrial thickness in the proliferative phase and improve immune tolerance in the secretory phase[8].

The meta-analysis conducted by Maged et al. demonstrated the low quality of the available evidence for the effects of PRP on implantation, clinical pregnancy, and live birth rates in patients with RIF[5]. In the meta-analysis conducted by Shalma et al. it was reported that the existing randomized controlled trials (RCTs) did not confirm that PRP had considerable effects on patients with RIF[4]. Anitua et al. also stated in their meta-analysis that there was insufficient evidence for the effects of PRP on patients with RIF[9]. The meta-analysis conducted by Deng et al. also showed that, although PRP had the potential to improve pregnancy outcomes in women with RIF, the existing evidence had to be cautiously interpreted because only a limited number of studies with varying quality had addressed this issue. Therefore, there is a need to conduct more RCTs[1]. Moreover, the European Society of Human Reproduction and Embryology (ESHRE) 2023 guideline posits that there is not sufficient evidence to support the use of intrauterine PRP infusion in patients with RIF[3]. Thus, this study aims to investigate the effects of intrauterine infusion of PRP on patients with u-RIF in fresh and frozen ET cycles.

## Methods

### Study design and population

The present RCT was conducted at a tertiary referral institute (ROYAN Institute, Tehran, Iran). This study retrospectively registered in http://www.clinicaltrial.gov (ID: NCT03996837) at 25/06/2019. A total of 80 participants who presented to the outpatient clinic from August 2017 to November 2021 were included in this study. The participants were randomly assigned to one of two groups with and without PRP intrauterine infusion according to a computer-generated, unconcealed randomization list. Each of the PRP and control groups were also divided into fresh and frozen ET subgroups.

Inclusion criteria included: infertile women with u-RIF (failure to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in a minimum of 3 fresh or frozen ET cycles)[3]; history of at least one blastocyst ET; age < 40 years; 19 < body mass index (BMI) < 29 Kg/m$^2$. Non-inclusion criteria included: endocrine, autoimmune and hematologic disorders; platelet count of less than 15010$^3$/µl; chromosomal and genetic abnormalities; cancer history; uterine anomalies; history of ovarian and uterine surgery; uterine fibroids; adenomyosis; endometriosis; hydrosalpinx; polycystic ovary syndrome (PCOS); moderate to severe male factor; gamete or embryo donation. Exclusion criteria included: endometrial thickness less than 7 mm at the triggering day in fresh ET or at the start of progesterone administration in frozen ET; no blastocyst formation; cervicitis; history of fever within one week before PRP infusion; use of steroid anti-inflammatories drugs at least two weeks before PRP infusion; use of non-steroid anti-inflammatories at least one week before PRP infusion; use of

anticoagulants at least one week before PRP infusion; use of antibiotics at least three days before PRP infusion; use of garlic, onion and omega 3 and 6 supplements at least three days before PRP infusion.

## Sampling

This study was conducted on a sample of 80 patients; 40 patients (20 Fresh ET candidates and 20 Frozen ET candidates) were assigned to the intervention group, six of whom were excluded from the study due to failed blastocyst formation (2 patients in fresh ET and 2 patients in frozen ET cycle) and endometrial thickness of less than 7 mm on the progesterone start day (2 patients in frozen ET). Therefore, the final sample size in the intervention group was 34 (18 patients in fresh ET and 16 patients in frozen ET). In addition, 40 patients (20 Fresh ET candidates and 20 Frozen ET candidates) were assigned to the control group, five of whom were excluded from the study due to failed blastocyst formation (2 patients in fresh ET and 1 patient in frozen ET) and endometrial thickness of less than 7 mm on the progesterone start day (2 patients in frozen ET). Thus, the final sample size in the control group was 35 (18 patients in fresh ET and 17 patients in frozen ET) (Fig. 1).

## Randomization

The participants eligible for enrollment were first stratified two strata of fresh and frozen ET. A randomization list, then, was generated with block sizes of four and six by an unbiased statistician for permuted block-

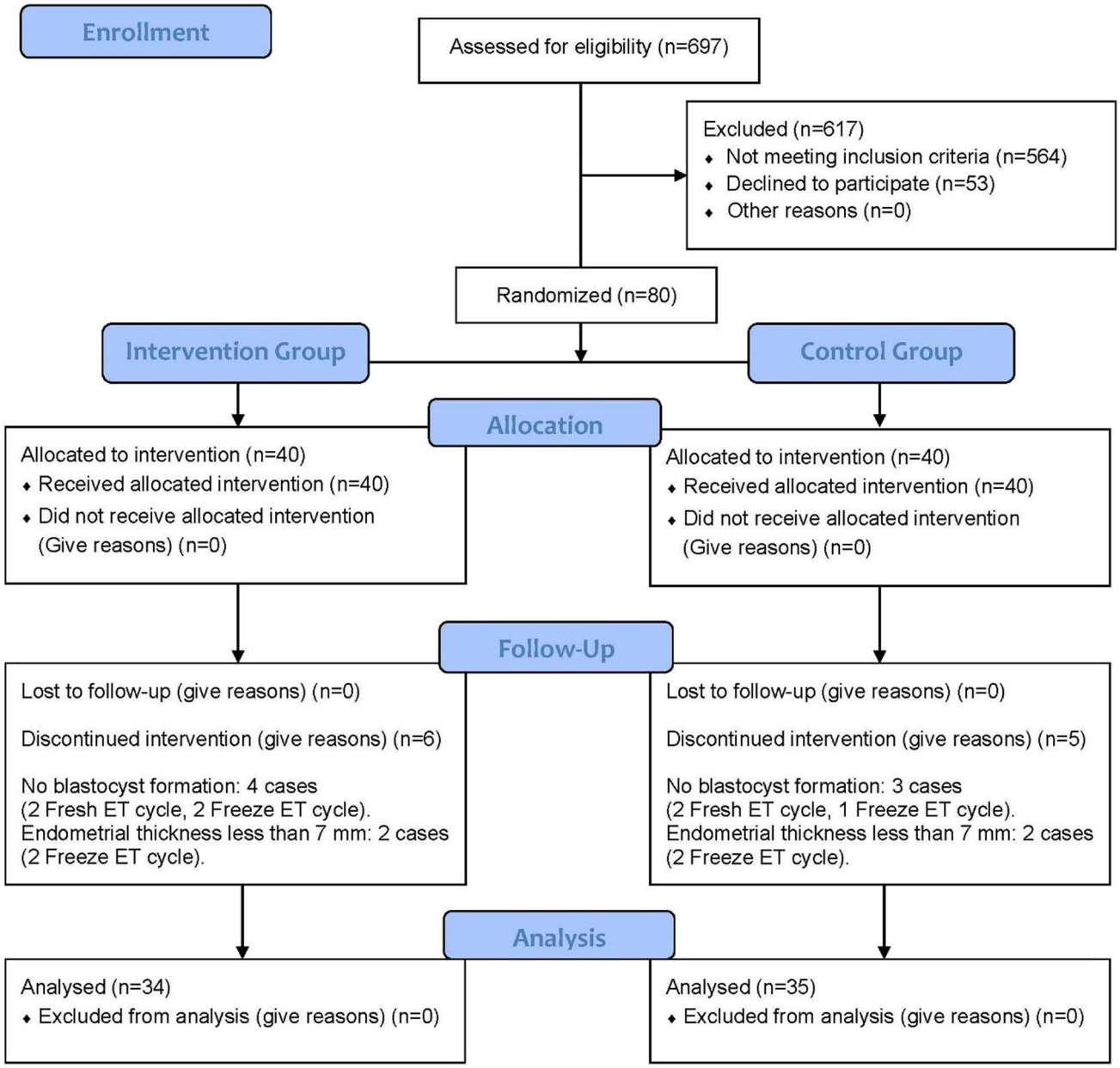


**Fig. 1**. The CONSORT flow diagram of the study.

randomization of the study participants in each stratum aiming for a proper balance of samples to intervention and control groups (in a 1:1 fashion).

### Ovarian stimulation

The ovarian stimulation was implemented through a standard long GnRH-agonist protocol[10]. All patients took one oral contraceptive pill (Ovocept LD Tablet, Aburaihan Pharmacy Company, Iran) per day for 21 days from the fifth day of their menstrual cycle. They were subcutaneously administered 500 µg of Suprefact (Buserelin, SANOFI AVENTIS, Germany) once a day for 14 days from the 17th day of the menstrual cycle. After confirming the hypothalamus-pituitary-ovary (HPO) axis suppression by vaginal ultrasound [Endometrial thickness < 5 mm, lead follicle < 12 mm] and serum estradiol levels < 50 (pg/ml), the Suprefact was reduced to 200 µg per day, and the patients were admonished 150 IU of Gonal-f (Follitropin alfa, Merck Serono, Germany) to stimulate the ovary. Depending on the ovarian response, its dose was increased or Merional (Highly Purified Menotropin, IBSA, Switzerland) was added, if necessary. When at least three follicles reached 18 mm in size, the patients were administered 500 µg of Ovitrelle (Choriogonadotropin alfa, Merck Serono, Germany) as a triggering final oocyte maturation. Ovum pick-up was performed 34–36 h after triggering.

For fresh ET candidates, the obtained embryos reached the blastocyst stage. For frozen ET candidates, the obtained embryos were frozen at the cleavage stage. Then at the time of ET thawed and reached the blastocyst stage.

### Endometrial preparation

The endometrial preparation was implemented through a standard long GnRH-agonist protocol[11]. All patients took one oral contraceptive pill per day for 21 days from the fifth day of their menstrual cycle. They were subcutaneously administered 500 µg of Suprefact once a day for 14 days from the 17th day of the menstrual cycle. After confirming the HPO axis suppression the Suprefact was reduced to 200 µg per day, the patients were also prescribed to take 6 mg of oral estradiol valerate tablets (Aburaihan Pharmacy Company, Iran) for 12 days to induce endometrial preparation. If the thickness of the endometrium measured more than 8 mm, the daily injection of Suprefact was discontinued, and the patients were prescribed to intramuscularly (IM) receive 50 mg of progesterone (Aburaihan Pharmacy Company, Iran) per day for 5 days.

### Preparation and PRP intrauterine infusion

In order to preparing PRP using the ROOYAGEN kit (Arya Mabna Tashkhis, Iran), 8.5 cc of peripheral venous blood and 1.5 cc of anticoagulant were centrifuged (Rotofix 32 A, Hettich, US) twice (1800 rpm for 10 min, and then, 3500 rpm for 6 min) on the day of the intrauterine infusion of PRP ($48 \pm 2$ h before ET). Finally, 1.5 cc of PRP was obtained, which was 4–6 times more concentrated than the baseline platelet. Then, a reproductive endocrinology and infertility physician infused 0.8–1 cc of PRP into the uterine cavity with ET catheter (Labotect Gmbh, Labor-Technik-Gottingen Kampweg 12, 37124 Rosdorf, Germany). It is noteworthy that the vaginal environment of all patients was disinfected with betadine vaginal gel 48 h before the intrauterine infusion of PRP. Furthermore, due to the effects of high serum glucose levels on the quality of PRP, patients were asked to follow a low-glucose diet for 3–5 days before PRP infusion.

### Fresh and frozen embryo transfer and hormonal support

The ET in blastocyst stage was performed around 120 h (5 days) after the start of progesterone injection in fresh or frozen ET cycles. 2–3 embryos in blastocyst stage was transferred by ET catheter via a standard technique[12] by reproductive endocrinology and infertility physician.

In fresh-ET cycles, the luteal phase support was provided with the 50 mg per day progesterone IM for two weeks. In frozen-ET cycles, the luteal phase support was provided with the 50 mg per day progesterone IM and daily 6 mg of the estradiol valerate for two weeks. Then, the serum β-hCG was checked (14 days after the ET). After a β-HCG positive result, the same dose of progesterone and estradiol was continued up 12 weeks of gestation.

### Outcome measures

The primary outcome was implantation rate. The secondary outcome was live birth rate. Implantation rate was defined as the number of gestational sacs per transferred embryos. The live birth was defined as an at least one live fetus delivery beyond 24 completed weeks of gestation.

### Statistical analysis

Qualitative and quantitative data were presented as the frequency and percentage, as well as mean and standard deviation (SD). The normality of data was assessed with the Kolmogorov-Smirnov test. Continuous variables between the two groups were compared using an independent t-test. Comparisons of means across subgroups were performed using one-way ANOVA, followed by Turkey's multiple comparisons test. On the other hand, categorical variables were compared using the chi-square test or Fisher's exact test when more than 20% of cells with expected counts of less than 5 were observable. All analyses were done using SPSS (version 23, SPSS Inc., Illinois, USA), and a P-value less than 0.05 was considered statistically significant.

## Results

The baseline and clinical characteristics of participants are shown in Table 1. Based on the results, there were no significant differences in terms of age, BMI, duration of infertility, previous failed cycles, menstrual pattern, type of infertility, and hormones (FSH, LH, Estradiol, and progesterone) between groups and between subgroups.

| Variable | Study Groups | | | | *P*-value |
|---|---|---|---|---|---|
| | PRP group ($n=34$) | | Control group ($n=35$) | | |
| | Fresh ET ($N=18$) | Freeze ET ($N=16$) | Fresh ET ($N=18$) | Freeze ET ($N=17$) | |
| Age (year) | 34.85 ± 3.19 | | 34.8 ± 3.43 | | 0.947 |
| | 34.88 ± 2.85 | 34.81 ± 3.64 | 34.94 ± 3.51 | 34.65 ± 3.44 | 0.994 |
| BMI (kg/m$^2$) | 24.77 ± 2.07 | | 24.73 ± 2.08 | | 0.930 |
| | 24.29 ± 1.88 | 25.32 ± 2.21 | 24.93 ± 2.05 | 24.51 ± 2.15 | 0.484 |
| Infertility duration (year) | 8.15 ± 2.41 | | 8.23 ± 2.37 | | 0.888 |
| | 8.11 ± 2.22 | 8.19 ± 2.69 | 8.17 ± 2.31 | 8.29 ± 2.49 | 0.997 |
| Previous failed cycles | 4.47 ± 1.02 | | 4.11 ± 0.90 | | 0.129 |
| | 4.39 ± 1.09 | 4.56 ± 0.96 | 4.22 ± 1.00 | 4.00 ± 0.79 | 0.391 |
| Menstruation | | | | | |
| Regular | 28 (82.4%) | | 32 (91.4%) | | |
| | 16 (88.9%) | 12 (75%) | 18 (100%) | 14 (82.4%) | 0.165 |
| Irregular | 6 (17.6%) | | 3 (8.6%) | | |
| | 2 (11.1%) | 4 (25%) | 0 (0.0%) | 3 (17.6%) | |
| Infertility | | | | | |
| Primary | 28 (82.4%) | | 33 (94.3%) | | |
| | 16 (88.9%) | 12 (75%) | 17 (94.4%) | 16 (94.1%) | 0.262 |
| Secondary | 6 (17.6%) | | 2 (5.7%) | | |
| | 2 (11.1%) | 4 (25%) | 1 (5.6%) | 1 (5.9%) | |
| FSH (IU/L) (Base) | 5.93 ± 1.67 | | 6.01 ± 1.93 | | 0.861 |
| | 6.35 ± 1.9 | 5.46 ± 1.22 | 5.83 ± 1.73 | 6.19 ± 2.16 | 0.484 |
| LH (IU/L) (Base) | 6.04 ± 1.95 | | 5.09 ± 2.06 | | 0.056 |
| | 6.28 ± 1.91 | 5.76 ± 2.02 | 5.2 ± 2.05 | 4.98 ± 2.13 | 0.235 |
| Estradiol (pg/ml) (Triggering day) | 2149.32 ± 286.25 | | 2107 ± 314.65 | | 0.563 |
| | 2054.78 ± 289.80 | 2255.69 ± 249.58 | 1998.89 ± 301.83 | 2221.82 ± 293.99 | 0.235 |
| Progesterone (ng/ml) (Triggering day) | 0.39 ± 0.26 | | 0.38 ± 0.24 | | 0.837 |
| | 0.36 ± 0.27 | 0.43 ± 0.28 | 0.35 ± 0.11 | 0.42 ± 0.32 | 0.712 |

**Table 1**. Comparison of demographic, clinical and laboratory characteristics between study groups. Values are presented as the mean ± SD and number (percent). P-value obtained by independent t-test and chi square test. Statistically significant level < 0.05.

Ovarian stimulation and endometrial preparation information displayed in Table 2. There were not any significant differences in endometrial preparation information (such as endometrial thickness and duration of endometrial preparation) between PRP cases and controls. The same pattern was observed when the variables were compared between four ET groups. Comparison of ovarian stimulation data between two groups of PRP and control revealed that no significant differences was found with regard to variables including oocyte retrieved, MII, 2PN, embryo obtained, number of ET, and fertilization rate. However, findings on ovarian stimulation showed that number of MII was significantly more in Frozen ET in PRP group than other subgroups ($p=0.036$). The number of 2PN ($p=0.003$) and embryo obtained ($p=0.009$) was significantly higher in Frozen ET in PRP group than fresh ETs; without any significant difference in terms of fertilization rate.

Results related to outcomes of ET displayed in Table 3. A significant difference was found in clinical pregnancy rate between PRP and control groups ($p<0.0001$). There was this similar finding when four subgroups were considered. Clinical pregnancy rate was significantly higher in Frozen ET in PRP group than other subgroups ($p<0.0001$). Miscarriage rate per cycle and miscarriage rate per pregnancy were significantly lower in PRP group than control group, with evidence on no miscarriage in fresh ET in PRP cases. Pregnancy complications (gestational hypertension and gestational diabetes) and preterm labor were significantly higher in PRP group than control group ($p<0.0001$). Also, placenta disorders (placenta accrete, percreta, and placenta previa) were evaluated, but no cases were observed in the intervention and control groups. Live birth rate (/cycle and /pregnancy) and healthy baby rate (/cycle and /pregnancy) were significantly higher in PRP group than control group ($p<0.0001$), with evidence on significant increase in frozen ET in PRP cases than other subgroups ($p<0.0001$).

Comparison of pregnant and non-pregnant patients following intrauterine infusion of PRP are shown in Table 4. There were no significant differences in terms of age, BMI, duration of infertility, previous failed cycles, type of infertility, FSH, LH, number of ET, and fertilization rate.

| Variable | Study groups | | | | *P*-value* |
|---|---|---|---|---|---|
| | PRP group ($n=34$) | | Control group ($n=35$) | | |
| | Fresh ET ($N=18$) | Freeze ET ($N=16$) | Fresh ET ($N=18$) | Freeze ET ($N=17$) | |
| Gonadotropin dose (IU) | 1793.38 ± 342.63 | | 1872.86 ± 407.75 | | 0.385 |
| | 1741.67 ± 288.25 | 1851.56 ± 396.59 | 1991.67 ± 346.30 | 1747.059 ± 439.54 | 0.158 |
| Stimulation duration (day) | 11.79 ± 1.20 | | 11.23 ± 1.33 | | 0.068 |
| | 11.89 ± 1.02 | 11.69 ± 1.40 | 11.06 ± 1.39 | 11.41 ± 1.28 | 0.245 |
| Endometrial thickness (mm) (Triggering day) | 9.56 ± 1.35 | | 9.67 ± 1.29 | | 0.725 |
| | 9.4 ± 1.10 | 9.74 ± 1.60 | 9.86 ± 1.36 | 9.47 ± 1.23 | 0.701 |
| Oocyte retrieved | 13.82 ± 1.45 | | 14.06 ± 1.61 | | 0.461 |
| | 13.33 ± 1.14 | 14.38 ± 1.59 | 13.78 ± 1.11 | 14.35 ± 1.17 | 0.050 |
| MII | 12.74 ± 1.56 | | 12.51 ± 1.27 | | 0.521 |
| | 12.28 ± 1.07 | 13.25 ± 1.88 | 12.06 ± 1.16 | 13.00 ± 1.22 | 0.036* |
| 2PN | 11.00 ± 1.63 | | 11.03 ± 1.64 | | 0.942 |
| | 10.5 ± 1.25 | 11.56 ± 1.86 | 10.22 ± 1.44 | 11.88 ± 1.41 | 0.003* |
| Embryo obtained | 10.50 ± 1.71 | | 10.62 ± 1.59 | | 0.747 |
| | 9.94 ± 1.26 | 11.13 ± 1.96 | 9.94 ± 1.39 | 11.35 ± 1.49 | 0.009* |
| Fertilization rate | 0.86 ± 0.09 | | 0.88 ± 0.09 | | 0.469 |
| | 0.86 ± 0.02 | 0.87 ± 0.02 | 0.85 ± 0.02 | 0.92 ± 0.02 | 0.109 |
| Estradiol dose (mg) | - | 107.25 ± 6.88 | - | 104.82 ± 6.04 | 0.290 |
| Duration of endometrial preparation (day) | - | 17.88 ± 1.15 | - | 17.47 ± 1.01 | 0.290 |
| Endometrial thickness (mm) (progesterone start day) | - | 9.64 ± 1.42 | - | 9.49 ± 1.29 | 0.753 |
| ET type | | | | | 0.232 |
| Easy | 32 (94.1%) | | 34 (97.1%) | | |
| | 18 (100%) | 14 (87.5%) | 18 (100%) | 16 (94.1%) | |
| Difficult | 2 (5.9%) | | 1 (2.9%) | | |
| | 0 (0%) | 2 (12.5%) | 0 (0%) | 1 (2.9%) | |
| No. ET | 2.29 ± 0.46 | | 2.2 ± 0.41 | | 0.372 |
| | 2.22 ± 0.43 | 2.36 ± 0.5 | 2.11 ± 0.32 | 2.29 ± 0.47 | 0.337 |

**Table 2**. Comparison of ovarian stimulation and endometrial preparation information between study groups. Values are presented as the mean ± SD and number (percent). P-value obtained by independent t-test and chi square test. Statistically significant level < 0.05 (*).

## Discussion

This RCT aimed to investigate the efficacy of the intrauterine infusion of PRP in patients with u-RIF at fresh and frozen ET cycles. The study findings demonstrated the considerable effectiveness of the intrauterine infusion of PRP in increasing pregnancy, live birth, and healthy baby rates and also reducing miscarriage in patients with u-RIF.

Although numerous studies have investigated the intrauterine infusion of PRP, the ESHRE guideline 2023 and British Fertility Society guideline 2021 find available evidence insufficient to support the use of this product for RIF patients[3,13]. Considering the quality of the available literature, meta-analyses conclude that further RCTs are needed to more accurately assess the efficacy of PRP in RIF patients[1,4,5,9].

Since the present study was conducted on u-RIF patients, studies on patients with RIF due to thin endometrium and patients with a history of less than 2 implantation failures, studies in which the control group received interventions such as GCSF injections, and studies that performed more than a PRP infusion or a sub endometrial injection were not discussed here.

Nazari et al.[14] conducted an RCT on 97 patients with RIF and infused 0.5 ml of PRP into the uterus of 49 of them 48 h before frozen ET; their studies revealed a statistically significant difference in the clinical pregnancy rate (44.9% in PRP group vs. 16.6% in control group). In our study, the clinical pregnancy rate was higher at frozen ET cycles (75% in PRP group vs. 29.4% in control group). Since Nazari et al. did not mention the embryonic stage (cleavage or blastocyst), the higher clinical pregnancy rate in our study can be attributed to blastocyst ET. Moreover, the volume of PRP infused into the uterus of patients was higher in our study. One of the weaknesses of the study conducted by Nazari et al. was no report of ongoing pregnancy rate and live birth rate. Zamaniyan et al.[15] conducted an RCT on 98 patients with RIF and infused 0.5 ml of PRP into the uterus of 55 of them 48 h before frozen ET; their results showed a statistically significant difference in the clinical pregnancy rate (52.7% in PRP group vs. 23.3% in control group) and ongoing pregnancy rate (50.9% in PRP group vs. 16.3% in control group). In our study, the live birth rate at frozen ET cycles was 68.8% in the PRP group and 11.7% in the control group. Zamaniyan et al. transferred blastocyst embryos, similar to our study, but infused less PRP into the uterus

| Variable | Study groups | | | | *P*-value |
|---|---|---|---|---|---|
| | PRP group ($n=34$) | | Control group ($n=35$) | | |
| | Fresh ET ($N=18$) | Freeze ET ($N=16$) | Fresh ET ($N=18$) | Freeze ET ($N=17$) | |
| Clinical pregnancy rate | 19/34 (55.8%) | | 7/35 (20%) | | 0.0001* |
| | 7/18 (38.8%) | 12/16 (75%) | 2/18 (11.1%) | 5/17 (29.4%) | 0.0001* |
| Implantation rate | 0.57 ± 0.21 | | 0.59 ± 0.29 | | 0.809 |
| | 0.64 ± 0.24 | 0.53 ± 0.19 | 0.75 ± 0.35 | 0.53 ± 0.27 | 0.501 |
| Miscarriage rate/ cycle | 1/34 (2.9%) | | 4/35 (11.4%) | | 0.033* |
| | 0/18 (0.0%) | 1/16 (6.3%) | 1/18 (5.5%) | 3/17 (17.6%) | 0.008* |
| Miscarriage rate/ pregnancy | 1/19 (5.3%) | | 4/7 (57.1%) | | 0.0001* |
| | 0/7 (0.0%) | 1/12 (8.3%) | 1/2 (50%) | 3/5 (60%) | 0.0001* |
| Pregnancy complications rate/ pregnancy | 3[1]/19 (15.8%) | | 0/7 (0.0%) | | 0.0001* |
| | 0/7 (0.0%) | 3/12 (25%) | 0/2 (0.0%) | 0/5 (0.0%) | 0.0001* |
| Twin pregnancy rate/ pregnancy | 5/19 (26.3%) | | 2/7 (28.6%) | | 0.686 |
| | 2/7 (28.6%) | 3/12 (25%) | 1/2 (50%) | 1/5 (20%) | 0.013* |
| Baby weight (kg) (singleton) | 2938.75 ± 529.99 | | 2516.67 ± 553.02 | | 0.225 |
| | 3007.17 ± 537.29 | 2885.56 ± 550.34 | 2000 ± 0.00 | 2775.00 ± 459.62 | 0.403 |
| Baby weight (kg) (twin) | 1950.00 ± 310.91 | | 1800.00 ± 0.0 | | 0.695 |
| | 2050.00 ± 70.71 | 1850.00 ± 494.97 | 1800.00 ± 0.00 | - | 0.812 |
| Preterm labor/ pregnancy | 7/19 (36.8%) | | 0/7 (0.0%) | | 0.0001* |
| | 2/7 (28.6%) | 5/12 (41.6%) | 0/2 (0.0%) | 0/5 (0.0%) | 0.123 |
| Live births rate/ cycle | 18/34 (52.9%) | | 3/35 (8.6%) | | 0.0001* |
| | 7/18 (38.8%) | 11/16 (68.8%) | 1/18 (5.5%) | 2/17 (11.7%) | 0.0001* |
| Live births rate/ pregnancy | 18/19 (94.7%) | | 3/7 (42.9%) | | 0.0001* |
| | 7/7 (100%) | 11/12 (91.6%) | 1/2 (50%) | 2/5 (40%) | 0.0001* |
| Healthy baby rate/ cycle | 16/34 (47.06%) | | 3/35 (8.6%) | | 0.0001* |
| | 7/18 (38.8%) | 9/16 (56.3%) | 1/18 (5.5%) | 2/17 (11.7%) | 0.0001* |
| Healthy baby rate/ pregnancy | 16/19 (84.2%) | | 3/7 (42.9%) | | 0.0001* |
| | 7/7 (100%) | 9/12 (75%) | 1/2 (50%) | 2/5 (40%) | 0.0001* |

**Table 3**. Comparison of embryo transfer outcome between study groups. Values are presented as the mean ± SD and number (percent). P-value obtained by independent t-test and chi square test. Statistically significant level < 0.05 (*). [1]One case of gestational hypertension, and two cases of gestational diabetes.

of patients. In another RCT on 120 patients with RIF, Safdarian et al.[16] infused 0.5 ml of PRP into the uterus of 60 patients 48 h before frozen ET; they reported a significant difference in clinical pregnancy rate (51.6% in PRP group vs. 26.6% in control group), live birth rate (58.3% in PRP group vs. 28.3% in control group), and preterm labor rate (25% in PRP group vs. 3.3% in control group). Our study also showed a significant difference between the intervention and control groups in preterm delivery rate (36.8% in PRP group vs. 0% in control group). Safdarian et al. transferred blastocyst embryos, similar to our study, but infused less PRP into the uterus of patients. Shah-bakhsh et al.[17] conducted an RCT on 100 patients with RIF and infused 0.5 ml of PRP into the uterus of 50 of them 48 h before frozen ET; their results demonstrated no significant difference between the intervention and control groups in pregnancy rate (20% in PRP group vs. 13.3% in control group). They used the same endometrial preparation protocol as this study and also transferred blastocyst embryos. However, they infused less PRP into the uterus of patients and also did not report ongoing pregnancy rate and live birth rate. In another RCT, Ershadi et al.[18] investigated 85 patients with RIF and infused 0.5 ml of PRP into the uterus of 40 of them 48 h before frozen ET; they reported a no significant difference in pregnancy rate (33% in PRP group vs. 24% in control group). They transferred embryos in the cleavage stage and infused less PRP into the uterus of patients, compared to our study. Weakness of their study was no report ongoing pregnancy rate and live birth rate. Zargar et al.[19] conducted an RCT on 80 patients with RIF and infused 1.5 ml of PRP into the uterus of 38 of them 48 h before frozen ET; their results showed no significant difference in pregnancy rate (12.5% in PRP group vs. 2.5% in control group) but a significant difference in live birth rate (12.5% in PRP group vs. 0% in control group). It is noteworthy that they did not address the embryonic stage. The high volume of PRP infusion, which decreased the success of the ET cycle, may be the primary reason for the discrepancy between the results of the above-mentioned study and those of similar studies. Also in a retrospective study, Xu et al.[20] investigated 288 patients with RIF and infused 1 ml of PRP into the uterus of 138 of them 48 h before frozen ET; they reported a significant difference in pregnancy rate (36.2% in PRP group vs. 24.6% in control group) and live birth rate (29.7% in PRP group vs. 14% in control group). It should be noted that they transferred embryos at the cleavage or blastocyst stage and also employed hormone-replacement or natural endometrial preparation protocols.

Furthermore, a few studies have investigated the effects of the intrauterine infusion of PRP at fresh ET cycles. Rageh et al.[21] conducted an RCT on 150 patients with RIF and infused 0.5-1 ml of PRP into the uterus of 75 of

| Variable | Pregnancy following PRP ($n=19$) | Non-pregnancy following PRP ($n=15$) | *P*-value |
|---|---|---|---|
| Age (year) | 35.00 ± 3.32 | 34.67 ± 3.13 | 0.768 |
| BMI (kg/m$^2$) | 25.03 ± 2.0 | 24.44 ± 2.18 | 0.421 |
| Infertility duration (year) | 8.26 ± 2.40 | 8.0 ± 2.51 | 0.758 |
| Previous failed cycles | 4.32 ± 0.89 | 4.67 ± 1.18 | 0.328 |
| Menstruation | | | 0.016* |
| Regular | 13 (68.4%) | 15 (100.0%) | |
| Irregular | 6 (31.6%) | 0 (0.0%) | |
| Infertility | | | 0.558 |
| Primary | 15 (78.9%) | 13 (86.7%) | |
| Secondary | 4 (21.1%) | 2 (13.3%) | |
| Platelet (10$^3$/μl) (base) | 276.53 ± 38.36 | 269.4 ± 38.54 | 0.595 |
| FSH (IU/L) (base) | 5.56 ± 1.48 | 6.40 ± 1.83 | 0.147 |
| LH (IU/L) (Base) | 6.09 ± 1.98 | 5.97 ± 1.97 | 0.858 |
| Fertilization rate | 0.87 ± 0.09 | 0.86 ± 0.09 | 0.684 |
| ET type | | | 0.863 |
| Easy | 18 (94.7%) | 14 (93.3%) | |
| Difficult | 1 (5.3%) | 1 (6.7%) | |
| No. ET | 2.63 ± 0.45 | 2.33 ± 0.49 | 0.667 |

**Table 4**. Comparison of pregnant and non-pregnant patients following intrauterine injection of PRP. Values are presented as the mean ± SD and number (percent). P-value obtained by independent t-test and chi square test. Statistically significant level < 0.05 (*).

them 48 h before fresh ET; their results showed a significant difference in pregnancy rate (43% in PRP group vs. 15% in control group). In our study, the pregnancy rate at fresh ET cycles was 38.8% in the PRP group and 11.1% in the control group. Rageh et al. employed GnRH-antagonist as the ovarian stimulation protocol and reported that the fertilization rate was almost significantly higher in the PRP group. In addition, similar to our study, they transferred blastocyst embryos. However, a weakness of their study was no report ongoing pregnancy rate and live birth rate. In another RCT, Elnafarawi et al.[22] investigated 74 patients with RIF and infused 1 ml of PRP into the uterus of 37 of them 48 h before fresh ET; their results indicated a significant difference between the PRP and control groups in pregnancy rate (48.6% in PRP group vs. 18.9% in control group), also endometrium thickness, number of oocytes and MII retrieved, blastulation rate, and fertilization rate. However, a weakness of their study was no report ongoing pregnancy rate and live birth rate. Dieamant et al.[23] conducted an RCT on 66 patients with RIF and infused 0.7 ml of PRP into the uterus of 33 of them 48 h before fresh ET, and they were subcutaneously administered 0.5 ml of GCSF. They reported no significant difference in clinical pregnancy rate (36.4% in PRP group vs. 30.3% in control group) and live birth rate (27.3% in PRP group vs. 27.3% in control group). In our study, the live birth rate at fresh ET cycles was 39% in the PRP group and 5.5% in the control group. Dieamant et al. employed GnRH-agonist as the ovarian stimulation protocol, similar to our study, but did not address the embryonic stage. Also, due to the simultaneous administration of PRP and GCSF, the PRP effect cannot be accurately judged.

The review of the existing studies demonstrated that the volume of PRP infused into the uterus of patients in frozen ET cycles was almost 0.5 ml, except a study conducted by Zargar et al.[19] who infused more than 1 ml of PRP and reported poor pregnancy outcomes. The volume of infused PRP can be one of the reasons behind the significant difference between our study and other similar studies in the clinical pregnancy rate. However, the results of studies conducted by Zamaniyan et al.[15] and Safdarian et al.[16] were comparable to the findings of our study on live birth rate. The volume of infused PRP at fresh ET cycles in all the above-mentioned studies was almost the same as that of our study. Except in the study conducted by Dieamant et al.[23] who reported no significant difference in clinical pregnancy rate, Rageh et al.[21] and Elnafarawi et al.[22] reported a clinical pregnancy rate more than that of our study. However, this may be probably due to the heterogeneity of intervention and control groups in their studies. Moreover, these two studies did not report ongoing pregnancy rate or live birth rate, whereas our study showed a promising live birth rate at fresh ET.

As mentioned earlier, some of the above-mentioned studies did not address the embryonic stage of transferred embryos. However, blastocyst ET and PRP infusion dose seem to play a major role in improving the pregnancy outcome of patients. The findings of Zamaniyan et al.[15], Safdarian et al.[16], and Rageh et al.[21] also corroborate this hypothesis.

None of the studies reported a significant difference in terms of miscarriage rate; Ershadi et al.[18] and Dieamant et al.[23] showed that the miscarriage rate was higher in the intervention group at frozen and fresh ET cycles, respectively, but its difference was not statistically significant. In our study reported no case of miscarriage in the PRP group at fresh ET and also a significantly lower miscarriage rate in the PRP group at frozen ET.

On the other hand, none of the studies reported pregnancy complications. In this study, no congenital anomalies were found, and pregnancy complications [gestational diabetes mellitus (GDM) and gestational hypertension

(GH)] were observed only in the PRP group, all of which were at frozen ET cycle. GDM and Hypertensive disorders in pregnancy associated with increased maternal and neonatal morbidity and mortality[24,25]. The pathophysiology of different types of GH can be narrowed to two different fundamental ground mechanisms following Ohm's law in hemodynamics: volume- and/or resistance-dominant hypertension. In normotensive individuals, a dominance in either direction can be present before conception or develops during the process of implantation[26]. The potential etiology of GDM includes insulin resistance or persistent glycemic intolerance resulting from alterations in pancreatic β cells during pregnancy. Furthermore, various placental hormones, e.g., estrogen and progesterone may cause GDM. GDM associated with some complications including preeclampsia, macrosomia, hypocalcemia, neonatal hypoglycemia, and congenital abnormalities[27]. Some studies have reported the role of oxidative stress in the pathogenesis of GH and GDM[28]. They have also shown that GH or GDM may influence the placenta's capacity to secrete vasoactive substances and its sensitivity to the contraction of placental blood vessels. However, further studies must be conducted on the physiological characteristics of human placental blood vessels and the pathological alterations associated with GH and GDM[29]. Recent studies have focused on the molecular mechanisms underlying pathological conditions during pregnancy, e.g., GH and GDM. Available data indicate that molecular mechanisms involved in the pathophysiology of GDM and GH are not fully known[25]. Additionally, understanding the epigenetic causes of these pathological conditions will facilitate their optimal prevention and management[27]. Although the existing literature has fully established the content of PRP, further studies must be conducted to reveal its exact mechanism of action. The results of this study showed that the prevalence of pregnancy complications, e.g., GH and GDM, was significantly higher in the PRP group. Accordingly, the question here is whether PRP intrauterine infusion was responsible for the increased prevalence of these complications. Since most previous studies have not reported pregnancy complications following PRP intrauterine infusion, future studies are recommended to analyze this relationship in larger samples. If future studies establish such a relationship, it is then necessary to conduct in-vitro studies to identify the reasons for the higher incidence of GH and GDM in patients with a history of PRP intrauterine infusion. If pregnancies following PRP intrauterine infusion are associated with a higher prevalence of GH and GDM; therefore, screening measures and specialized consultations should be prioritized during pregnancy to identify these complications as early as possible to mitigate the risk of maternal and fetal complications.

Only Safdarian et al.[16] reported the preterm labor rate, which was significantly higher in the intervention group. In our study, preterm labor was observed only in the PRP group and higher in frozen ET cycles, which can be associated with the higher pregnancy rate at these cycles. Studies have shown that the preterm labor rate is higher at ET cycles (fresh or freeze)[30]. As stated earlier, ongoing pregnancy rate and live birth rate were not reported in half of the studies, which makes it difficult to assess the effectiveness of PRP in patients with RIF. However, our study reported the healthy baby rate in addition to the live birth rate. The live birth rate was equal to the healthy baby rate in the control group, whereas the healthy baby rate was lower than the live birth rate in the intervention group (in the frozen ET subgroup) due to the death of two babies after birth caused by preterm delivery under 30 weeks of pregnancy.

There were also differences between our study and similar previous studies in terms of ovarian stimulation protocol (in fresh ET cycles) and endometrial preparation protocol (in frozen ET cycles). In addition, unlike most similar studies that investigated RIF patients with different causes of infertility, this study specifically focused on u-RIF patients. Another strength of this study was reporting PRP outcomes at both fresh and frozen ET cycles. Moreover, this study reported pregnancy complications, and infant health status after birth. In this study, placenta disorders (Placenta accrete, percreta, and placenta previa) were also evaluated, but no cases were observed in the intervention and control groups. Nevertheless, a weakness of this RCT was the lack of blinding.

The study results demonstrated the effectiveness of PRP at fresh ET cycles, which significantly increased the clinical pregnancy rate and live birth rate. The existing evidence suggests that the freezing procedure may negatively affect IVF outcomes. Fresh ET appears to be associated with better birth outcomes[31]. Roeca et al. demonstrated that fresh ET is more likely to result in a live birth and has a significantly higher probability of good obstetric outcome (GBO) than frozen transfer [GBO defined as a singleton, term, live birth with appropriate for gestational age birth weight][32], which is consistent with the findings of this study. Our study results indicated that the clinical pregnancy rate was equal to the live birth rate at fresh ET cycles, whereas the live birth rate was lower than the clinical pregnancy rate at frozen ET cycles. Therefore, considering the superiority of fresh cycles over freeze cycles, the infusion of PRP into the uterus of patients with RIF is recommended to be done at fresh ET cycles.

Generally, it is challenging to compare the risks of ET approaches and identify the most preferable method. Evidence suggests that each method entails particular risks; therefore, no singular approach can be definitively deemed safer[33]. The adverse effects of ovarian stimulation on endometrial acceptance have led to the emergence of the freeze-all policy as an alternative to fresh ET to improve IVF outcomes. The implantation rate is presumed to increase when embryos are transferred to a receptive endometrium devoid of the detrimental effects of gonadotropins[34]. Some studies have reported that fresh ET is associated with higher rates of preterm delivery and low birth weight, whereas frozen ET may increase the risk of GH and fetal macrosomia[35]. The incidence of ectopic pregnancy and the risk of placenta previa and placental abruption are lower in frozen ET. This is attributed to supra-physiological concentrations of sex steroids on the endometrium during ovarian stimulation, which may result in defective implantation and/or placentation. Studies have also reported an association between hypertensive disorders during pregnancy in frozen ET cycles and endometrial preparation protocol. Reportedly, the risk of preeclampsia is significantly higher in artificially prepared frozen ET cycles, which lack corpus luteum, than in frozen ET cycles that contain one or more corpus luteum. On the other hand, epigenetic changes caused by the freeze/thaw process may cause fetal macrosomia[33]. Some studies indicate that prolonged storage duration of frozen embryos can adversely affect both embryo survival rate and clinical pregnancy rate[36]. Vitrification has been reported to induce alterations in the cytoskeleton that adversely affect embryo

viability. It can also modify transcription in the embryo, potentially hindering its development. Although it is commonly believed that frozen ET occurs in a more physiological endometrium, endometrial preparation cycles utilizing estradiol and progesterone may still exert varying effects on the endometrium, placenta, and fetal development[32]. In the present study, the effects of freezing may lead to the superiority of fresh cycles in the more reliable achieving live birth following clinical pregnancy.

Since many studies have reported the anti-inflammatory effects of PRP[2,8,37], future studies are recommended to investigate the effects of the intrauterine infusion of PRP on RIF patients who suffer from PCOS, endometriosis, and fibromatous uterus. The transfer of euploid embryos can be another area of research for future studies to achieve more accurate and reliable results on the efficacy of PRP.

## Conclusions

The study findings demonstrated that the intrauterine infusion of 0.8-1 ml of PRP 48 h before blastocyst ET at fresh and frozen cycles can be an efficient treatment option for u-RIF patients.

## Data availability

All data generated or analyzed during this study are included in this published article.

Received: 30 July 2024; Accepted: 23 October 2024
Published online: 29 October 2024

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## Acknowledgements
Authors would like to thank the staff of ROYAN Institute for their assistance in this study and the women who participated in this study.

## Author contributions
A.Y.: Project development, study design, data collection/management, and manuscript writing; T.M.: Provided clinical expertise and supervision, and manuscript editing; S.V.: Data analysis, and manuscript editing; M.M.: Provided clinical expertise and supervision, protocol/project development, and manuscript editing.

## Funding
No financial support has been granted.

## Declarations

## Competing interests
The authors declare no competing interests.

## Ethical approval and consent to participate
The study was approved by the Institutional Review Board of the Affiliated Research Ethics Committee of Royan institute (IR.ACECR.ROYAN.REC.1396.99), (15/08/2017). All methods were performed in accordance with the relevant guidelines and regulations. All patients signed the written informed consent form before participation in the study.

## Additional information
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