Autologous peripheral blood mononuclear cells intrauterine instillation to improve pregnancy outcomes after recurrent implantation failure: a systematic review and meta-analysis

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Abstract

Purpose Recurrent implantation failure (RIF) is a common cause of disappointment and a big challenge after assisted reproduction technology treatments. The objective of this study was to evaluate the existing literature to explore whether peripheral blood mononuclear cells' (PBMCs) instillation could improve pregnancy outcomes among patients with RIF.

Methods We conducted a comprehensive search including PubMed, EMBASE, Cochrane library and various databases in China. Three randomized controlled trials (RCTs) and three non-randomized controlled trials (non-RCTs) were included. We included subgroup and sensitivity analyses using Stata 12.0.

Results The results of the three RCTs showed that PBMC improved outcomes in all patients compared with placebo or no-treatment [clinical pregnancy rate (CPR): odds ratio (OR) 2.45, 95% confidence interval (CI) 1.53–3.91; implantation rate (IR): OR 2.46, 95% CI 1.48–4.09; live birth rate (LBR): OR 2.43, 95% CI 1.32–4.49]. However, the results of the three non-RCTs indicated that there were no statistically significant differences in the outcomes and that the heterogeneity was higher ($I^2 > 0\%$). Subgroup analysis further suggested that PBMCs treatment significantly increased the CPR, IR and LBR in the three or more implantation failure subgroups (CPR: OR 2.83, 95% CI 1.29–6.22; IR: OR 3.74, 95% CI 1.71–8.19; LBR: OR 3.03, 95% CI 1.15–7.98).

Conclusions Among patients with three or more implantation failures, this treatment improved IR, LBR, and CPR compared to that in controls, due to the limited data available, PBMCs' intrauterine instillation should only be used in the context of clinical trials.

Keywords Recurrent implantation failure · Peripheral blood mononuclear cell · In vitro fertilization · Assisted reproductive technology

Introduction

Despite a gradual improvement in in vitro fertilization (IVF) success rates over the last 2 decades, there is still a class of patients who may undergo more than one cycle of treatment and may remain childless even after successive attempts. The Canadian Assisted Reproductive Technology Registry (CARTR) showed that the overall live birth rate (LBR) after IVF was about 30% per cycle in 2010 [1]. The Human Fertilization and Embryo Authority (HFEA) reported that the

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LBR was 21–28% in 2016 [2]. In the Unites States of America (USA), the pregnancy rate and LBR were about 39% and 32%, respectively [3]. These substantial rates indicate that IVF failures are still matters of great concern. Failures could be caused by many different factors including inappropriate ovarian stimulation, poor embryo quality, and implantation failure. Recurrent implantation failure (RIF) is one of the most common intractable reproductive disorders observed with assisted reproductive technology (ART) [4]. Since its potential pathogenesis is poorly understood and the clinical features are complex, there are no unanimously agreed upon definitions of RIF in terms of the number of implantation failures or the quality and total number of transferred embryos that do not implant successfully. In the European Society of Human Reproduction and Embryology (ESHRE) preimplantation genetic diagnosis (PGD) consortium document, it is stated that RIF is consistent with more than three high-quality embryo transfers (ETs) or implantation failures after a cumulative transfer of more than ten good embryos [5]. Polanski's systematic review defined RIF as the absence of implantation after two consecutive cycles of IVF, intracytoplasmic sperm injection (ICSI), or frozen embryo replacement cycles, wherein the cumulative number of transferred embryos was no less than four cleavage-stage embryos or no less than two blastocysts, with all embryos being of good quality and of appropriate developmental stage [6].

To achieve a successful implantation, functional embryos and a receptive endometrium are indispensable. More importantly, synchronizing and coordinating crosstalk between the embryo and the maternal endometrium is crucial in this intricate process. This synchronization and coordination effort includes timely development of maternal tolerance to the embryo and an appropriate level of invasiveness [7]. The window of implantation is presented in a hypothesis that defines the 6–10-day period after ovulation as the optimal timeframe for the period of receptivity. An asynchrony of greater than 3.0 ± 1.5 days results in infertility [8, 9]. To improve the outcomes in women with RIF, various treatments, including peripheral blood mononuclear cells' (PBMCs) instillation have been studied. Because of the proportional difference in the visible components of peripheral blood, PBMCs (isolated using Ficoll–Hypaque centrifugation) consist primarily of mononuclear macrophages (10–30%), lymphocytes (70–90%), dendritic cells (1–2%), and a few other cell types. Early in 2002, the research of Egawa et al. showed that PBMCs can improve BeWo cell invasion in co-culture with the endometrium by secreting more soluble factors [10]. It was later reported that PBMCs could ameliorate endometrial receptivity by enhancing interactions between human endometrial epithelial cells [11]. Subsequent to these reports, a series of randomized controlled trials (RCTs) [12, 13], case–control trials [14], and case reports [15, 16] were conducted on this subject. The

published studies reported inconsistent effects of PBMCs' instillation in patients with RIF. Hence, it became necessary to conduct a persuasive meta-analysis that stratified patients according to the number of previous implantation failures. The aim of our study was to evaluate the safety and benefit of PBMC instillation among patients with RIF and to provide theoretical basis for selection of appropriate RIF patients for autologous PBMC intrauterine instillation. Therefore, in an attempt to collect all current studies on the topic, the present research included studies of patients with at least one implantation failure. To the best of our knowledge, this is the first meta-analysis to explore whether PBMCs instillation could improve pregnancy outcomes among patients with RIF.

Materials and methods

Literature search

In this systematic review, the following electronic databases were searched by two authors (YW and LFL.): PubMed, EMBASE, the Cochrane library, China Biomedicine (CBM), China National Knowledge Infrastructure (CNKI), China Wan Fang data, and the VIP Database for Chinese Technical Periodicals (VIP). The following keywords and/or medical subject heading terminology were used: recurrent implantation failure, repeat implantation failure, RIF, peripheral blood mononuclear cells, peripheral blood monocytes, peripheral monocytes, and PBMCs. We examined cited articles in the reference lists of all known primary and review articles to identify additional eligible studies that may not have been not captured by the electronic searches. The last retrieval of data was in April 2018. The selection process is reported in a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) flow diagram.

Ethical considerations

There were no human or animal participants directly participating in this review.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) studies focused on treatment with PBMCs compared with placebo or no adjuvant treatment among women with RIF undergoing ART (fresh or frozen cycles), (2) studies in which the patients had experienced at least one implantation failure (so chosen because the current definition of RIF is ambiguous), (3) RCTs or prospective non-randomized studies, and (4) studies

wherein PBMCs were instilled into the uterine cavities of patients from the study group.

The exclusion criteria were as follows: (1) a lack of raw data collection in case or control groups, (2) additional interventions imposed, and (3) duplicate studies, (4) master's or doctoral theses that were not published in a peer-reviewed journal.

Data extraction

The included RCTs were assessed for methodological quality using the Cochrane risk of bias tool [17], and the non-randomized prospective studies were assessed for quality using the Newcastle–Ottawa Quality Assessment Scales [18]. For each eligible publication, the following information was obtained: author, year of publication, study design, case and control participants, average age, length of infertility, number of previous implantation failures, number of embryos transferred, and interventions. The outcome measures were: clinical pregnancy rate (CPR), implantation rate (IR), LBR, miscarriage rate (MR), and endometrial thickness on the day of ET. Two authors (YW and LFL) independently performed the study selection and data extraction. Where there was a lack of information, the corresponding authors were contacted for further details. After independent assessments of the manuscripts, any disagreement between the two reviewers was resolved in consultation with a third reviewer (PJY).

Statistical analysis

The primary outcomes were CPR, IR, and LBR. The secondary outcomes were MR and endometrial thickness on ET day. CPR was defined as gestational sac and fetal heart activity seen on transvaginal ultrasound scan after 2 weeks of gestation, per woman. IR was defined as the number of sacs seen divided by the number of embryos transferred. LBR was defined as pregnancy that attained at least 25 weeks of gestational age per woman. MR was defined as the loss of clinical pregnancy after confirmation of clinical pregnancy per woman.

The effect estimate was expressed as an odds ratio (OR) with a 95% confidence interval (CI). For continuous data, the mean difference and 95% CI were calculated. When the same outcome was measured using different scoring scales, we used the weighted mean difference (WMD) with 95% CI. When the I^2 statistic showed heterogeneity of 0%, a fixed-effect model was used, and when the I^2 statistic showed heterogeneity of $> 0\%$, a random effects model was applied. Statistical heterogeneity was examined using a visual inspection of the forest plot and the I^2 statistic was used to describe the percentage of variation across the included studies due to heterogeneity instead of random chance. We performed a subgroup analysis to explore the source of heterogeneity according to the number of implantation failures. A further sensitivity analysis was performed to investigate the clinical and methodological variations in the studies. All statistical tests were performed using Stata 12.0 (Stata Corporation, College Station, TX, USA).
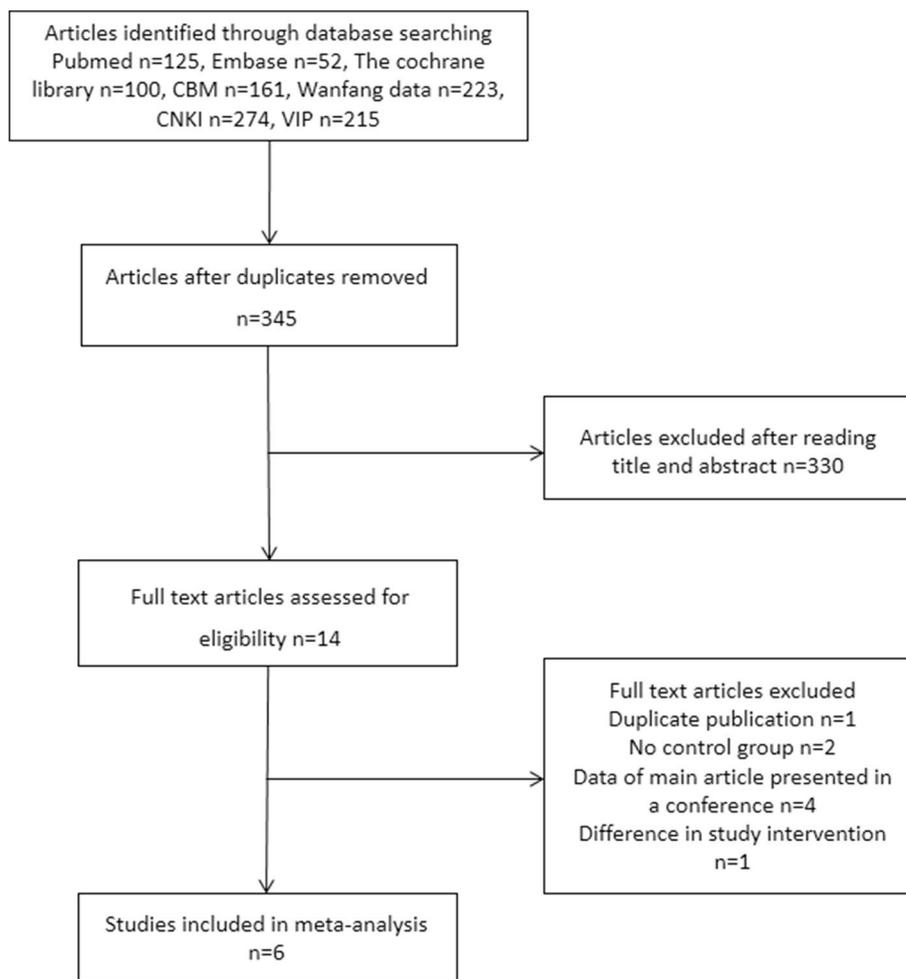
Results

The literature search process and selection of studies for the meta-analysis are shown in Fig. 1. A total of 345 potentially relevant studies were systematically identified. After reviewing the titles and abstracts, 330 studies were excluded, and we obtained full manuscripts for the remaining 14 studies. According to the inclusion and exclusion criteria, eight studies were excluded for the following reasons: two did not have a control group [14, 19], the main article data of four studies were presented in a conference [20–23], one was a duplicate [24], and two had differences in study interventions [25, 26]. Ultimately, six studies of 1361 patients were included in our analysis [12, 13, 27–30]. The patient characteristics included in the analysis are summarized in Tables 1 and 2. In addition, we performed a stratified analysis to explore the effect of PBMCs in patients with different numbers of implantation failures. In all eligible studies, we classified patients with at least one previous implantation failure into one group, and greater than or equal to three failures into another group. All of the results are shown in Tables 3 and 4. The risk of bias summary for RCTs is shown in Fig. 2 and that for non-randomized prospective trials is shown in Table 5. We did not develop a funnel plot to detect publication bias since the number of trials was less than 10.

Study quality assessment

Studies from 2006 onwards were included in this meta-analysis; however, they were especially clustered over 2011–2017 as intrauterine perfusion of autogenous PBMCs is a relatively new adjuvant therapy and has gained popularity in this decade. The quality of the three non-RCTs [27–29] was assessed based on the Newcastle–Ottawa scale that ranges between 0–9 and evaluates 8 aspects of trials. The three non-RCTs received a good score, suggesting a low risk of bias. The follow-up duration of every study was long enough, and the studies reported a major outcome indicator of LBR. However, the design of the control group was flawed in three non-RCTs: the intrauterine perfusion saline or the culture medium was not administered to the patients in the control group. In the study by Yoshika et al., the sample

Fig. 1 Study selection process for the systematic review


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┌─────────────────────────────────────────────┐
│ Articles identified through database searching│
│ Pubmed n=125, Embase n=52, The cochrane       │
│ library n=100, CBM n=161, Wanfang data n=223, │
│ CNKI n=274, VIP n=215                          │
└─────────────────────────────────────────────┘

┌─────────────────────────────────────────────┐
│ Articles after duplicates removed             │
│ n=345                                          │
└─────────────────────────────────────────────┘
                    │
                    │──────────────►┌──────────────────────────┐
                    │               │ Articles excluded after reading│
                    │               │ title and abstract n=330  │
                    │               └──────────────────────────┘

┌─────────────────────────────────────────────┐
│ Full text articles assessed for               │
│ eligibility n=14                               │
└─────────────────────────────────────────────┘
                    │
                    │──────────────►┌──────────────────────────┐
                    │               │ Full text articles excluded │
                    │               │ Duplicate publication n=1 │
                    │               │ No control group n=2      │
                    │               │ Data of main article presented in│
                    │               │ a conference n=4          │
                    │               │ Difference in study intervention│
                    │               │ n=1                       │
                    │               └──────────────────────────┘

┌─────────────────────────────────────────────┐
│ Studies included in meta-analysis             │
│ n=6                                            │
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size was too small to be representative. The quality of the three RCTs [13, 24, 30] was evaluated using the Cochrane risk of bias tool. All three RCTs were at low risk of selective reporting, other bias, or attrition bias. In contrast, allocation concealment was unclear in every RCT; none of the RCTs explicitly stated blinding implementation and whether the individuals assessing the outcome were blinded to the trial or not. All these risks of bias have a potential impact on the results. Thus, we must explain the results with caution.

Clinical pregnancy rate (CPR)

The impact of PBMCs on CPR was estimated in six studies. In the three RCTs trials, the fixed-effects model showed a higher CPR in the PBMCs group (48.00%, 72/150) compared to the control group (19.88%, 33/166) ($P=0$; OR 2.45, 95% CI 1.53–3.91, $I^2=0\%$, Fig. 3a). In the subgroup analysis, PBMCs treatment increased the CPR ($P=0.002$; $P=0.024$, Fig. 3a). In the three non-RCTs, the random-effects model was used, and no significant differences between the case and control groups were identified (OR

1.33, 95% CI 0.97–1.81, $I^2=14.7\%$, Fig. 4a). In the subgroup analysis, treatment with PBMCs increased the CPR among patients in the group that underwent three or more IVF cycles ($P=0.009$; OR 2.83, 95% CI 1.29–6.22, $I^2=0\%$, Fig. 4a).

Implantation rate (IR)

The impact of PBMCs on IR was estimated in seven studies. In the four RCTs, the fixed-effects model showed a higher IR in the PBMCs group (23.94%, 57/238) than in the control group (10.12%, 25/247) ($P=0$; OR 2.46, 95% CI 1.48–4.09, $I^2=0\%$, Fig. 3b). The subgroup analysis demonstrated PBMCs treatment increased the IR ($P=0.003$; $P=0.010$, Fig. 3b). In the three non-RCTs, the random-effects model was used, and no significant differences were found between the case and control groups (OR 1.58, 95% CI 0.92–2.70, $I^2=62.8\%$, Fig. 4b). In the subgroup analysis, PBMCs treatment increased the IR in the group that underwent three or more IVF cycles ($P=0.001$; OR 3.74, 95% CI 1.71–8.19, $I^2=0\%$, Fig. 4b).

Table 1 Characteristics of the RCTs included in the systematic review

Trial	Setting	Design	Participant characteristics		Inclusion and exclusion criteria	Control	Outcomes analysed	Intervention
			Cases	Controls				
Nan Yu 2016 No. of RIF ≥ 3	China	Randomized controlled trial	No. of participant: 93, age: 31.08 ± 3.95 years, infertile time: 4.42 ± 3.50 years, no. of implantation failures: 3.36 ± 1.28	No. of participant: 105, age: 31.22 ± 5.12 years, infertile time: 4.00 ± 2.52 years, no. of implantation failures: 3.44 ± 2.12	IC*: age < 35 years, ≥ 3 implantation failures; EC*: poor ovarian reserve, chromosomal abnormalities, hydrosalpinx, dysontogenesis, Asherman syndrome, severe endometriosis, endometrial organic disease	No treatment	Clinical pregnancy rate, endometrial thickness on day of ET, implantation rate, miscarriage rate, live birth rate	PBMCs were isolated by Ficoll–Hypaque centrifugation as described in Hashii' study*, then incubated with HCG for 24 h. On day 2 of oocyte retrival, PBMCs were administrated to the cavity
Madkour 2015 1 No. of RIF ≥ 2	Morocoo	Randomized controlled trial	No. of participant: 27, age: 34.74 ± 4.17 years, no. of implantation failures: 3.19 ± 1.75	No. of participant: 27, age: 34.44 ± 3.86 years, no. of implantation failures: 3.63 ± 1.76	IC*: ≥ 2 implantation failures, primary infertility, BMI < 30, endometrial thickness < 6 mm in ovation induction, regular menstrual cycles, age < 40 years, infectious negative balance; EC*: polycystic ovary syndrome and uterine pathology	No treatment	Clinical pregnancy rate, miscarriage rate, implantation rate	PBMCs were isolated by Ficoll–Hypaque centrifugation, then incubated with HMG for 72 h. Two day before embyro transfer, PBMCs were administrated to the cavity
Madkour 2015 2 No. of RIF ≥ 3			No. of participant: 10, age: 37.20 ± 2.57 years, no. of implantation failures: 4.50 ± 1.96	No. of participant: 17, age: 34.65 ± 3.76 years, no. of implantation failures: 4.59 ± 1.54				

Table 1 (continued)

Trial	Setting	Design	Participant characteristics		Inclusion and exclusion criteria	Control	Outcomes analysed	Intervention
			Cases	Controls				
Qi Lin 2013 No. of RIF≥2	China	Randomized controlled trial	No. of participant: 20, age: 33.0±4.6 years, no. of implantation failures: 4.9±1.7, no. of embryos transferred: 2.4±0.6	No. of participant: 17, age: 34.7±3.0 years, no. of implantation failures: 4.6±1.5, no. of embryos transferred: 2.8±0.4	IC*: ≥2 implantation failures and cumulative nubmer of transferred embryo ≥ 10 or ≥3 implantation failures and good ambryo transfered per cycle; EC*: FSH ≥15 IU/L, hydrosalpinx chromosomal abnormalities, presence of antibodies or uterine organic disease	No treatment	Clinical pregnancy rate, endometrial thickness on day of ET, implantation rate, live birth rate	PBMCs were isolated by Ficoll–Hypaque centifugation as described in Hashii' study* Three days before Frozen/thawed embyro transfer, PBMCs were administrated to the cavity

The values of age, infertile time, number of embryos transferred and endometrial thickness on day of ET are expressed as mean ± standard deviation; Hashii' study*: Ref. [38]

No. number, *IC** inclusion criterion, *EC** exclusion criterion, *RCTs* randomized controlled trials

Table 2 Characteristics of non-RCTs included in the systematic review

Trial	Setting	Design	Participant characteristics		Inclusion and exclusion criteria	Control	Outcomes analysed	Intervention	Score*
			Cases	Controls					
Saijiao Li 2017 1 No. of RIF≥1	China	Prospective cohort study	No. of participant: 294, age: 30.83+4.10 years, infertile time: 4.68+3.31 years, endometrial thickness on day of ET: 8.5+1.4 mm, no. of embryos transferred: 2.07+0.47	No. of participant: 339, age: 30.51+4.08 years, infertile time: 4.61+3.29 years, endometrial thickness on day of ET: 8.2+1.3 mm, no. of embryos transferred: 2.18+0.38	IC*: ≥1 implantation failures; EC*: poor ovarian reserve, thin endometrium, chromosomal abnormalities, presence of antibodies or mutatious involving parts of the coagulation system	No treatment	Clinical pregnancy rate, implantation rate, live birth rate	PBMCs were isolated by Ficoll–Hypaque centifugation as described in Hashii' study*, then incubated with HCG for 24 h. One day before frozen/ thawed embyro transfer, PBMCs were administrated to the cavity	9
Saijiao Li 2017 2 No. of RIF≥4			No. of participant: 48, age: 31.31+4.53 years, infertile time: 5.13+3.69 years, endometrial thickness on day of ET: 7.9+1.1 mm, no. of embryos transferred: 2.08+0.40	No. of participant: 21, age: 30.10+3.15 years, infertile time: 5.50+4.65 years, endometrial thickness on day of ET: 7.9+1.6 mm, no. of embryos transferred: 1.95+0.22					
Okitsu 2011 1 No. of RIF≥1	Japan	Prospective cohort study	No. of participant: 83, age: 35.7±5.01 years, no. of implantation failures: 2.07±2.12, no. of embryos transferred: 1.61±0.62	No. of participant: 170, age: 35.7±4.67 years, no. of implantation failures: 1.95±1.51, no. of embryos transferred: 1.56±0.59	IC*: ≥1 implantation failures; EC*: poor ovarian reserve, chromosomal abnormalities, presence of antibodies or mutatious involving parts of the coagulation system	No treatment	Clinical pregnancy rate, implantation rate, live birth rate	PBMCs were isolated by Ficoll–Hypaque centifugation as described in Hashii' study*. On day 1 of the embyro at the early cleavage stage or on day 2 of the embyro at the blastocyst stage, PBMCs were administrated to the cavity	9
Okitsu 2011 2 No. of RIF≥3			No. of participant: 19, age: 37.4±5.33 years, no. of implantation failures: 4.47±3.42, no. of embryos transferred: 1.79±0.79	No. of participant: 36, age: 38.3±4.20 years, no. of implantation failures: 4.31±1.73, no. of embryos transferred: 1.78±0.59					

Table 2 (continued)

Trial	Setting	Design	Participant characteristics		Inclusion and exclusion criteria	Control	Outcomes analysed	Intervention	Score*
			Cases	Controls					
Yoshioka 2006 No. of RIF≥4	Japan	Prospective cohort study	No. of participant: 17, age: 37.5 ± 4.4 years, no. of embryos transferred: 2.76 ± 0.56, endometrial thickness on day of oocyte retrieval: 9.59 + 1.47 mm	No. of participant: 18, age: 36.6 ± 4.4 years, no. of embryos transferred: 2.72 ± 0.58, endometrial thickness on day of oocyte retrieval: 10.7 + 1.97 mm	IC*: ≥4 implantation failures, FSH < 15 mIU/ml; EC*: poor ovarian reserve	No treatment	Clinical pregnancy rate, implantation rate, live birth rate	PBMCs were isolated by Ficoll–Hypaque centifugation as described in Hashii' study*, then incubated with HCG for 48 h, 2 days later, fresh PBMCs were obtained and combined with 2-day cultured PBMCs. On day 2 of oocyte retrival, PBMCs were administrated to the cavity	9

The values of age, infertile time, number of embryos transferred and endometrial thickness on day of ET are expressed as mean ± standard deviation; Hashii' study*: Ref. [38]

No. number, *IC** inclusion criterion, *EC** exclusion criterion, *Score** Newcastle–Ottawa Quality Assessment Scale Score, *non-RCTs* non-randomized controlled trials

Table 3 Comparing PBMCs treatment versus control/placebo in RCTs

Outcomes	Subgroup analysis	Analysis mode	Positive/total in case group	Positive/total in control group	OR/WMD	95% CI	I^2 (%)	P
Clinical pregnancy rate	Overall	Fixed	48% (72/150)	19.88% (33/166)	2.45	1.53, 3.91	0%	0.000
	≥ 1 cycles	Fixed	46.8% (22/47)	15.9% (7/44)	2.93	1.14, 7.53	0%	0.024
	≥ 3 cycles	Fixed	48.5/% (50/103)	21.3% (26/122)	2.3	1.34, 3.95	0%	0.002
Implantation rate	Overall	Fixed	23.94% (57/238)	10.12% (25/247)	2.46	1.48, 4.09	0%	0.000
	≥ 1 cycles	Fixed	22.1% (27/122)	7.6% (8/105)	2.91	1.27, 6.69	0%	0.010
	≥ 3 cycles	Fixed	25.9% (30/116)	12.0% (17/142)	2.19	1.15, 4.18	0%	0.016
Live birth rate	Overall	Fixed	36.28% (41/113)	14.75% (18/122)	2.43	1.32, 4.49	0%	0.004
	≥ 1 cycles	Fixed	45% (9/20)	17.6% (3/17)	2.55	0.59, 10.96	–	0.204
	≥ 3 cycles	Fixed	34.4% (32/93)	14.3% (15/105)	2.41	1.23, 4.72	–	0.009
Miscarriage rate	Overall	Fixed	19.35% (12/62)	43.33% (13/30)	0.45	0.18, 1.11	0%	0.083
	≥ 1 cycles	Fixed	16.7% (2/12)	75% (3/4)	0.22	0.03, 1.85	–	0.157
	≥ 3 cycles	Fixed	20% (10/50)	38.5% (10/26)	0.53	0.19, 1.44	0%	0.216
Endometrium thickness on ET day	Overall	Fixed	–	–	− 0.36	− 0.75, 0.03	0%	0.073
	≥ 1 cycles	fixed	–	–	0.00	− 1.22, 1.22	–	1.000
	≥ 3 cycles	Fixed	–	–	− 0.40	− 0.81, 0.01	–	0.059

OR odds ratio, *WMD* weighted mean difference, *ET* embryo transfer, *CI* confidence interval, *PBMCs* peripheral blood mononuclear cell, *RCTs* randomized controlled trials

Table 4 Comparing PBMCs treatment versus control/placebo in non-RCTs

Outcomes	Subgroup analysis	Analysis mode	Positive/total in case group	Positive/total in control group	OR/WMD	95% CI	I^2 (%)	P
Clinical pregnancy rate	Overall	Random	44% (203/461)	34.2% (200/584)	1.33	0.97, 1.81	14.7	0.074
	≥ 1 cycles	Random	44.8% (169/377)	37.1% (189/509)	1.18	0.92, 1.51	0	0.201
	≥ 3 cycles	Random	40.5% (34/84)	14.7% (11/75)	2.83	1.29, 6.22	0	0.009
Implantation rate	Overall	Random	26.3% (236/898)	23.4% (257/1097)	1.58	0.92, 2.70	62.8	0.095
	≥ 1 cycles	Random	27.1% (195/719)	26.2% (247/943)	1.01	0.82, 1.25	0	0.928
	≥ 3 cycles	Random	22.9% (41/179)	6.5% (10/154)	3.74	1.71, 8.19	0	0.001
Live birth rate	Overall	Random	30.6% (141/461)	25.9% (151/584)	1.23	0.82, 1.87	23.60	0.318
	≥ 1 cycles	Random	30.5% (115/377)	28.3% (144/509)	1.04	0.78, 1.37	0	0.811
	≥ 3 cycles	Random	31.0% (26/84)	9.3% (7/75)	3.03	1.15, 7.98	0	0.025

OR odds ratio, *WMD* weighted mean difference, *ET* embryo transfer, *CI* confidence interval, *PBMCs* peripheral blood mononuclear cell, *non-RCTs* non-randomized controlled trials

Live birth rate (LBR)

Five studies were included in the comparison of LBR. In two RCTs, the fixed-effects model showed a higher LBR in the PBMCs group (36.28%, 41/113) than in the control group (14.75%, 18/122) ($P = 0.004$; OR 2.43, 95% CI 1.32–4.49, $I^2 = 0$%, Fig. 3c). In the subgroup analysis, there was only one study in each subgroup. PBMCs treatment increased the LBR in the group that underwent three or more IVF cycles ($P = 0.009$; OR 2.41, 95% CI 1.23–4.72, Fig. 3c). In the three non-RCTs, the random-effects model was used, and no significant differences were found between the case and control groups (OR 1.23, 95% CI 0.82–1.87, $I^2 = 23.6$%, Fig. 4c). In

the subgroup analysis, the PBMCs treatment increased the LBR in the group that underwent three or more IVF cycles ($P = 0.025$; OR 3.03, 95% CI 1.15–7.98, $I^2 = 0$%, Fig. 4c).

Miscarriage rate (MR)

Three studies were included in the comparison of MR. Miscarriages occurred in 12 of 62 (19.35%) events in the PBMCs group and in 13 of 30 (43.33%) events in the control group. There were no significant differences between the two groups (OR 0.45, 95% CI 0.18–1.11, $I^2 = 0$%, Fig. 3d) in the fixed-effects model. The subgroup analysis showed no significant differences.

Fig. 2 Risk of bias summary for the randomized controlled trials

could potentially benefit from PBMCs instillation. Higher CPR, IR, and LBR, despite the absence of an apparent benefit in increasing endometrial thickness following PBMCs perfusion, suggests the possibility that an immunological mechanism aiding embryo implantation may be responsible for the improved rates, rather than an increase in endometrial thickness. The mechanism behind autologous PBMCs treatments is complex and not entirely clear, but it is probably related to an upregulation of endometrial receptivity, thus enhancing embryo invasion and increasing "cross-talk" at the maternal–fetal interface.

PBMCs could coordinate the entire implantation process and maintain the embryo through local immune cells and hormonal induction of endometrial receptivity in the implantation micro-environment. The physiological balance between T-helper 1 (Th1) and T-helper 2 (Th2) cells is very important for embryo implantation and maintenance of early pregnancy [31, 32]. The ratio of Th1/Th2 cytokines in the peripheral blood of patients with RIF is significantly higher compared with that of normal women [33, 34], and successful pregnancy is related to a predominance of Th2 cytokines. The Th1 cytokine profile is actually detrimental to pregnancy [35]. PBMCs could facilitate the secretion of Th2 type cytokines, such as, IL-4, IL-6 and IL-10

Table 5 Appraisal of methodological quality (Newcastle–Ottawa Scale) of non-randomized controlled trials

Study	Case-cohort representative	Selection of non-explosed control	Assessment of explosure	Outcome negative at start	Comparability by design	Comparability by analysis	Outcome assessment	Duration of follow-up	Score
Saijiao Li 2017	*	*	*	*	×	*	*	*	8
Okitsu 2011	*	*	*	*	×	*	*	*	8
Yoshioka 2006	×	*	*	*	×	*	*	*	7

Endometrial thickness on ET day

Three studies were included in the comparison of the endometrial thickness on ET day. The differences between the PBMCs and control groups did not reach statistical significance for the endometrial thickness on ET day. The WMD was -0.36 (95% CI -0.75 to 0.03, $I^2 = 0\%$, Fig. 3e) in the fixed-effects model. In the subgroup analysis, there were no significant differences between the case and control groups.

Discussion

Interpretation

The aforementioned evidence suggests that autologous PBMCs could play a role in improving implantation among patients with RIF. It would appear that patients with RIF

[36]. Moreover, IL-4 and IL-10 could promote progesterone production in the luteal phase of early pregnancy, which suggests that PBMCs enhance the function and differentiation of the corpus luteum via Th-2 type cytokines, which leads to a successful pregnancy. Hashii et al. testified to this theory [37]. Progesterone induces the estrogen-primed endometrium to become suitable for embryo implantation [38]. Furthermore, PBMCs can boost endometrial receptivity by increasing the concentration of leukemia inhibitory factor (LIF) and integrinαVβ3 in endometrial cells in vitro, and may also enhance the expression of the LIF receptor on the embryo under co-culture with the endometrium [39].

PBMCs can initiate and improve invasion and establish hemochorial placentation, while simultaneously regulating the immune system's tolerance for the embryo at the time of implantation [40, 41]. PBMCs notably increased the expression of matrix metalloproteinase-2 (MMP-2),
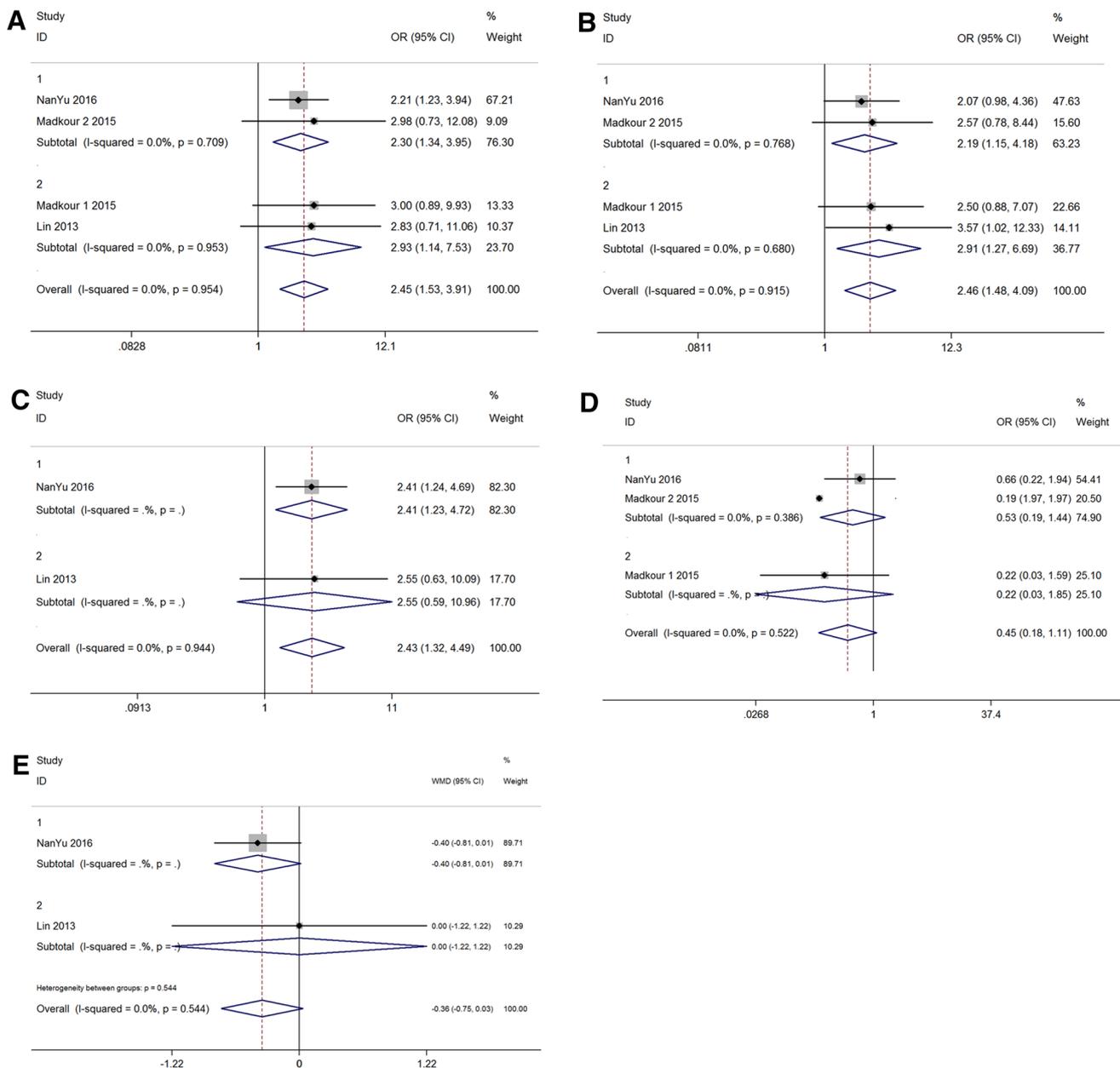
Fig. 3 Forest plot for pregnancy outcomes in women with RIF of the PBMCs versus control/placebo groups in randomized controlled trials, including the clinical pregnancy rate (**a**), implantation rate (**b**), live birth rate (**c**), miscarriage rate (**d**), endometrial thickness on ET day (**e**). *ID* identification, *OR* odds ratio, *CI* confidence interval, *WMD* the weighted mean difference

MMP-9, and vascular endothelial growth factor (VEGF) and decreased the expression levels of tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2. MMP-2 and MMP-9 are the key enzymes in trophoblast invasion leading to successful implantation. TIMP-1 and TIMP-2 are considered to be MMP inhibitors [42, 43]. VEGF not only stimulates angiogenesis, but also regulates trophoblast differentiation and invasion [44, 45]. MMP-2, MMP-9, and VEGF work together to regulate trophoblastic invasion via microRNAs, such as miR-346 and miR-583-3p [46]. Human chorionic

gonadotropin (HCG) might be an important signaling factor that activates PBMCs to facilitate embryo invasion and endometrial receptivity by altering PBMC functions. Moreover, it could enhance this effect immensely by increasing the production of chemo-attractive factors [12]. Circulating mononuclear cells derived from early pregnant women were shown to enhance trophectoderm invasion of the murine embryo. After PBMCs derived from non-pregnant women were co-cultured with hCG, the production of chemo-attractive factors increased to promote murine embryo and BeWo
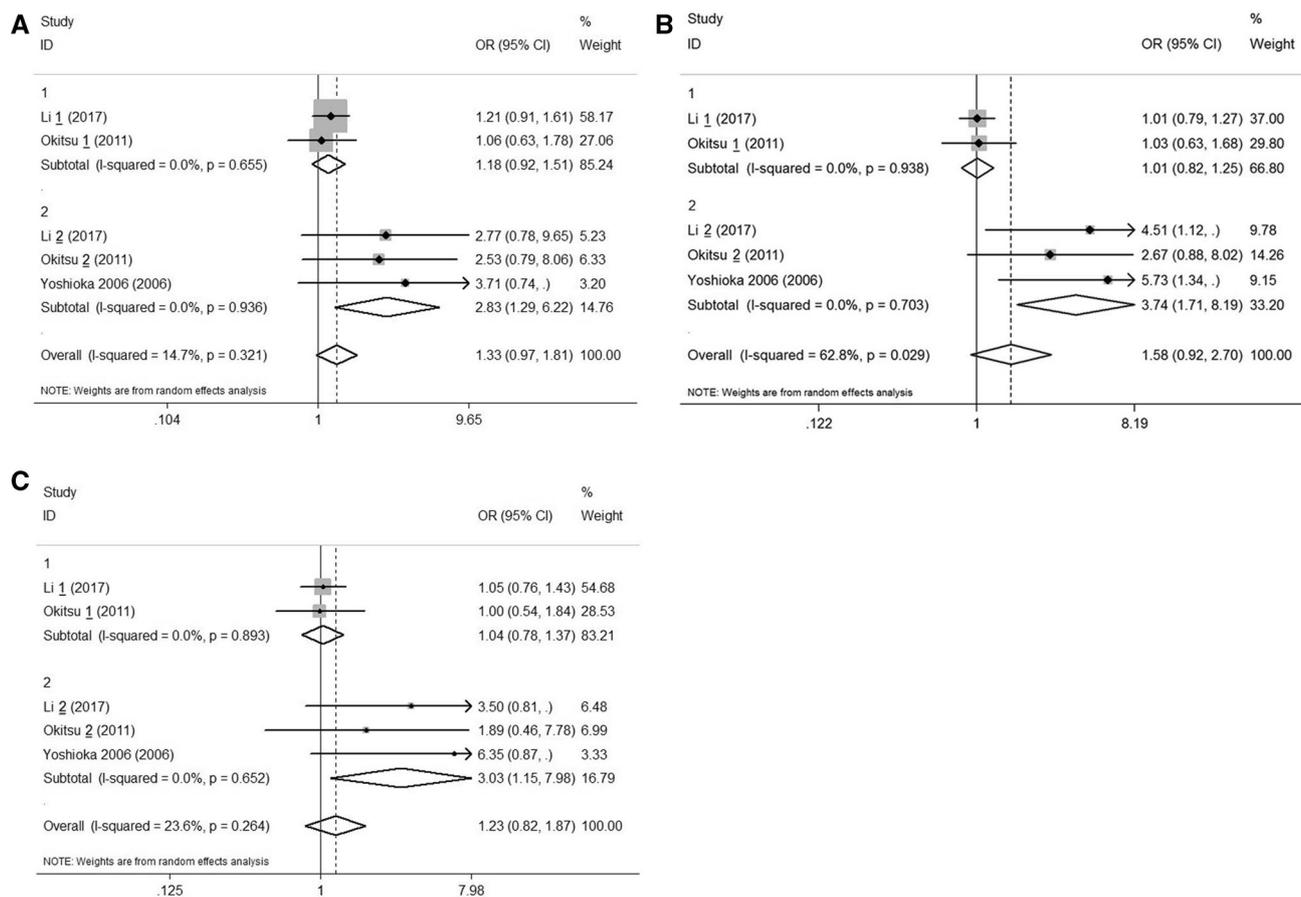
Fig. 4 Forest plot for pregnancy outcomes in women with RIF of the PBMCs versus control/placebo groups in non-randomized controlled trials, including the clinical pregnancy rate (**a**), implantation rate (**b**), live birth rate (**c**). *ID* identification, *OR* odds ratio, *CI* confidence interval

cell invasion [10, 47]. The study by NanYu et al. showed that PBMC activated by hCG could significantly stimulate trophoblast invasion; however, hCG only or PBMC only did not show similar results [42]. However, intrauterine perfusion of HCG is an effective adjunctive treatment in patients with RIF, we should not exclude the direct effect of hCG on embryo implantation.

Our results of subgroup analysis confirm that PBMCs' instillation could improve pregnancy outcomes including IR, CPR, and LBR among those patients with three or more implantation failures. However, we did not identify a consistent conclusion among patients with at least one implantation failure in a statistical heterogeneity analysis. This may be because there were only a few eligible studies, and they all provided insufficient data. Another reason may be that the immunological dysfunction of the uterine cavity micro-environment was not severe in patients with one or two implantation failures. In those cases, the therapeutic effect of PBMCs instillation was not obvious. Owing to the financial pressures and clinical heterogeneity among patients with RIF, it is suggested that we should select appropriate patients for

treatment with PBMC. The optimal time to consider PBMC instillation might be after two implantation failures.

Strengths and weakness

Our analysis had the following advantages. Although PBMCs are increasingly being used in clinical practice, ours, to the best of our knowledge, is the first meta-analysis exploring whether this therapy is curative, and which groups of patients with RIF will have the most effective therapeutic benefit. Moreover, we conducted an extensive search strategy, as well as used valid and cautious data synthesis methods. If the I^2 statistic showed heterogeneity of more than 0%, a random effects model was applied. We used different quality evaluation tools for different types of studies, the Newcastle–Ottawa Quality Assessment Scale was used to rate the quality of the non-RCTs, and the Cochrane collaboration tool for assessing the risk of bias was used to rate the quality of the eligible RCTs.

There were also some limitations to our meta-analysis. First, there is no worldwide-accepted definition of RIF. This

might have led to our overlooking eligible studies despite the extensive literature search. Another major limitation of our study is the small sample size of patients and the imperfect control. The small sample size included in this study restricted the generalizability of our findings. The procedure for the preparation and intrauterine administration of PBMCs has not been standardized (i.e., the timing and number of blood samples needed, the PBMCs incubation time with HCG, the occasion for administration into the uterine cavity, and so on) and may have differed among the included studies. Additionally, the therapeutic schedules for IVF or ICSI were different, as were the types, dosages, and durations of drugs used, and the class and number of embryos transferred. Thus, implementation and measurement biases were inevitable.

Conclusion

The results of this meta-analysis showed that among patients with three or more implantation failures, PBMCs' instillation as an adjunct to IVF treatment significantly improved IR, LBR, and CPR. Although the uterine instillation of PBMCs in patients with RIF is described both locally and globally, detailed assessment of the perinatal and neonatal outcomes should be considered in the future. Hence, more adequately powered trials with ethical approval are warranted to explore the effects of PBMC instillation in patients with RIF. These future studies would be most beneficial if they could be multi-centered and use standardized criteria to define RIF; meanwhile, a control group treated with placebo would be important and essential.

Author contributions YW, LFL, and XHZ conceived and designed the study. YW, LFL, and XY analysed the data. YW and LL wrote the "Introduction and Discussion". YW and PJY wrote the "Materials and methods" and "Results". KHY and XHZ provided significant advice for the manuscript. YW and XY were primarily responsible for writing and modifying the draft.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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