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Optimized gene-enhanced ERA improves in vitro fertilization outcomes in patients with repeated implantation failure

A meta-analysis

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Abstract

Background: Assisted reproductive technology still presents a significant challenge when it comes to repeated implantation failure (RIF). To address this issue, personalized embryo transfer (pET) has been suggested as a potential solution. pET involves guiding the embryo transfer process based on an analysis of endometrial receptivity, known as endometrial receptivity analysis (ERA). However, the clinical value of this approach remains uncertain.

Methods: We conducted a comprehensive analysis of 14 studies pertaining to pET guided by ERA in patients with RIF. The outcomes evaluated in these studies encompassed the clinical pregnancy rate (CPR), implantation rate, and live birth rate (LBR). In addition, we performed subgroup analysis by considering different definitions of RIF and utilizing various ERA techniques, such as traditional and optimized gene-enhanced approaches.

Results: In general, the utilization of ERA-guided pET did not have a substantial impact on CPR (relative risk [RR], 1.25 [95% CI, 0.85–1.84]), implantation rate (RR, 1.59 [95% CI, 0.89–2.82]), or LBR (RR, 1.55 [95% CI, 0.96–2.50]) compared with standard transfer. However, the implementation of optimized gene-enhanced ERA methods demonstrated significant enhancements in CPR (RR, 2.04 [95% CI, 1.53–2.72]) and LBR (RR, 2.61 [95% CI, 1.58–4.31]).

Conclusion: While ERA-guided pET shows limited efficacy in improving pregnancy outcomes in patients with RIF, there lies potential in optimizing gene-enhanced ERA techniques to augment both clinical pregnancy rates and LBRs.

Abbreviations: CPR = clinical pregnancy rate, CRP = cumulative pregnancy rate, ERA = endometrial receptivity analysis, ET = embryo transfer, IR = implantation rate, IVF = in vitro fertilization, LBR = live birth rate, pET = personalized embryo transfer, PGT = preimplantation genetic testing, PR = pregnancy rate, RIF = repeated implantation failure, RR = relative risk, rsERT = RNA-Seq-based endometrial receptivity test.

Key Words: clinical pregnancy rate, endometrial receptivity analysis, personalized embryo transfer, repeated implantation failure, window of implantation

1. Introduction

Assisted reproductive technology has proven to be highly beneficial for individuals dealing with infertility. However, a significant portion (≈5%–10%) of patients still face the challenge of repeated implantation failure (RIF).[1] In clinical practice, RIF is generally defined as the failure of ≥2 embryo transfers (ETs), involving at least 2 high-quality embryos.[2,3] It is important to note that there is no universally accepted formal definition for RIF. Several potential factors have been identified in

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The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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the pathogenesis of RIF, including maternal age, uterine factors, adnexal lesions, thrombophilia, immune factors, and unexplained RIF.[4,5] While preimplantation genetic testing (PGT) has emerged as a valuable tool to improve clinical pregnancy outcomes by identifying and eliminating noneuploid embryos, it is worth mentioning that ≈1/3 of transfers still fail to result in successful implantation.[6] Therefore, the focus has shifted toward understanding and enhancing endometrial receptivity during the window of implantation to improve embryo implantation rates (IRs).[7]

Endometrial receptivity analysis (ERA) is a technique that utilizes a gene-expression microarray to evaluate the expression of 238 different genes associated with endometrial receptivity.[8,9] This analysis, coupled with an algorithm, allows for the identification of the window of implantation by assessing progesterone exposure. This enables personalized embryo transfer (pET) to take place at the optimal time.[10–12] The impact and efficacy of ERA on ET outcomes have been debated for many years, with increasing numbers of studies questioning its routine use in patients with good prognosis for infertility.[13–15] While it is believed that ERA can be used to detect patients with unexplained implantation failure or RIF, there is still a lack of extensive investigation and consensus on this matter. Therefore, it is crucial to gather robust real-world evidence in order to fully understand the relevance of ERA in populations with RIF.

The efficacy of ERA in improving pregnancy and birth outcomes has been a topic of debate in recent years, especially among patients with a history of RIF. Several meta-analyses, conducted by Liu et al[16] and Glujovsky et al[17], have provided support for the potential of ERA to enhance the clinical pregnancy rate (CPR) in the RIF population. However, contrasting viewpoints have been presented by Huy et al[18] and Arian et al,[19] who argue that ERA does not lead to significant improvements. This disagreement may arise from the lack of a standardized definition for RIF and the heterogeneity of different ERA techniques. Some authors define RIF as ≥2 unsuccessful cycles or transferred embryos, while others set the threshold at ≥3 unsuccessful cycles or embryos transferred.

In addition, due to the increasing popularity of RNA-Seq over microarray analysis, He et al[20] proposed a new RNA-Seq-based endometrial receptivity test (rsERT). This test incorporates 175 biomarker genes and has shown improved clinical outcomes in patients with RIF.[20] Similarly, Ohara et al[21] used real-time quantitative polymerase chain reaction to develop a test called ERPeakSM, which includes 48 biomarker genes.

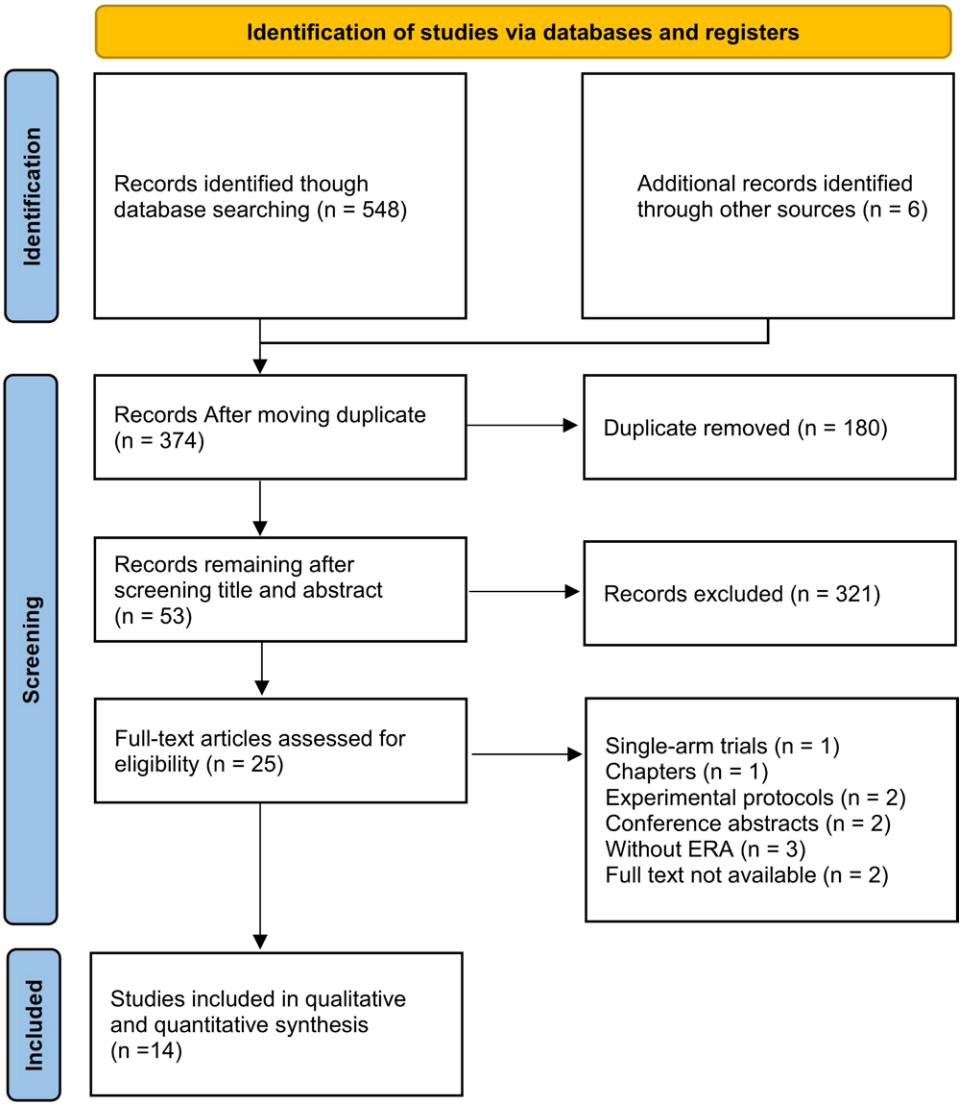

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart. Diagram illustrating study selection for meta-analysis of personalized embryo transfer guided by endometrial receptivity analysis (ERA).

Table 1

Characteristics of the selected studies.

Authors	Year	Country	Study design	Group	Number (pET/IVF or R/NR)	Age, yr	Technology	Outcomes	RIF
Bergin et al[22]	2021	United States	Cohort	ERA	45	N/A	ERA:iGenomix; 238 genes	LBR	≥3 prior ETs (RIF ≥ 3)
				IVF	120				
Cozzolino et al[23]	2020	Spain	Cohort	ERA	134	38.77 ± 1.43	ERA:iGenomix; 238 genes	IR/CPR	Patients who failed to achieve a clinical pregnancy after transfers of at least 3 good-quality embryos in different single fresh or frozen embryo transfers were considered RIF (RIF ≥ 2)
				IVF	2248	38.00 ± 0.38			A moderate RIF (M-RIF) group consisted of patients who first received at least 3 embryos transferred. Severe RIF (S-RIF) patients underwent at least 5 embryos transferred summed across consecutive cycles. M-RIF (RIF ≥ 2); S-RIF (RIF ≥ 3)
Edimiris et al[24]	2023	Germany	Cohort	ERA	67 (*R* = 18; NR = 49)	35 ± 3	ERA:iGenomix; 238 genes	CPR/MR/LBR	No clinical pregnancy occurred after at least 2 embryo transfers with a total of 3 good-quality embryos (RIF ≥ 2)
				IVF	32	36 ± 3			
Fodina et al[25]	2021	Latvia	Cohort	ERA	22	34.90 ± 4.36	ERA:iGenomix; 238 genes	CPR/MR	Patients who failed to achieve a clinical pregnancy after transfers of at least 3 good-quality embryos in different single fresh or frozen embryo transfers were considered RIF (RIF ≥ 2)
				IVF	72	34.35 ± 3.78			
He et al[20]	2021	China	Non-RCT	ERA (rsERT)	56 (*R* = 39; NR = 17)	32.71 ± 4.14	rsERT: RNA-Seq; 175 genes	CPR/IR/LBR	After the transfer of at least 4 morphologically high-quality cleavage-stage embryos or 2 high-quality blastocysts in a minimum of 2 fresh or frozen cycles (RIF ≥ 2)
				IVF	86	32.90 ± 3.79			
Jia et al[26]	2022	China	Cohort	ERA	140 (*R* = 49; NR = 91)	32.01 ± 2.99	ERA:iGenomix; 238 genes	CPR/IR/MR	At least 2 cycles of embryo transfer or transfer of at least 3 good-quality blastocysts with a Gardner score of 4 BB or higher (RIF ≥ 2)
				IVF	141	31.87 ± 3.21			
Mathew et al[27]	2022	India	Cohort	ERA	47	N/A	ERA:iGenomix; 238 genes	CPR/IR/MR/LBR	≥2 failed ET (couples with a minimum of 2 failed transfers were recruited; RIF ≥ 2)
				IVF	189	N/A			
Neves et al[28]									≥2 failed donor-ET (RIF ≥ 2)
				IVF	158	43.40 ± 4.13			
Ohara et al[21]	2022	Japan	Cohort	ERA (ERPeak^{SM})	215	38.5 ± 4.1	ERPeakSM: RT-qPCR; 48 genes	CPR/LBR/MR	Failed to achieve clinical pregnancy with ≥3 IVF cycles in which 1 or 2 morphologically good-quality blastocysts were transferred to the patient (RIF ≥ 3)
				IVF	215	38.2 ± 4.3			
Doyle et al[14]	2022	United States	Cohort	ERA	15 (*R* = 5; NR = 10)	N/A	ERA:iGenomix; 238 genes	LBR	≥3 failed ETs
						N/A			
Hashimoto et al[29]	2017	Japan	Cohort	ERA	50 (*R* = 38; NR = 12)	38.42 ± 3.40	ERA:iGenomix; 238 genes	MR/CPR/IR	Patients with RIF and a history of RIF with at least 3 good-quality embryo transfers were eligible for this study (RIF ≥ 2)
						40.08 ± 5.16			
Patel et al[30]	2019	India	Cohort	ERA	248 (*R* = 204; NR = 44)	33.67 ± 5.12	ERA:iGenomix; 238 genes	CPR/IR/MR	≥3 failed ETs
						34.11 ± 4.49			Women having ≥3 unsuccessful embryo transfer cycles each with 1 or 2 morphologically high-grade embryos using self or donor oocytes (RIF ≥ 3)
Ruiz-Alonso et al[10]	2013	Spain	Cohort	ERA	37 (*R* = 29; NR = 8)	N/A	ERA:iGenomix; 238 genes	CPR/IR/MR	≥3 failed ETs
						N/A			
Shu et al[31]	2023	China	Cohort	ERA (rsERT)	52 (*R* = 28; NR = 24)	N/A	rsERT: RNA-Seq; 175 genes	CPR/LBR	≥2 failed ETs
						N/A			

CPR = clinical pregnancy rate, ERA = endometrial receptivity analysis, ET = embryo transfer, IR = implantation rate, IVF = in vitro fertilization, LBR = live birth rate, MR = miscarriage rate, N/A = not available, NR = nonreceptive endometrium, pET = personalized embryo transfer, R = receptive endometrium, RCT = randomized controlled trial, RIF = repeated implantation failure, rsERT = RNA-Seq-based endometrial receptivity test, RT-qPCR = real-time quantitative polymerase chain reaction.

Table 2

Quality assessment of retrospective cohort studies.

	Selection of cohort				Comparability	Outcome			
Author	Representativeness of the exposed cohort	Representativeness of the nonexposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at the start of study	Comparability of the exposed cohort and nonexposed cohort	Assessment of outcome	Was follow-up long enough	Adequacy of follow-up	Total score
Jia et al[26]	0	0	1	1	0	1	1	0	4
Ohara et al [21]	0	1	1	1	1	1	1	0	6
Fodina et al[25]	1	1	1	1	0	1	1	1	7
Bergin et al[22]	1	1	1	1	1	1	1	1	8
Cozzolino et al[23]	1	1	1	1	0	1	1	0	6
Patel et al[30]	1	1	1	1	0	1	1	0	6
Hashimoto et al[29]	1	1	1	1	0	1	1	0	6
Neves et al[28]	1	1	1	1	1	1	1	0	7
Mathew et al[27]	0	0	1	1	0	1	1	0	4
Edimiris et al[24]	1	1	1	1	1	1	1	0	7
Doyle et al[14]	1	1	1	1	1	1	1	0	7
Ruiz-Alonso et al[10]	1	1	1	1	1	1	1	0	7
Shu et al[31]	1	1	1	1	0	1	1	1	7

Their study also demonstrated enhanced CPR and live birth rate (LBR) in patients with RIF. In our study, we performed a comparative analysis between traditional ERA techniques and optimized gene-enhanced ERA techniques in patients with RIF. In addition, we analyzed different subgroups based on various definitions of RIF.

2. Materials and methods

The meta-analysis adhered to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist. To maintain transparency and accountability, the study protocol was registered in PROSPERO under the unique registration number CRD42023385704.

2.1. Search strategy

We conducted a thorough search in PubMed, The Cochrane Library, Web of Science, and Embase from inception to August 2023. The search was focused on keywords related to ERA, pET, and RIF. We excluded case reports, editorials, expert opinions, perspectives, and nonhuman articles.

2.2. Inclusion and exclusion criteria

We included randomized and nonrandomized controlled trials and retrospective comparative studies that compared pregnancy rates (PRs) in in vitro fertilization (IVF)-ET cycles with or without endometrial receptivity array testing. RIF was defined as the inability to achieve a clinical pregnancy after at least 2 ET attempts. The clinical outcomes assessed were CPR, IR, and LBR. We excluded review articles, editorials, letters to the editor, case reports, conference abstracts, and animal studies in our analysis.

2.3. Outcome measures

The CPR was identified as the primary outcome, while the IR and LBR were considered secondary outcomes. Clinical pregnancy was confirmed by visualizing intrauterine gestational sacs using ultrasonography. The IR was determined by counting the number of gestational sacs observed through vaginal ultrasound at the fifth week of pregnancy. Live birth referred to the delivery of ≥1 live babies after a gestational period of ≥23 weeks.

Table 3

Bias risk of the non-RCT studies.

Evaluation field	He et al[20]
Confounding bias	Moderate
Subject selection bias	Low
Interventional classification bias	Low
Bias of deviation from established intervention	Low
Data loss bias	Moderate
Outcome measurement bias	Low
Selective reporting bias	Low
Overall bias	Moderate

RCT = randomized controlled trial.

2.4. Data extraction

Data from the included studies were independently extracted by 3 investigators. The extracted data consisted of study characteristics, population details, intervention (ERA trial), and IVF results. In case of any disagreements, they were resolved through discussion and consultation with the trial authors, if necessary.

2.5. Quality assessment

The studies underwent an evaluation of the risk of bias utilizing both the modified Newcastle-Ottawa scale for cohort studies and the Risk of Bias in Non-Randomized Studies of Interventions tool for observational studies. A Newcastle-Ottawa scale score exceeding 6 was indicative of high quality.

2.6. Statistical analysis

Statistical analysis was performed using Stata software version 15.0. To express the study results, the relative risk (RR) along with a 95% CI was utilized. Statistical significance was defined as a P value of <0.05. The choice between random-effects or fixed-effects models was determined based on heterogeneity, specifically if the I2 statistic was greater than or equal to 50%

and the *P* value was <0.1. In cases of high heterogeneity, subgroup analysis was conducted based on prior failures and ERA techniques. Sensitivity analysis was carried out as necessary. Publication bias analysis was performed using Begg and Egger tests when there were ≥10 studies reporting the outcomes.

3. Results

3.1. Study selection

The study selection process is depicted in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart

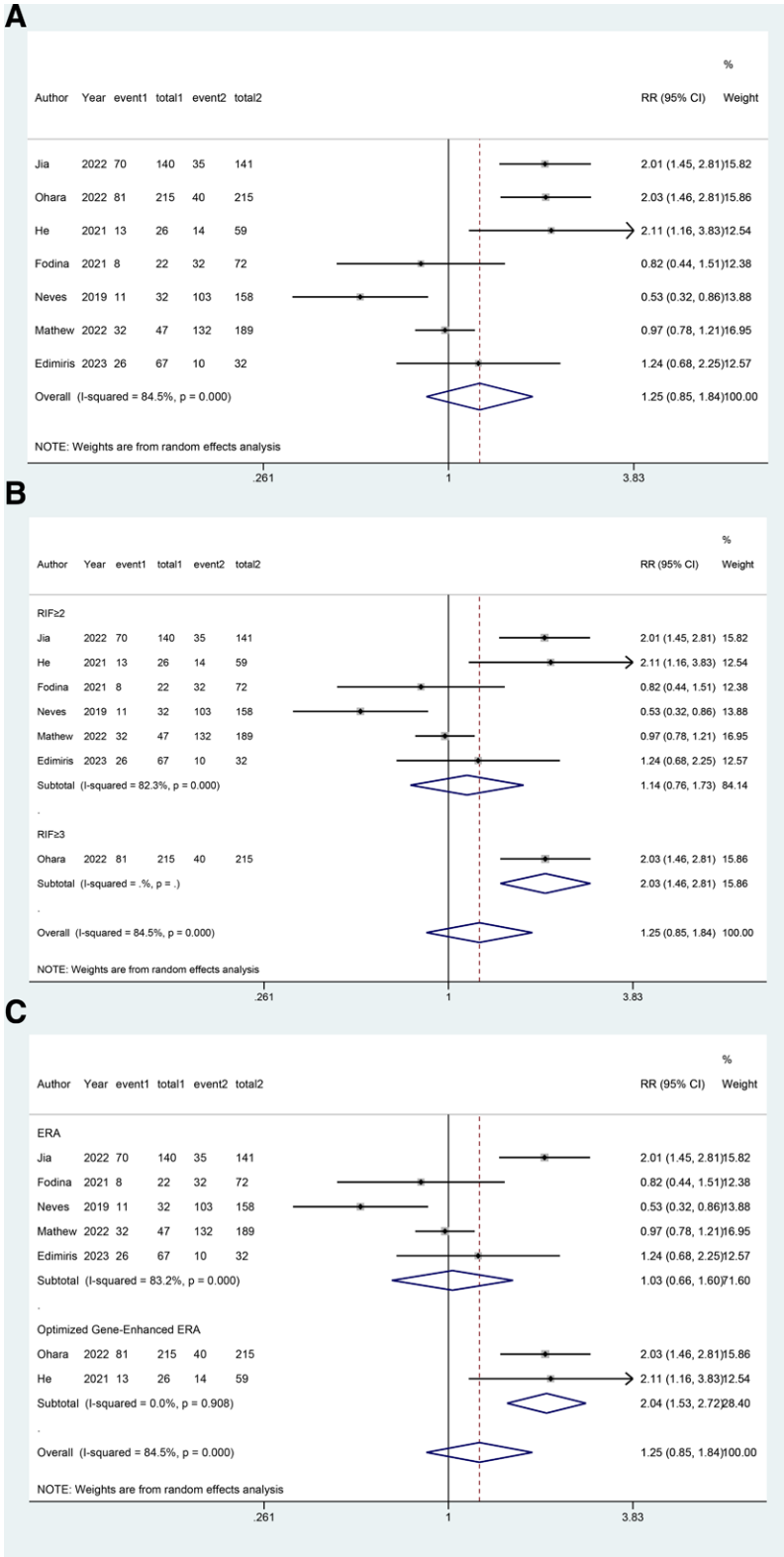

Figure 2. (A) Forest plot of endometrial receptivity analysis (ERA) vs in vitro fertilization (IVF) for the clinical pregnancy rate (CPR) outcome on the repeated implantation failure (RIF). (B) Forest plot of ERA vs IVF for CPR outcome based on RIF definitions. (C) Forest plot of ERA vs IVF for CPR outcome based on ERA design. RR = relative risk.

(Fig. 1). Initially, a total of 548 articles were identified from the target databases, with an additional 6 studies found from other sources. After removing 180 duplicate studies, 374 articles were left for review. Following the review process, 25 studies were chosen for further analysis. Subsequently, we excluded 1 single-arm trial, 1 chapter, 2 trial protocols, 2 conference summaries, 3 studies without ERA intervention, and 2 studies with unavailable full texts. Eventually, a total of 14 studies that met all the predetermined criteria were included in the analysis.

3.2. Characteristics of the included studies

The characteristics of the studies included in the meta-analysis are presented in Table 1. Of the 14 studies included, 13 were cohort studies. Ruiz-Alonso et al, Hashimoto et al, Patel et al, Cozzolino et al, Neves et al, Cohen et al, Bergin et al, Fodina et al, Ohara et al, Jia et al, Mathew et al, Doyle et al,[14] and Edimiris et al conducted these cohort studies.[6,21–32] One study was a nonrandomized controlled clinical trial conducted by He et al.[20] Of the included studies, 9 focused on applying ERA to patients with a history of RIF compared with control patients. Five studies compared outcomes between nonreceptive and receptive subjects undergoing ERA-timed preimplantation embryo transfer (pET) in patients with RIF. The pregnancy outcomes assessed in the studies included CPR, IR, and LBR.

3.3. Quality assessment of the included studies

In this study, we included a total of 13 cohort studies and 1 nonrandomized controlled clinical trial. To evaluate the quality of the cohort studies, we utilized the Newcastle-Ottawa Risk of Bias Scoring System.[33] In addition, the risk of bias in the nonrandomized study was assessed using the Risk of Bias in Non-Randomized Studies of Interventions tool.[34] We have assessed the bias in the 13 cohort studies, and the results are presented in Table 2. It is worth noting that only 2 studies[26,27] scored below 6, indicating a slightly lower quality. In contrast, the remaining studies were considered to be of high quality as their scores exceeded 6. Notably, the study conducted by Bergin et al[22] obtained the highest quality score of 8.

Table 3 displays the scores for the risk of bias assessment in the nonrandomized controlled clinical trial conducted by He et al.[20] In regards to subject selection, He et al provided a clear description of the inclusion/exclusion criteria and received a low-risk grade. However, due to the intricacies of pregnancy and potential confounding factors such as the timing for endometrial biopsy and the specific luteal support protocol, the trial was assigned a medium risk compared with randomized controlled trials. The interventional classification specified ERA-guided pET and, hence, was considered low risk. It is worth noting that of the planned enrollment of 56 patients in the test group, 5 patients did not undergo further transfer due to poor embryo quality, and there were 3 patients lost to follow-up, resulting in a medium risk of bias in data loss. The outcome measure in the trial was the objective PR, which led to a low-risk assessment for outcome measurement. In addition, there was no evidence of selective reporting in the trial. Taking all these factors into consideration, the overall bias risk for this nonrandomized controlled clinical trial was determined to be medium.

3.4. Meta-analysis of ERA vs non-ERA

3.4.1. Clinical pregnancy rate. There was no significant difference in the primary efficacy indicator CPR between the ERA group and the non-ERA group (7 studies: RR, 1.25 [95% CI, 0.85–1.84]; $I^2 = 84.5\%$; $P = .25$; Fig. 2A). Subgroup analysis revealed that ERA improved CPR in the RIF ≥ 3 groups, while the pooled data from RIF ≥ 2 groups did not show a significant difference in CPR (6 studies: RR, 1.14 [95% CI, 0.76–1.73]; $I^2 = 82.3\%$; $P = .00$; Fig. 2B). Furthermore, we conducted a stratified analysis based on different methods of ERA, which demonstrated that the novel ERA technique (He 2021;

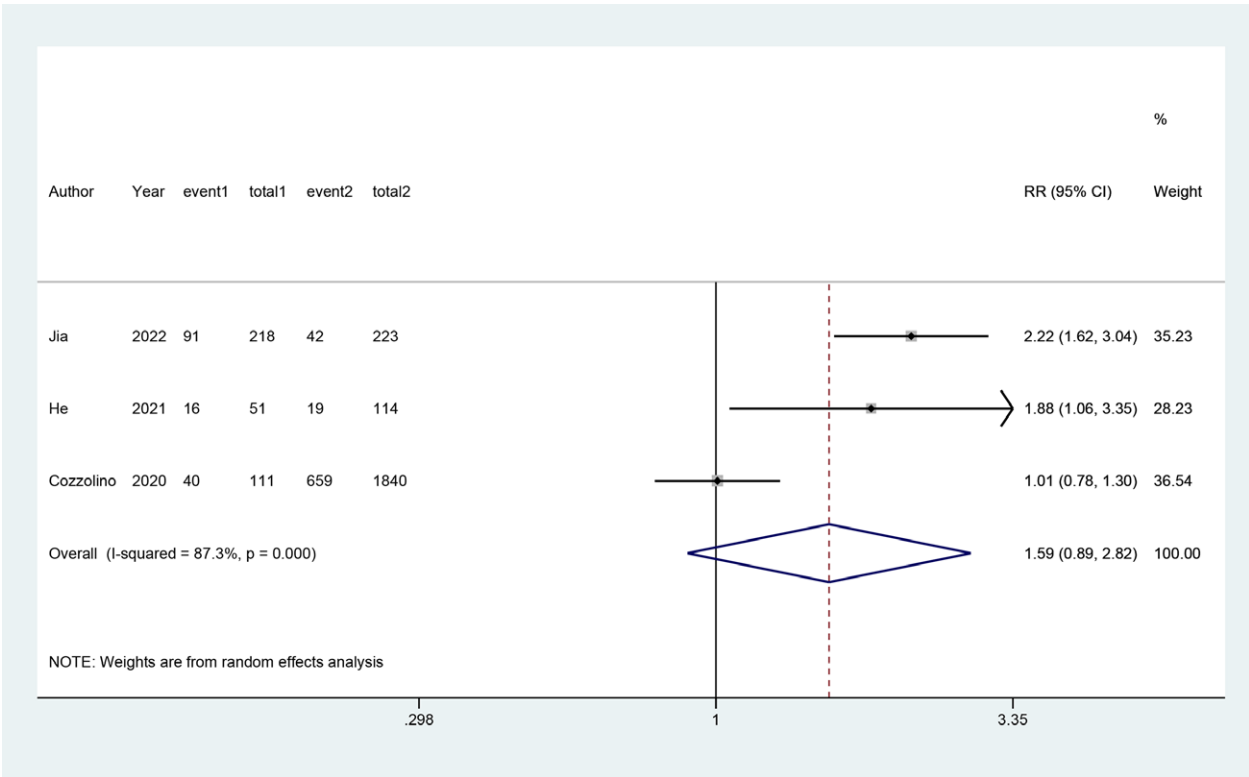

Figure 3. Forest plot for implantation rate in endometrial receptivity analysis (ERA) vs in vitro fertilization (IVF; control). RR = relative risk.

Ohara 2022) resulted in a significantly higher CPR in the RIF population compared with the non-ERA group (RR, 2.04 [95% CI, 1.53–2.72]; I^2 = 0.0%; *P* = .91; Fig. 2C).

3.4.2. Implantation rate. No significant differences in IR were found for the secondary outcome between patients undergoing ERA and those not undergoing ERA (3 studies: RR, 1.59 [95% CI, 0.89–2.82]; I^2 = 87.3%; *P* = .12; Fig. 3).

3.4.3. Live birth rate. Figure 4A displayed the LBR outcome from 5 studies. In general, the use of ERA did not result in an improvement in LBR (5 studies: RR, 1.55 [95%

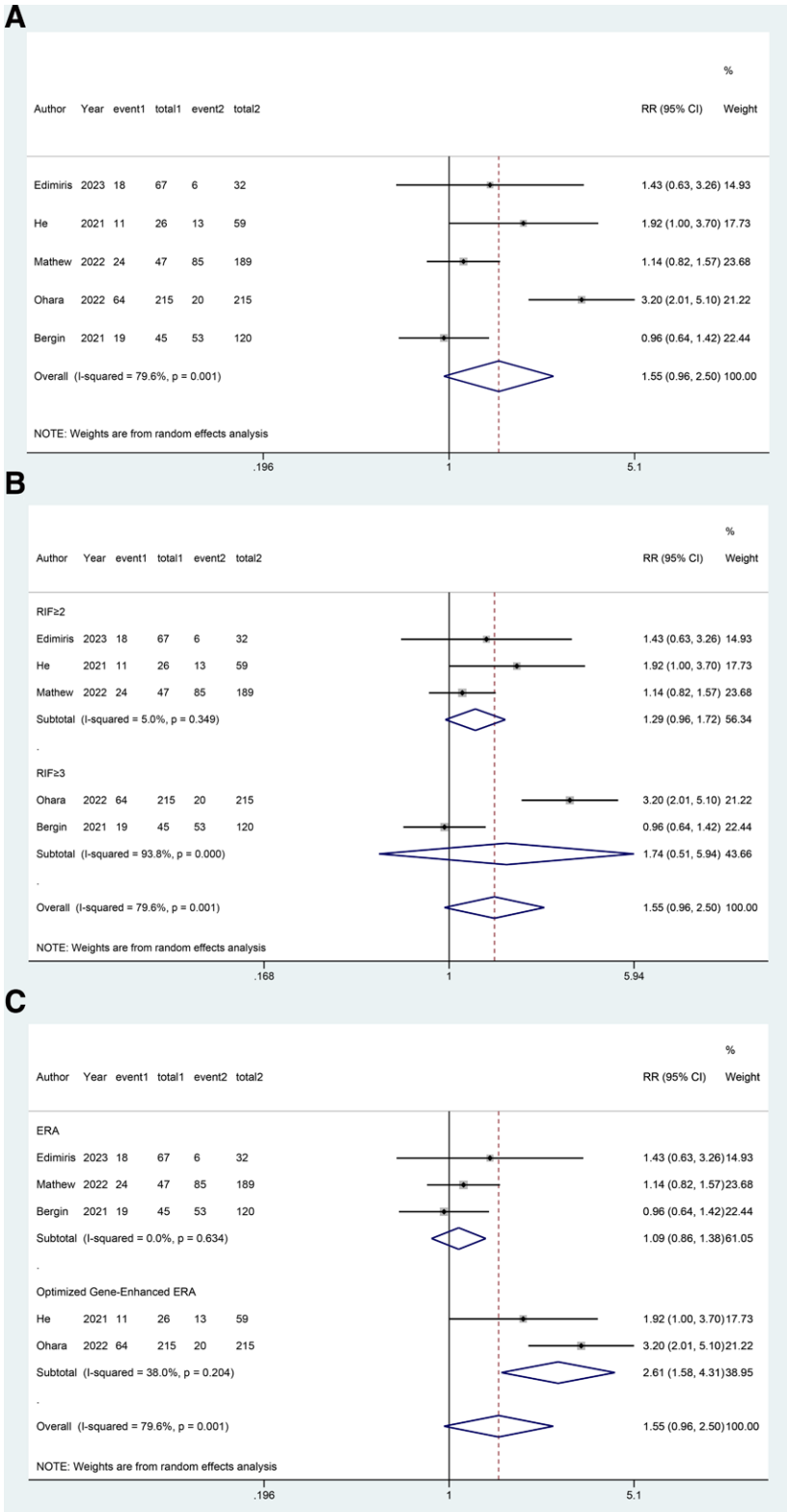

Figure 4. (A) Forest plot of endometrial receptivity analysis (ERA) vs in vitro fertilization (IVF) for the LBR outcome on the repeated implantation failure (RIF). (B) Forest plot of ERA vs IVF for live birth rate (LBR) outcome based on RIF definitions. (C) Forest plot of ERA vs IVF for LBR outcome based on ERA design. RR = relative risk.

CI, 0.96–2.50]; $I^2 = 79.6\%$; $P = .001$). Subgroup analysis revealed that there was no significant difference in LBR between patients belonging to the RIF ≥ 2 group or RIF ≥ 3 group (RR, 1.29 [95% CI, 0.96–1.72] and RR, 1.74 [95% CI, 0.51–5.94], respectively; Fig. 4B). In addition, further subgroup analysis based on ERA design indicated that RNA-seq-based ERA (He 2021; Ohara 2022) significantly enhanced LBR (RR, 2.61 [95% CI, 1.58–4.31]), whereas traditional ERAs showed no significant impact (RR, 1.09 [95% CI, 0.86–1.38]; Fig. 4C).

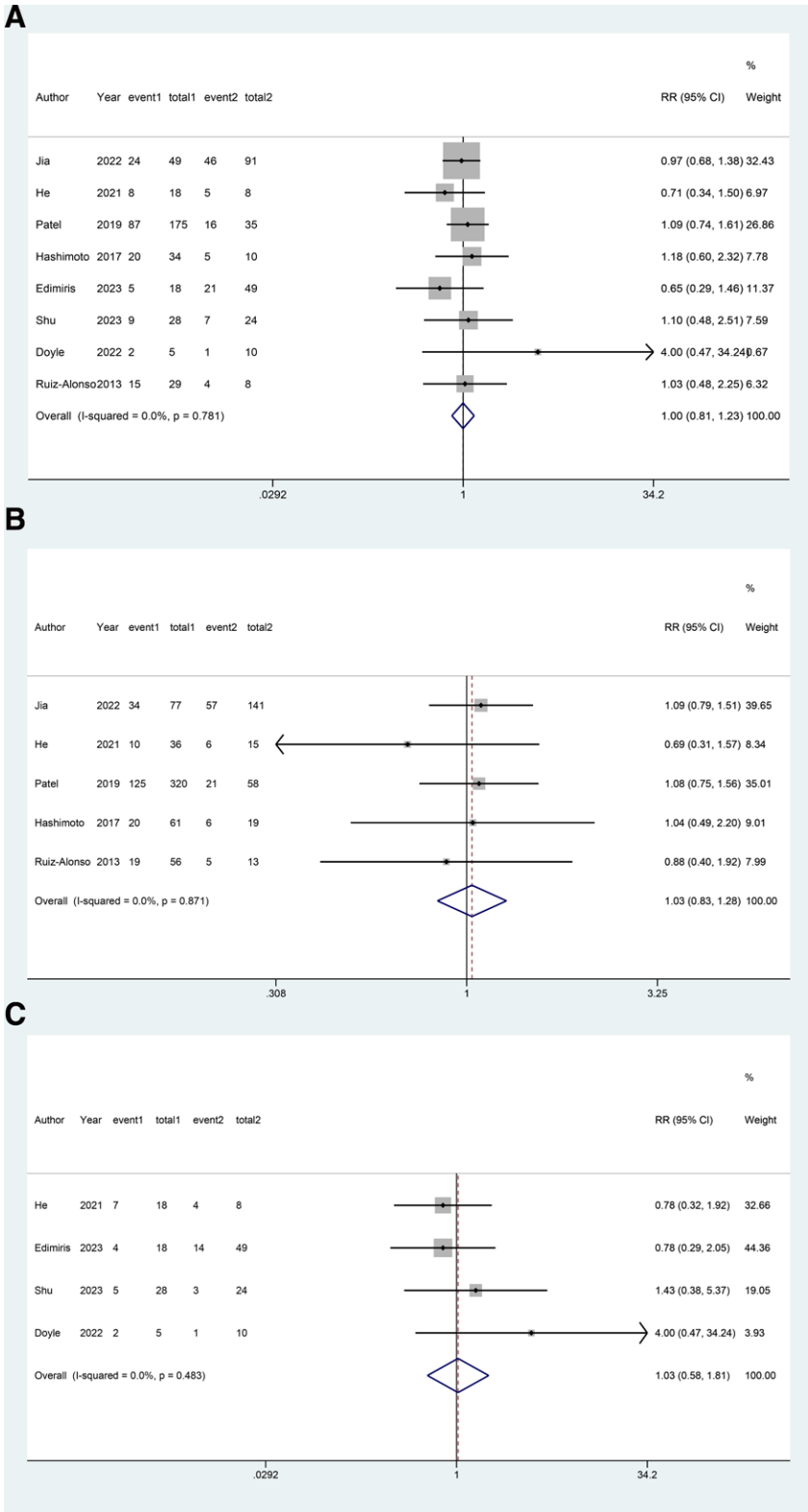

Figure 5. Forest plot for (A) clinical pregnancy rate, (B) implantation rate, and (C) live birth rate in endometrial receptivity array vs nonendometrial receptivity array (control). RR = relative risk.

3.5. ERA results: receptivity vs nonreceptivity

Statistical analysis was performed in this study to examine the clinical outcomes of patients with different endometrial receptivity statuses (receptive and nonreceptive) in the ERA group. The findings revealed that there was no significant difference, as supported by the data, between patients with nonreceptive and receptive endometrium in terms of CPR, IR, and LBR. Specifically, the results from multiple studies indicated that there was no significant variation in CPR (8 studies: RR, 1.00 [95% CI, 0.81–1.23]; $P = .78$; $I^2 = 0.0\%$), IR (5 studies: RR, 1.03 [95% CI, 0.83–1.28]; $P = .87$; $I^2 = 0.0\%$), and LBR (4 studies: RR, 1.03 [95% CI, 0.58–1.81]; $P = .48$; $I^2 = 0.0\%$) between the 2 groups. These results are visually represented in Figure 5A through 5C.

3.6. Sensitivity analysis and publication bias

We performed a sensitivity analysis on the aggregated findings of the aforementioned indicators. The analysis revealed that eliminating articles individually did not result in a significant alteration in the combined effect size. This suggests that the results of the meta-analysis are robust. For a detailed description of the sensitivity analysis procedure, refer to Supplemental Digital Content 1, http://links.lww.com/MD/O419. Given that each outcome analysis included <10 articles, we did not conduct a publication bias test.

4. Discussion

This study presents a thorough examination focused on patients with RIF undergoing PGT for aneuploidies (pET) guided by ERA. Although the overall effectiveness of the ERA approach in enhancing IVF pregnancy outcomes appears to be limited, RNA sequencing-based ERA demonstrates effectiveness in improving LBR and CPR in patients with RIF.

All patients participating in this study were diagnosed with RIF although its precise definition continues to be a topic of debate. The most commonly accepted definition considers RIF as the inability to achieve a clinical pregnancy following the transfer of at least 3 high-quality embryos across 3 fresh or frozen cycles in women aged under 40 years. Nonetheless, certain definitions encompass failure after 2 cycles. Researchers such as Bergin et al, Doyle et al, Patel et al, and Ruiz-Alonso et al characterize RIF as ≥3 failed ETs, whereas Shu et al,[31] Neves et al, Mathew et al, and Edimiris et al classify it as ≥ 2 failed ETs. However, some of these studies did not specify whether embryos were chosen through PGT-A or if they were of high quality. Other studies, including the study by Cozzolino et al, define RIF as the failure to achieve a clinical pregnancy after the transfer of at least 3 good-quality embryos in separate fresh or frozen cycles, while Chen et al stipulate ≥4 high-quality cleavage embryos or ≥2 high-quality blastocysts in a minimum of 2 transfer cycles. It is important to note that these studies incorporated the count of transferred embryos and defined RIF based on the total number of failed transfers, irrespective of whether they originated from the same or different oocyte stimulation cycles.[35]

In this study, we categorized patients based on the frequency of RIF occurrences to evaluate the influence of ERA on the severity of RIF. This method reduces inconsistencies in baseline data and diminishes statistical bias. Upcoming studies should strive for more precise definitions of RIF, encompassing factors such as the number of unsuccessful transfers, embryo quality, and the application of PGT-A screening. Furthermore, it is essential to clarify whether RIF results from repeated failures within a single oocyte retrieval cycle or across separate cycles. Such precision would facilitate a more accurate evaluation of pregnancy outcomes, including the use of the COMFFETI index[36] to assess success rates in each stimulation cycle. This matter deserves further examination to attain a more clear and accurate comprehension of RIF.

The complexity of endometrial receptivity is highlighted by the divergence among candidate genes selected by different methods of ERA. Diaz-Gimeno et al[8] first introduced ERA in 2011, incorporating 238 genes, of which 134 exhibited specific transcriptomic signatures for receptivity. The rsERT, developed by Mantione et al,[37] identified 175 biomarker genes using high-throughput sequencing. In contrast, the 2021 ERPeakSM test utilizes real-time quantitative polymerase chain reaction to analyze 184 genes. From this analysis, 85 genes with significant variations in receptivity expression profiles were chosen, constituting a set of 48 genes that collectively explain over 99.5% of the variability observed in endometrial receptivity.[21] Interestingly, only 22 genes were found to overlap between the traditional ERA's 238 genes and the rsERT's 175 genes (genes from ERPeakSM are undisclosed). Such a substantial disparity in the selection of candidate genes by different ERA methods indicates an ongoing debate regarding their relevance to endometrial receptivity.

Our investigation has observed that He et al and Ohara et al utilized the rsERT and ERPeakSM test methods, respectively. It is worth noting that their cumulative pregnancy rates (CRPs) and LBRs exhibited statistically significant improvements when compared with the control groups (CRP: RR, 2.04 [95% CI, 1.53–2.72]; LBR: RR, 2.61 [95% CI, 1.58–4.31]). This implies that traditional ERA methods may exhibit lower accuracy, possibly due to their reliance on transcriptomic approaches, which may lack the precision offered by RNA sequencing technology. Furthermore, the significant differences in candidate genes selected by traditional ERA and rsERT imply that the gene selection process of traditional ERA may not adequately reflect endometrial receptivity, leading to suboptimal outcomes in IVF procedures.

We conducted a comparison between patients in the receptive and nonreceptive groups and observed no variations in pregnancy outcomes, specifically the CPR, IR, and LBR. It is worth noting that nonreceptive patients achieved comparable results to their receptive counterparts, which is consistent with findings from previous studies.[10,16,31] However, recent research has presented a challenge to this notion, suggesting that there is no discernible difference in outcomes between nonreceptive and receptive patients (LBR: 62.5% vs 62.4%).[38] This raises doubts about the accuracy of the endometrial receptivity assay (ERA) in determining the ideal implantation window, thereby emphasizing the need for caution when utilizing this technology.

This meta-analysis has uncovered an additional finding, indicating that the severity of RIF does not have a significant impact on the effectiveness of ERA. When stratifying RIF studies, it was found that there were no notable differences in LBRs between the RIF ≥ 2 group and the RIF ≥ 3 group. In a study conducted by Cozzolino et al,[23] we delved deeper into the data and obtained similar results. After performing further subgroup analysis, no significant enhancement associated with ERA was observed in terms of ongoing PRs between the severe RIF group (≥5 embryos transferred) and the moderate RIF group (≥3 embryos transferred; RR, 1.24 [95% CI, 0.88–1.74] vs RR, 1.02 [95% CI, 0.85–1.22]; Supplemental Digital Content 2, http://links.lww.com/MD/O419).

Upon an investigation into the uncertainties surrounding the effectiveness of ERA, several potential explanations become apparent. First, there is a lack of standardization in the collection of uterine tissue, as well as inconsistencies in the detection platforms and laboratories used, both contributing to variability in the quantification of gene expression. Second, the absence of paired samples leads to increased heterogeneity between patients. Third, while specific biomarkers of endometrial receptivity have been identified within the natural menstrual cycle, the use of ovarian stimulation delays the genomic response of receptive endometrial genes, potentially affecting their predictive

value in ovulation induction cycles.[39] In addition, elevated concentrations of estradiol resulting from gonadotropin stimulation may prematurely induce progesterone receptors, triggering premature endometrial secretion.[40] Finally, the studies included in our analysis did not universally incorporate PGT screening prior to ET, and research focusing on PGT-A or donor cycles remains sparse. Consequently, some implantation failures could be linked to aneuploidy instead of endometrial receptivity. Thus, a more holistic approach is essential, especially by incorporating further studies that involve PGT-A or donor cycles to tackle these clinical intricacies.

However, our meta-analysis of ERA has some inherent limitations due to the nature of the available data. First, it is important to note that the majority of studies included in our analysis are retrospective in nature. This can potentially introduce bias compared with prospective trials, as retrospective studies rely on previously gathered data and may be subject to confounding variables. Second, our subgroup analysis reveals significant differences between RNA-seq-based ERA and traditional ERA in terms of CRPs and LBRs. However, it is worth mentioning that all the RNA-seq-based ERA data used in our analysis are sourced from East Asia. This may introduce a publication bias as it limits the generalizability of our findings to other geographical regions.

In conclusion, our analysis suggests that ERA-guided pET shows limited efficacy in improving pregnancy outcomes in patients with RIF. However, RNA-seq-based ERA, in comparison to traditional transcriptomic ERA, demonstrates the potential to enhance IVF outcomes for patients with RIF to a certain degree. Randomized controlled trials comparing these 2 ERA approaches are essential to evaluate their effectiveness for patients with RIF, particularly in regions outside of East Asia. Furthermore, it is crucial to conduct comprehensive multicenter investigations with substantial sample sizes.

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