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REVIEW ARTICLE

Pre-Operative Factors on Prognosis of Regenerative Endodontic Procedures: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Although regenerative endodontics has advanced in recent years, the influence of pre-operative factors on treatment outcomes remains poorly understood.

Objective: To evaluate the effect of pre-operative factors—including age, gender, tooth type, aetiology of pulp necrosis, stage of root development, clinical signs/symptoms and periradicular status—on the treatment outcomes of regenerative endodontic procedures (REPs) in immature permanent teeth.

Methods: A literature search was conducted on six electronic databases and grey literature to identify studies investigating the effect of pre-operative factors on REP outcomes. The risk of bias was assessed using the Newcastle-Ottawa Scale for observational studies and the Cochrane Risk-of-Bias 2 tool for randomised clinical trials (RCTs). Meta-analyses of proportions were conducted to estimate pooled rates for the outcomes 'clinical and radiographic healing', 'root development' and 'apical closure' across different pre-operative factors. Associations between these factors and treatment outcomes were assessed through meta-analyses of effect sizes. The certainty of evidence was evaluated using the GRADE approach.

Results: Twenty studies were included, comprising 13 observational studies and seven RCTs. Most studies presented a moderate to high risk of bias. The pooled success rate for clinical and radiographic healing exceeded 81% across all pre-operative factors. Moreover, root development was achieved in 52%–95% of cases, whereas complete apical closure ranged from 32% to 91%. No significant association was found between pre-operative factors and clinical and radiographic healing. It was found that teeth with pulp necrosis due to trauma presented 3.59 times higher relative risk of root development failure compared to those with necrosis due to anatomic anomaly (RR = 3.59, 95% CI 1.21–10.67, $p = 0.02$). Incisors presented 1.90 times higher relative risk of root development failure (RR = 1.90, 95% CI: 1.37–2.63, $p < 0.01$) and 1.98 times higher relative risk of incomplete apical closure (RR = 1.98, 95% CI: 1.34–3.13, $p = 0.02$) compared to premolars. The presence of an apical lesion increased the relative risk of root development failure by 2.55 times (RR = 2.55, 95% CI: 1.63–4.86, $p = 0.01$). The certainty of evidence was rated as very low.

Conclusion: Pre-operative factors were not significantly associated with clinical and radiographic healing in REPs. However, trauma-related pulp necrosis, tooth type (incisors) and the presence of apical lesions were associated with an increased risk of root development failure. These findings should be interpreted with caution due to between-study heterogeneity, the moderate to high risk of bias and the very low certainty of the evidence.

1 | Introduction

Traditional management of necrotic immature permanent teeth includes apexification by inducing an apical barrier with prolonged calcium hydroxide application or using a mineral trioxide aggregate (MTA) apical plug (Galler et al. 2016; Sabeti et al. 2024). However, these methods do not ensure root maturation, leading to thin and fragile radicular walls (Huang 2009). Therefore, regenerative endodontics is an evolving field that has gained increasing attention from researchers and clinicians due to its potential to promote continued root development (Murray 2023; Reis-Prado et al. 2025). This approach is founded on the core principles of tissue engineering, comprising growth factors, stem cells and three-dimensional scaffolds (Smith et al. 2016). Regenerative endodontic procedures (REPs) can promote the repair of damaged dental structures and cells within the pulp-dentin complex, thereby facilitating the resolution of clinical signs and symptoms, including the healing of apical lesions, while restoring normal tooth function (Wei et al. 2022). With a success rate exceeding 80%, REPs involve minimal instrumentation, copious irrigation, use of intracanal medication, induction of a blood clot or use of an autologous scaffold within the root canal and placement of a biologically active capping material (Ong et al. 2020; Rahul et al. 2023).

Currently, various novel materials and recent reviews in clinical guidelines provide essential support for the effective implementation of REPs (American Association of Endodontists 2021; Galler et al. 2016). However, to optimise clinical management and improve treatment prognosis, continuous research efforts are underway to refine protocols and develop new materials (Dos Reis-Prado et al. 2024). While advancements in these aspects are essential, treatment outcomes can be influenced by several patient-related factors that must also be considered in clinical decision-making (Kim et al. 2018). Regarding age, REPs are primarily recommended for permanent teeth in younger patients (Kim et al. 2018). This recommendation is supported by evidence indicating that the regenerative capacity of pulp tissue diminishes with increasing age (Kim et al. 2018). Such decline may be attributed to a more robust immune response in younger individuals and an age-related reduction in the proliferative, survival and differentiation potential of stem cells (Iohara et al. 2014; Kellner et al. 2014).

Regarding root morphology, REPs are indicated for teeth with up to two-thirds of root development and an open apex, demonstrating the highest clinical success rate in teeth with apical diameters between 0.5 and 1.0 mm, as a closed apical foramen may provide insufficient blood supply to the canal (Fang et al. 2018). Beyond age and anatomical configurations, other factors such as the aetiology of pulp necrosis and the presence of apical lesions also appear to influence treatment outcomes (Hu et al. 2023; Kateb and Fata 2022). Dental trauma, particularly in anterior teeth, is the most frequently reported cause of pulp necrosis in candidates for REPs (Kahler et al. 2024). However, cases associated with developmental malformations have shown more favourable root development than those resulting from trauma (Hu et al. 2023). Moreover, while REPs are recognised for promoting the healing of apical periodontitis, the size of the periradicular lesions seems to impair complete periapical tissue healing, especially in cases involving large lesions (Kateb and Fata 2022).

Despite the increasing number of reviews examining the impact of different treatment protocols on REP outcomes, limited attention has been given to the influence of pre-operative factors that may act as prognostic indicators. Although the influence of the aetiology of pulp necrosis has been explored in a previous systematic review (Koç and DEL Fabbro 2020), other potential factors remain poorly investigated, and the available evidence needs to be updated. A comprehensive understanding of patient demographics, clinical factors and radiographic parameters is essential for effective individualised treatment planning. Assessing these variables may support case selection and clinical decision-making, ultimately enhancing treatment outcomes and improving long-term tooth survival. Therefore, the purpose of this systematic review and meta-analysis was to evaluate the effect of pre-operative factors—including age, gender, tooth type, aetiology of pulp necrosis, stage of root development, clinical signs/symptoms and periradicular status—on the treatment outcomes of REPs in immature permanent teeth with pulp necrosis.

2 | Methods

2.1 | Protocol and Registration

The present study was reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement (Page et al. 2021). A systematic review protocol was previously registered at the International Prospective Register of Systematic Reviews (PROSPERO) database (code CRD42024600746) (Booth et al. 2011).

2.2 | Research Question and Eligibility Criteria

This study was conducted to address the following research question: In patients with necrotic immature teeth treated with REPs, do pre-operative factors—such as patient age, gender, tooth type, aetiology of pulp necrosis, stage of root development, clinical signs and symptoms and periradicular status—affect treatment outcomes?

Eligibility criteria were defined based on the PECO acronym, as follows:

2.2.1 | Population

Patients with necrotic immature permanent teeth treated with REPs.

2.2.2 | Exposure/Comparison

Pre-operative factors, including age, gender, tooth type, aetiology of pulp necrosis, stage of root development, clinical signs and symptoms and periradicular status. In this context, the factors themselves served as both exposure and comparison. Comparisons were performed as follows (exposure vs. comparison): age (up to 10 years vs. 11–16 years), gender (male vs. female), tooth type (incisors vs. premolars, incisors vs. molars), aetiology of pulp necrosis (trauma vs. caries, anatomic anomaly vs. caries, trauma vs. anatomic anomaly), stage of root development (half-formed vs. more than two-thirds formed), clinical signs and symptoms (present vs. absent) and periradicular status (presence vs. absence of apical lesion).

2.2.3 | Outcomes

The outcomes were defined as follows: (1) 'clinical and radiographic healing', defined as the resolution of clinical signs and symptoms of infection accompanied by radiographic evidence of periradicular healing; (2) 'continued root development', characterised by thickening of root canal walls and/or increased root length; (3) 'complete apical closure' and (4) 'pulp responsiveness to sensitivity tests'.

Studies were excluded if they involved mature teeth, retreatment cases or only included traumatised teeth, as these scenarios could introduce confounding variables. Additionally, studies that did not clearly differentiate between REP outcomes and apexification were excluded to avoid outcome misclassification. Furthermore, case reports, case series, conference abstracts, letters, editorials, reviews, and in vitro or animal studies were also excluded.

2.3 | Information Sources and Search Strategy

A librarian with expertise in systematic reviews and the review team collaborated to develop a comprehensive search strategy. Initially, potentially relevant terms were identified using controlled vocabularies (Medical Subject Headings [MeSH], Embase Subject Headings [Emtree], Health Sciences Descriptors [DeCS] and Index terms) and after reading topic-related articles for additional free terms. These terms were then combined into a single search strategy adapted to the specific requirements of each selected database or repository (Table S1).

The literature search was conducted on November 1st, 2024, across the following electronic databases: Cochrane Library, Embase, Latin American and Caribbean Health Sciences (LILACS), PubMed, Scopus and Web of Science. Grey literature was searched through Google Scholar (the first 100 results) and ProQuest Dissertations and Theses databases. No filters or restrictions were applied. To ensure comprehensive coverage of the literature, the reference lists of the included studies were also checked. After completing the search, the identified references were imported into EndNote 21 software (Clarivate Analytics, London, UK), where duplicates were removed.

2.4 | Selection Process

The selection of studies was conducted in two distinct phases. Initially, two independent reviewers (F.C.V. and A.H.R.P.) screened the titles and abstracts of the retrieved records using a web-based systematic review tool (Rayyan, Qatar Computing Research Institute, Al-Rayyan, Qatar). Subsequently, articles meeting the inclusion criteria were independently assessed through full-text reading by the same reviewers. Disagreements during the selection process were resolved through discussion and consensus. If consensus could not be reached, a third reviewer (P.S.S.) was consulted to make the final decision.

2.5 | Data Collection

Data collection was conducted using a specific form developed by the research team. To ensure consistency, two reviewers (F.C.V. and A.H.R.P.) were initially trained in a pilot study. Subsequently, these reviewers independently collected key data and cross-checked all information. In cases of inconsistencies, the full text was reaccessed for data verification and consensus.

Data extraction included bibliographic details (authors, year and country), study design, sample size (including total number of participants and group allocation), pre-operative factors (age, gender, tooth type, aetiology of pulp necrosis, stage of root development, clinical signs and symptoms, periradicular status, and their respective absolute frequencies), REP protocol (number of visits, irrigation, intracanal medication, scaffold, matrix and capping material) and outcomes assessment (follow-up period, method, evaluation criteria and proportion of success/failure for each pre-operative condition of interest).

2.6 | Risk of Bias Assessment

The risk of bias was assessed according to the study design. Observational studies were evaluated using the Newcastle-Ottawa Scale (NOS), while randomised clinical trials were assessed with the second version of the Cochrane risk-of-bias (RoB 2) tool (Stang 2010; Sterne et al. 2019). Two reviewers (F.C.V. and A.H.R.P.) were previously trained to use these tools, including discussions on the criteria that guided the evaluation. Subsequently, the reviewers independently assessed the risk of bias in each study. Any disagreements were resolved through discussion and consensus, and unresolved cases were referred to by a third reviewer (P.S.S.) for a final decision.

The NOS establishes a scoring system based on the attribution of stars, with a maximum of nine stars distributed across three domains: participant selection (up to four stars), comparability (up to two stars) and outcome assessment (up to three stars). Studies scoring up to four stars were classified as having a 'high risk of bias', those scoring five to six stars as 'moderate risk of

bias' and those scoring seven or more stars as 'low risk of bias' (Lo et al. 2014; Stang 2010). RoB 2 tool is structured into five domains, including bias related to the randomisation process, deviations from intended interventions, missing outcome data, measurement of outcomes and selection of the reported results. Each domain was rated as 'low risk of bias', 'some concerns' or 'high risk of bias'. After reaching a consensus on the five domains, the overall risk of bias was determined via Cochrane guidance (Sterne et al. 2019).

2.7 | Data Synthesis and Analysis

All collected data were analysed both quantitatively and qualitatively. For the quantitative analysis, the outcome 'clinical and radiographic healing' was categorised as 'success' or 'failure', where 'success' was defined as the resolution of clinical signs and symptoms of infection, accompanied by radiographic evidence of periradicular healing, after a minimum follow-up of 1 year. The outcomes 'root development' and 'complete apical closure' were categorised as 'yes' or 'no', with 'yes' indicating an increase in root length/thickness and the presence of a closed apex, respectively. Pre-operative factors were classified based on the nature of the condition and the potential for grouping studies: gender (female or male), age (up to 10 years or 11–16 years), aetiology of pulp necrosis (caries, trauma or anatomic anomaly), tooth type (incisors, premolars or molars), stage of root development (half-formed or more than two-thirds formed), pre-operative signs/symptoms (present or absent, including pooled data of pain, swelling, abscess or sinus tract) and apical lesion (present or absent).

Initially, pooled estimates for the outcomes were calculated based on different pre-operative factors using a meta-analysis of proportions. The pooled proportion (%) and corresponding 95% confidence interval (CI) were used as the summary measure. Subsequently, a meta-analysis was conducted to assess the association between pre-operative factors and the outcomes. The risk ratio (RR) was used as the effect measure, with pooled RRs and their 95% CIs calculated using the restricted maximum likelihood model (Ranganathan et al. 2015). Furthermore, subgroup analyses were conducted based on the study design (observational studies and randomised clinical trials) to assess potential methodological sources of heterogeneity. Additionally, meta-regression models explored the effect of age and follow-up duration (as continuous moderating variables) on the pooled study outcomes, including risk of failure, root development and apical closure. Subgroup analyses were also planned based on treatment protocols and patient demographics; however, these analyses could not be performed due to insufficient data. Likewise, data on pulp responsiveness to sensitivity tests were compiled and reported narratively, as a meta-analysis was not feasible due to the impossibility of grouping studies.

All analyses were conducted using Stata statistical software (version 18.5; StataCorp, College Station, Texas, USA). A random effects method was adopted to account for methodological heterogeneity identified among studies. Heterogeneity was quantified using the I^2 statistic (Higgins et al. 2003). Moreover, publication bias was assessed by generating funnel plots and performing regression-based Egger tests for association models that included more than 10 studies. This threshold follows current methodological guidance (Egger et al. 1997; Higgins et al. n.d.; Sterne et al. 2011), which discourages the use of funnel plots and related statistical tests when fewer than 10 studies are available, due to the high risk of misleading results.

In cases of uncertainty or missing data, corresponding authors were contacted twice via email for clarification. When data remained unavailable after this step, the affected outcomes were excluded from the respective meta-analytic models. No statistical imputation methods were applied to avoid introducing bias into the pooled estimates.

2.8 | Certainty of Evidence Assessment

The certainty of evidence for the effect of pre-operative factors on REP outcomes was assessed using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach (Atkins et al. 2004). Two reviewers (F.C.V. and A.H.R.P.) independently conducted the assessment. Disagreements were resolved through discussion, and when consensus could not be reached, a third reviewer (P.S.S.) was consulted. The certainty assessment started 'high' for randomised clinical trials and 'low' for observational studies. This assessment could be downgraded due to concerns identified in the domains of 'risk of bias,' 'indirectness,' 'imprecision', 'inconsistency' and 'publication bias' or upgraded based on the magnitude of the effect and the presence of plausible residual confounding (Zhang et al. 2018). Risk of bias referred to the potential for systematic errors in the included studies and was evaluated using the RoB 2 tool for randomised clinical trials and the NOS for observational studies, as previously described (Guyatt, Oxman, Vist, Kunz, et al. 2011). Inconsistency was assessed based on variability in results across studies, including the I^2 statistic and the degree of overlap in confidence intervals (Guyatt, Oxman, Kunz, Woodcock, et al. 2011b). Indirectness considered differences in population, interventions or outcomes relative to the review question (Guyatt, Oxman, Kunz, Woodcock, et al. 2011a). Imprecision was evaluated based on the width of confidence intervals and whether sample sizes were adequate to produce reliable estimates (Guyatt, Oxman, Kunz, Brozek, et al. 2011). Publication bias was assessed either qualitatively or, when applicable, through funnel plot analysis and Egger's regression test (Guyatt, Oxman, Montori, Vist, et al. 2011). The overall certainty of the evidence was rated as 'very low', 'low', 'moderate' or 'high' using the GRADEpro Guideline Development Tool (McMaster University, Hamilton, Canada 2020).

3 | Results

3.1 | Literature Search

Figure 1 summarises the study selection process. The electronic search identified 5543 references. After removing 2515 duplicates, 3028 references were screened by title and abstract reading, resulting in 108 articles for full-text screening. Eighty-eight articles were excluded for different reasons (Table S2), leading to the inclusion of 20 studies (Alagl et al. 2017; Alfahadi et al. 2022; Bezgin et al. 2015; Botero et al. 2017; Bukhari et al. 2016;

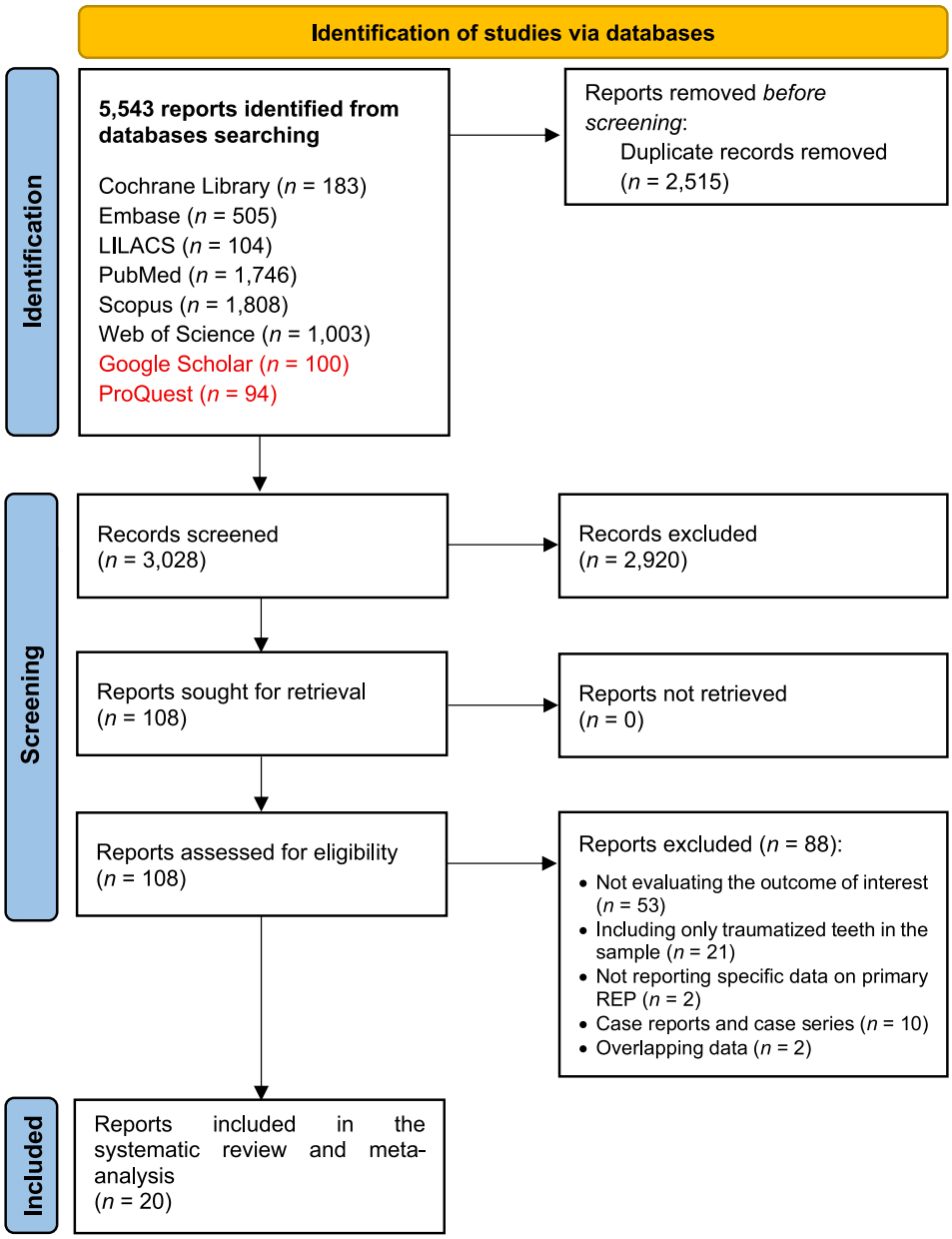

FIGURE 1 | PRISMA flow diagram of the study search and selection.

Caleza-Jiménez et al. 2022; Chatha 2017; Chrepa et al. 2020; Elheeny and Tony 2024; Estefan et al. 2016; Hu et al. 2023; Jeeruphan et al. 2012; Jiang and Liu 2023; Lin et al. 2017; Linsuwanont et al. 2017; Song et al. 2017; Theekakul et al. 2024; Yang et al. 2023; Yoshpe et al. 2022; Zeng et al. 2022).

3.2 | Characteristics of Included Studies

Table 1 presents the bibliographic details, sample characteristics and pre-operative factors reported in the included studies. Seven studies were randomised clinical trials, while 13 were observational (retrospective cohort) studies. The studies included 1250 patients recruited from university clinical settings. The age of patients ranged from 6 to 31 years, with 55% of studies including only patients under 16 years of age. A total of 1356 immature teeth underwent REPs, with the majority being incisors (50.8%). The most frequently reported causes of pulp necrosis were anatomic anomalies (46.5%), followed by trauma (32.8%) and dental caries (16.6%). The most reported stage of root maturation was characterised by roots that had formed more than half of their final length (86.9%) and exhibited an apical lesion of endodontic origin (89.5%). Additionally, among the studies that reported the presence of pre-operative clinical signs or symptoms, at least 86.1% of cases exhibited some form of involvement, with abscess or swelling being the most common signs (33.6%).

The REPs protocol varied across the included studies. Table 2 presents the main aspects of the clinical protocols and details about outcome assessment. Most studies (80%) followed the recommendations of the American Association of Endodontists (2021). Overall, variations were observed in irrigation protocols (sodium hypochlorite concentrations ranging from 1.5% to 6%), types of intracanal medicaments (double or triple antibiotic

TABLE 1 | Bibliographic details, sample characteristics and pre-operative conditions reported in the included studies.

Authors (year), country	Study design	Sample			Tooth type	Aetiology of pulp necrosis: n	Stage of root development	Perirradicular lesion: *n*	Clinical signs and symptoms: *n*
		Total	Gender	Age range					
Alagl et al. (2017), Saudi Arabia	RCT	15 patients, 30 teeth	6 females 9 males	8–11 y	24 incisors 6 premolars	Caries: 6 Trauma: 24	NR	No lesion: 5 Lesion < 2 mm: 9 Lesion 2–4 mm: 16	Pain: 26 Abscess: 9
Alfahadi et al. (2022), Saudi Arabia	RTP	27 patients, 27 teeth	12 females 15 males	10–22 y	14 incisors 13 posteriors (NS)	Caries: 12 Trauma: 15	NR	No lesion: 7 Lesion (NS): 16	Asymptomatic: 19 Pain: 7 Abscess: 1
Bezgin et al. (2015), Turkey	RCT	18 patients, 20 teeth	8 females 10 males	7–13 y	14 incisors 6 premolars	Caries: 6 Trauma: 14	NR	No lesion: 4 Lesion in the apical region only: 7 Lesion involving one third to one half of the root: 5 Lesion involving more than one half of the root: 4	Pain: 16 Abscess: 7
Botero et al. (2017), USA	RCT	25 patients, 28 teeth	17 females 8 males	6–25 y	22 incisors 5 premolars 1 M	Caries: 3 Trauma: 22 NS: 3	Nolla's stage 9: 9 Others: NR	NR	NR
Bukhari et al. (2016), USA	RTP	23 patients, 28 teeth	NR	8–31 y	21 incisors 5 premolars 2 M	Caries: 5 Trauma: 20 Anatomic anomaly: 3	NR	No lesion: 6 Lesion (NS): 22	Generic (NS): 18
Caleza-Jiménez et al. (2022), Spain	RCT	9 patients, 9 teeth	NR	7–10 y	3 incisors 6 M	Caries: 6 Trauma: 3	Cvek's stage 2: 2 Cvek's stage 3: 5 Cvek's stage 4: 2	NR	NR
Chatha (2017), USA	RTP	28 patients, 28 teeth	5 females, 23 males	7–15 y	21 incisors 5 premolars 2 M	NR	NR	NR	Generic (NS): 19

(Continues)

TABLE 1 | (Continued)

Authors (year), country	Study design	Sample			Tooth type	Aetiology of pulp necrosis: n	Stage of root development	Perirradicular lesion: *n*	Clinical signs and symptoms: *n*
		Total	Gender	Age range					
Chrepa et al. (2020), USA	RTP	51 patients, 51 teeth	25 females 26 males	7–26 y	38 incisors, 11 premolars, 2 M	Caries: 3 Trauma: 36 Anatomic anomaly: 12	NR	NR	NR
Elheeny and Tony (2024), Egypt	RCT	55 patients, 66 teeth	25 females, 30 males	Over 7 y (NS)	66 incisors	NR	NR	No lesion: 11 Lesion (NS): 55	Tenderness: 30 Sinus tract or swelling: 33 Tooth mobility: 32
Estefan et al. (2016), USA	RCT	35 patients, 35 teeth	15 females, 20 males	9–18 y	36 incisors	NR	NR	NR	NR
Hu et al. (2023), China	RTP	52 patients, 55 teeth	27 females, 28 males	6–14 y	25 incisors 30 premolars	Trauma: 22 Anatomic anomaly: 33	Cvek's stage 3: 25 Cvek's stage 4: 27 Cvek's stage 5: 3	No lesion: 20 Lesion (NS): 35	NR
Jeeruphan et al. (2012), Thailand	RTP	20 patients, 20 teeth	10 females, 10 males	8–24 y	7 incisors, 13 premolars	Caries: 1 Trauma: 7 Anatomic anomaly: 12	NR	No lesion: 1 Lesion (NS): 19	Generic (NS): 17
Jiang and Liu (2023), China	RTP	408 patients, 436 teeth	196 females, 212 males	6–16 y	136 incisors, 300 premolars	Caries: 8 Trauma: 121 Anatomic anomaly: 307	Nolla's stage 6: 2 Nolla's stage 7: 39 Nolla's stage 8: 291 Nolla's stage 9: 104	No lesion: 33 Lesion (NS): 403	NR
Lin et al. (2017), China	RCT	69 patients, 69 teeth	NR	8–16 y	21 incisors, 48 premolars	Trauma: 21 Anatomic anomaly: 48	NR	Lesion (NS): 69	NR

(Continues)

TABLE 1 | (Continued)

Authors (year), country	Study design	Sample			Tooth type	Aetiology of pulp necrosis: n	Stage of root development	Perirradicular lesion: *n*	Clinical signs and symptoms: *n*
		Total	Gender	Age range					
Linsuwanont et al. (2017), Thailand	RTP	15 patients, 15 teeth	9 females 6 males	7–23 y	5 incisors, 8 premolars, 2 M	Caries: 2 Trauma: 5 Anatomic anomaly: 8	NR	NR	Pain: 2 Abscess: 8
Song et al. (2017), South Korea	RTP	29 patients, 29 teeth	12 females, 17 males	8–18y	6 incisors, 21 premolars, 2 M	Caries: 2 Trauma: 6 Anatomic anomaly: 14 NS: 7	NR	No lesion: 4 Lesion (NS): 25	Pain: 15 Sinus tract: 12 Swelling: 2
Theekakul et al. (2024), Thailand	RTP	108 patients, 120 teeth	60 females, 54 males	Mostly 12 y (NS)	53 incisors, 67 posteriors (NS)	Caries: 72 Trauma: 48	Moorrees' stage 3: 18 Moorrees' stage 4: 70 Moorrees' stage 5: 32	No lesion: 4 Lesion < 5 mm: 61 Lesion > 5 mm: 55	Swelling: 64 Sinus tract: 42
Yang et al. (2023), China	RTP	107 patients, 121 teeth	62 females, 59 males	6–14 y	53 incisors, 62 premolars, 6 M	Caries: 8 Trauma: 51 Anatomic anomaly: 62	Nolla's stage 7: 11 Nolla's stage 8: 55 Nolla's stage 9: 55	NR	NR
Yoshpe et al. (2022), Israel	RTP	46 patients, 50 teeth	23 females, 23 males	7.6–16 y	36 incisors, 1 premolar 13 M	Caries: 14 Trauma: 16	Cvek's Class 3–5 (NS)	NR	NR
Zeng et al. (2022), China	RTP	110 patients, 116 teeth	58 females, 52 males	7–13 y	34 incisors, 82 premolars	Trauma: 34 Anatomic anomaly: 82	NR	NR	Abscess: 21 Pain: 23

Abbreviations: *n*, number; NR, not reported; NS not specified; RCT, randomised clinical trial; RTP, retrospective study; y, year.

TABLE 2 | Regenerative endodontic protocol and treatment outcome assessment.

	REP protocol						Outcome assessment		
Study	**Number of visits (interval)**	**Irrigation**	**Intracanal medication**	**Scaffold**	**Matrix**	**Capping material**	**Follow-up period**	**Method**	**Outcomes assessed**
Alagl et al. (2017)	Multiple (3 w)	First visit: 2.5% NaOCl (20 mL), saline (20 mL) and 0.12% CHX (10 mL). Second visit: 17% EDTA and saline (20 mL each)	TAP (metronidazole, ciprofloxacin and minocycline)	BC or PRP	None	MTA	1 y	CBCT scans	Success/failure Root development Pulp responsiveness to electric and cold tests
Alfahadi et al. (2022)	Multiple (NR)	2.5% NaOCl (20 mL), saline solution and/or EDTA	CH or TAP (metronidazole, ciprofloxacin and minocycline)	BC	None	MTA	6 m to 3 y	Periapical radiography	Success/failure Root development and apical closure
Bezgin et al. (2015)	Multiple (3 w)	First visit: 2.5% NaOCl (20 mL), saline (20 mL), and 0.12% CHX (10 mL). Second visit: 5% EDTA and saline (20 mL each)	TAP (metronidazole, ciprofloxacin and cefaclor)	BC or PRP	None	MTA	1.5 y	Periapical radiography	Success/failure Root development and apical closure Pulp responsiveness to electric and cold tests
Botero et al. (2017)	One or multiple (NR)	2.5% NaOCl (20 mL), saline (3 mL), and 17% EDTA (3 mL)	CH	BC	Collagen	MTA	1 y	Periapical radiography	Success/failure Root development and apical closure
Bukhari et al. (2016)	Three (NR)	First visit: 3% NaOCl. Second visit: 3% NaOCl and 17% EDTA	TAP (metronidazole, ciprofloxacin and clindamycin)	BC	None or collagen	Bioceramic putty (Endosequence) or MTA	7 m to 6 y	Periapical radiography	Success/failure
Caleza-Jiménez et al. (2022)	Two (NR)	1.5%–2.5% NaOCl and 17% EDTA	TAP (metronidazole, ciprofloxacin and minocycline)	BC	None	MTA	1–5.5 y	Periapical radiography	Success/failure Root development

(Continues)

TABLE 2 | (Continued)

	REP protocol						Outcome assessment		
Study	**Number of visits (interval)**	**Irrigation**	**Intracanal medication**	**Scaffold**	**Matrix**	**Capping material**	**Follow-up period**	**Method**	**Outcomes assessed**
Chatha (2017)	Two (2–4 w)	First visit: 1.5% NaOCl (20 mL), saline or EDTA (20 mL). Second visit: 17% EDTA (20 mL)	CH, DAP (metronidazole and ciprofloxacin) or TAP metronidazole, ciprofloxacin and minocycline)	BC	None	Bioceramic putty (NS) or MTA	3 m to 3y	Periapical radiography	Success/failure Root development
Chrepa et al. (2020)	One or two (2–4w)	First visit: 1.5%–6% NaOCl or NaOCl/saline and 2% CHX. Second visit: 6% NaOCl or 17% EDTA and/or 17% EDTA/saline	TAP (metronidazole, ciprofloxacin and minocycline), DAP (metronidazole and ciprofloxacin) or CH	BC	Collagen	MTA or Biodentine	1–8 y	Periapical radiography and CBCT	Success/failure Root development Pulp responsiveness to electric and cold tests
Elheeny and Tony (2024)	Multiple (3 w)	First visit: 1.5% NaOCl and saline (20 mL each). Second visit: 1.5% NaOCl, 0.9% saline and 17% EDTA (20 mL each)	CH	PRF or CGF	Collagen	Biodentine	1 y	Periapical radiography and CBCT	Success/failure Root development and apical closure Pulp responsiveness to electric and cold tests
Estefan et al. (2016)	Multiple (3w)	First visit: 2.6% NaOCl (10 mL). Second visit: 2.6% NaOCl and 17% EDTA (10 mL each)	TAP (metronidazole, ciprofloxacin and doxycycline)	BC	None	MTA	1 y	Periapical radiography	Success/failure Root development
Hu et al. (2023)	Two (2–3 w)	3% NaOCl and saline	TAP (metronidazole, ciprofloxacin and cefaclor)	BC	None	iRoot BP Plus	1–7.1 y	Periapical radiography	Success/failure Root development and apical closure

(Continues)

TABLE 2 | (Continued)

	REP protocol						Outcome assessment		
Study	**Number of visits (interval)**	**Irrigation**	**Intracanal medication**	**Scaffold**	**Matrix**	**Capping material**	**Follow-up period**	**Method**	**Outcomes assessed**
Jeeruphan et al. (2012)	Two (≈1 m)	2.5% NaOCl	TAP (metronidazole, ciprofloxacin and minocycline)	BC	Collagen	NR	≈21 m	Periapical radiography	Success/failure Root development
Jiang and Liu (2023)	Two (2–4 w)	First visit: 1.5%–3% NaOCl (20 mL) or NaOCl/saline (20 mL). Second visit: 17% EDTA (20 mL) or EDTA/saline	TAP (NS), DAP (NS) or CH	BC or PRF	Collagen	MTA or iRoot BP or glass ionomer	4 m to ≈4.5 y	Periapical radiography	Success/failure Root development and apical closure
Lin et al. (2017)	Two (3w)	First visit: 1.5% NaOCl (20 mL), saline, and 17% EDTA (20 mL). Second visit: saline and 17% EDTA (20 mL)	TAP (metronidazole, ciprofloxacin and clindamycin)	BC	Collagen	MTA	1y	Periapical radiography and CBCT	Success/failure Root development and apical closure
Linsuwanont et al. (2017)	Two (NR)	NaOCl and EDTA (NE)	TAP (metronidazole, ciprofloxacin and minocycline) or CH	BC	None	MTA	1–8 y	Periapical radiography and CBCT	Success/failure Root development and apical closure
Song et al. (2017)	Multiple (NR)	2.5% NaOCl	TAP (metronidazole, ciprofloxacin and minocycline), DAP (metronidazole, ciprofloxacin) or CH	BC	None	MTA	1–6y	Periapical radiography and CBCT	Success/failure Root development and apical closure
Theekakul et al. (2024)	Multiple (NR)	First visit: 1.5%–5.25% NaOCl (20 mL) and saline. Second visit: 17% EDTA and saline (20 mL each)	TAP (metronidazole, ciprofloxacin and minocycline) or CH	BC	Collagen	MTA or Biodentine	1–12 y	Periapical radiography	Success/failure Root development and apical closure Pulp responsiveness to electric and cold tests

(Continues)

TABLE 2 | (Continued)

	REP protocol						Outcome assessment		
Study	**Number of visits (interval)**	**Irrigation**	**Intracanal medication**	**Scaffold**	**Matrix**	**Capping material**	**Follow-up period**	**Method**	**Outcomes assessed**
Yang et al. (2023)	Multiple (NR)	0.5%–1.5% NaOCl and saline	TAP (metronidazole, ciprofloxacin and minocycline) or CH	BC or CGF	None	MTA or iRoot BP Plus	More than 6 m (NE)	Periapical radiography	Success/failure Root development Pulp responsiveness to electric and cold tests
Yoshpe et al. (2022)	Two (3–4 w)	First visit: 4% NaOCl. Second visit: 17% E DTA	TAP (metronidazole, ciprofloxacin and cefuroxime axetil)	PRF	Collagen	MTA or Biodentine	1–3y	Periapical radiography	Success/failure Root development and apical closure
Zeng et al. (2022)	Two (NR)	1.5% NaOCl and 17% EDTA	TAP (metronidazole, ciprofloxacin and clindamycin)	BC	Collagen	MTA	1 y	CBCT	Success/failure Root development and apical closure

Abbreviations: BC, blood clot; CBCT, cone-beam computed tomography; CGF, concentrated growth factor; CH, calcium hydroxide paste; CHX, chlorhexidine; DAP, double antibiotic paste; EDTA, ethylenediaminetetraacetic acid; m, month; MTA, Mineral Trioxide Aggregate; NaOCl, sodium hypochlorite; NR, not reported; NS, not specified; PRF, platelet-rich fibrin; PRP, platelet-rich plasma; TAP, triple antibiotic paste; w, week; y, year.

paste or calcium hydroxide paste), scaffolds [blood clot, platelet-rich fibrin (PRF), platelet-rich plasma (PRP) or concentrated growth factor], use of a collagen matrix and types of capping materials (MTA, Biodentine, Endosequence or iRoot BP Plus). Regarding outcome assessment, all studies evaluated clinical and radiographic healing. Additionally, 19 studies assessed root development, 12 studies reported complete apical closure information, and 6 studies evaluated pulp responsiveness to sensitivity tests. The methods used for outcome assessment included periapical radiography (65%), cone beam computed tomography (CBCT) (10%) and a combination of periapical radiography and CBCT (25%).

3.3 | Risk of Bias Assessment

Figure 2 summarises the risk of bias assessment for the included studies. Among the observational studies, 38.5% were classified as 'low risk of bias', 46% as 'moderate risk of bias' and 15.5% as 'high risk of bias'. All studies exhibited inconsistencies in specific domains, particularly in participant selection, where no study achieved the maximum score, and outcome assessment, where only 31% attained the highest score. Considering the randomised clinical trials, 71.5% were classified as 'high risk of bias', whereas 28.5% as 'low risk of bias'. The main reasons for a lower-quality assessment were

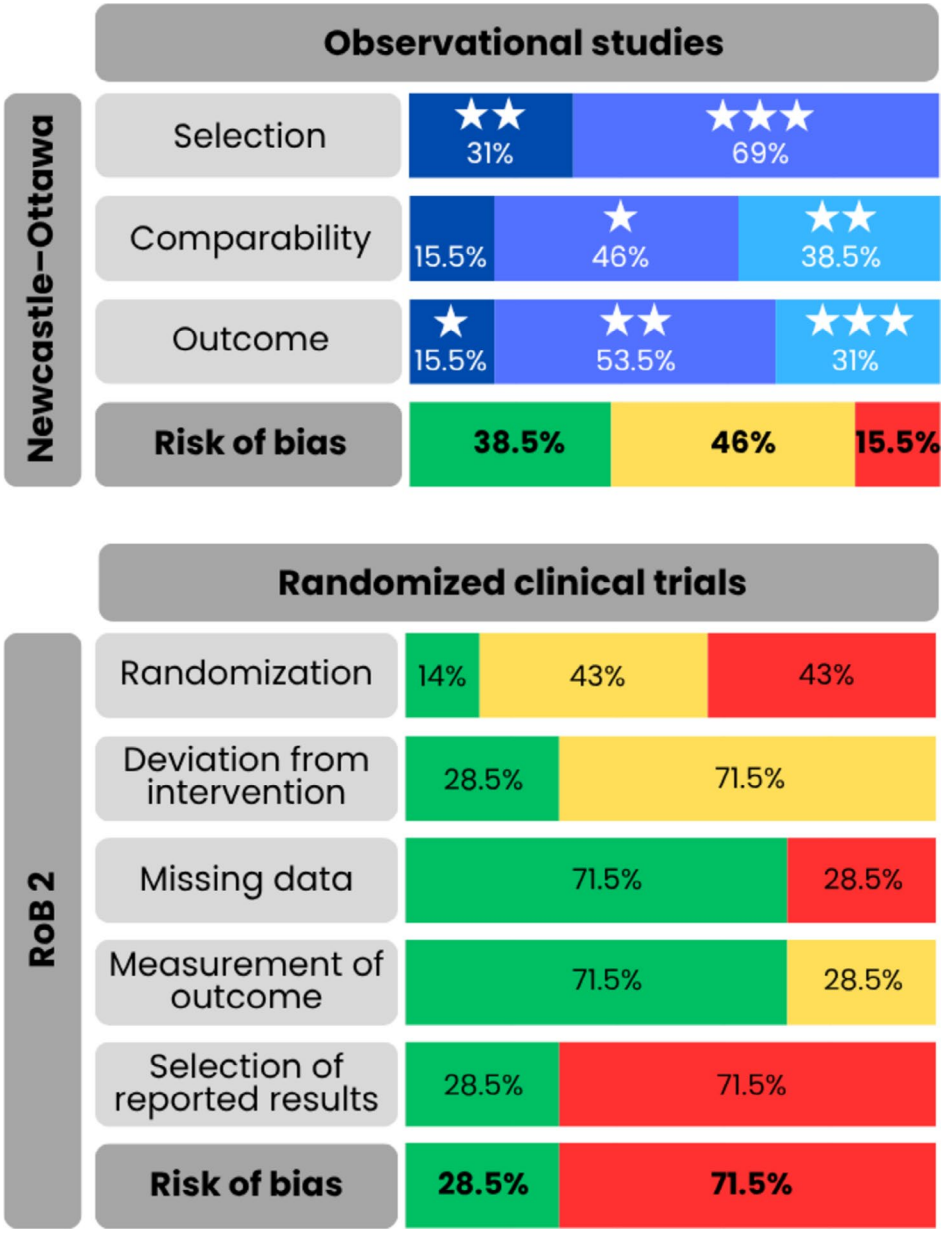

FIGURE 2 | Summary of the risk of bias assessment for observational studies [using the Newcastle–Ottawa Scale (NOS)] and randomised clinical trials [using the Cochrane Risk-of-Bias 2 tool (RoB 2)]. For observational studies, the risk of bias was evaluated based on three domains: Selection (maximum of 4 stars), comparability (maximum of 2 stars) and outcome assessment (maximum of 3 stars). Studies scoring 0–4 stars were classified as having a high risk of bias, 5–6 stars as moderate risk and 7–9 stars as low risk of bias. For randomised trials, the RoB 2 assesses five domains: The randomiSation process, deviations from intended interventions, missing outcome data, measurement of the outcome and selection of reported results. The overall risk of bias was categorised as low (green), some concerns (yellow) or high (red) for each domain.

potential biases identified in the domains of randomisation (14% of studies had a low risk of bias), deviations from the intended intervention (28.5% of studies had a low risk of bias) and selection of reported outcomes (28.5% of studies had a low risk of bias). The detailed evaluation of each study is available in Tables S3 and S4.

3.4 | Synthesis of Results

All included studies evaluated the outcome 'clinical and radiographic healing'. A total of 17 studies were included in the meta-analysis of proportions (Alagl et al. 2017; Alfahadi et al. 2022; Bezgin et al. 2015; Botero et al. 2017; Bukhari et al. 2016; Caleza-Jiménez et al. 2022; Chatha 2017; Chrepa et al. 2020; Elheeny and Tony 2024; Estefan et al. 2016; Hu et al. 2023; Jiang and Liu 2023; Lin et al. 2017; Linsuwanont et al. 2017; Song et al. 2017; Theekakul et al. 2024; Yang et al. 2023). Table 3 summarises the overall pooled estimates of clinical and radiographic healing according to different pre-operative factors, whereas Figures S1–S10 present the forest plots for each analysis. Overall, clinical and radiographic healing was consistently high across all analysed subgroups, with pooled proportions exceeding 81%.

A total of 15 studies were included in the association meta-analysis (Alagl et al. 2017; Alfahadi et al. 2022; Bezgin et al. 2015; Botero et al. 2017; Bukhari et al. 2016; Caleza-Jiménez et al. 2022; Chatha 2017; Chrepa et al. 2020; Elheeny and Tony 2024; Estefan et al. 2016; Hu et al. 2023; Jiang and Liu 2023; Linsuwanont et al. 2017; Song et al. 2017; Yang et al. 2023). This meta-analysis assessed the risk of failure of clinical and radiographic healing according to different pre-operative factors. Table 4 summarises the meta-analysis results, whereas Figures S11–S20 present the funnel plots for each analysis. Gender, age, aetiology of pulp necrosis, tooth type, stage of root development, clinical signs/symptoms and the presence of an apical lesion were not associated ($p > 0.05$) with the risk of failure of clinical and radiographic healing. Substantial heterogeneity ($I^2 = 73.86\%$) was observed in the

TABLE 3 | Overall pooled estimates (proportion [%] and 95% confidence intervals) of outcomes of regenerative endodontic procedures according to different pre-operative factors.

Subgroup	Clinical and radiographic healing	Root development	Apical closure
Gender			
Male	94% (92%–97%)	85% (74%–96%)	65% (36%–94%)
Female	96% (94%–99%)	76% (56%–95%)	47% (18%–66%)
Age			
< 10 years	96% (92%–100%)	95% (88%–100%)	58% (17%–98%)
11–16 years	96% (92%–100%)	86% (72%–100%)	32% (0%–78%)
Aetiology of pulp necrosis			
Caries	88% (80%–96%)	88% (76%–99%)	84% (67%–100%)
Trauma	89% (85%–94%)	72% (66%–78%)	61% (50%–71%)
Anatomic anomaly	81% (64%–98%)	92% (84%–99%)	77% (61%–93%)
Tooth type			
Incisors	93% (90%–97%)	52% (56%–87%)	48% (29%–68%)
Premolars	97% (96%–99%)	83% (79%–87%)	73% (46%–100%)
Molars	89% (78%–100%)	89% (74%–100%)	60% (16%–100%)
Stage of root development			
Half-formed	87% (64%–100%)	—[a]	—[a]
More than 2/3 formed	94% (91%–97%)	—[a]	—[a]
Clinical signs/symptoms			
Presence	96% (92%–100%)	75% (56%–93%)	65% (50%–80%)
Absence	96% (92%–100%)	86% (72%–99%)	88% (68%–100%)
Apical lesion			
Presence	96% (94%–98%)	77% (73%–81%)	76% (71%–81%)
Absence	97% (93%–100%)	90% (81%–100%)	91% (82%–100%)

[a]No data available for grouping.

TABLE 4 | Meta-analysis of the association between pre-operative predictors and failure of the primary outcome (clinical and radiographic healing) in regenerative endodontic procedures according to the different subgroups' characteristics.

Subgroup	RR (95% CI)	*p*	I^2
Gender			
Male vs. Female	1.48 (0.83–2.65)	0.19	0%
Age			
< 10 year vs. 11–16 year	0.60 (0.09–4.13)	0.61	26.79%
Aetiology of pulp necrosis			
Trauma vs. caries	0.65 (0.31–1.37)	0.26	19.37%
Anatomic anomaly vs. caries	0.58 (0.24–1.40)	0.22	15.35%
Trauma vs. anatomic anomaly	1.23 (0.37–4.03)	0.74	73.86%
Tooth type[a]			
Incisors vs. premolars	2.08 (0.79–5.47)	0.14	0%
Incisors vs. molars	0.58 (0.19–1.80)	0.35	0%
Stage of root development			
Half-formed vs. more than 2/3 formed	0.62 (0.31–1.25)	0.18	0%
Clinical signs/symptoms			
Presence vs. absence	1.81 (0.57–5.70)	0.31	0%
Apical lesion			
Presence vs. absence	0.70 (0.19–2.60)	0.59	0%

Abbreviations: CI, confidence interval; RR, risk ratio.
[a]Comparison between premolars and molars was not performed due to insufficient data available for grouping.

'aetiology of pulp necrosis' (trauma vs. anatomic anomaly). The subgroup analysis revealed that part of the heterogeneity was attributed to the study design (Figure S15). Across other analyses, no substantial heterogeneity was detected ($I^2 < 26.79\%$).

Seventeen studies assessed the outcomes 'root development' and 'complete apical closure'. A total of 13 studies were included in the meta-analysis of proportions (Alagl et al. 2017; Alfahadi et al. 2022; Bezgin et al. 2015; Caleza-Jiménez et al. 2022; Chatha 2017; Elheeny and Tony 2024; Hu et al. 2023; Jiang and Liu 2023; Lin et al. 2017; Linsuwanont et al. 2017; Song et al. 2017; Yoshpe et al. 2022; Zeng et al. 2022). Table 3 presents the pooled estimates of both outcomes according to different pre-operative factors, and Figures S21–S34 present the forest plots for each analysis. Overall, root development was achieved in 52%–95% of cases, while complete apical closure ranged from 32% to 91%.

A total of eight studies were included in the association meta-analysis (Alagl et al. 2017; Alfahadi et al. 2022; Bezgin et al. 2015; Caleza-Jiménez et al. 2022; Chatha 2017; Jiang and Liu 2023; Linsuwanont et al. 2017; Song et al. 2017). The meta-analysis assessed the risk of failure of root development or not completing apical closure after REP based on the available pre-operative factors. Table 5 summarises the results, and Figures S35–S46 present the funnel plots for each analysis. A significant association was observed in the comparison between trauma versus anatomic anomalies, where teeth with pulp necrosis due to trauma presented 3.59 times higher relative risk of root development failure than teeth with pulp necrosis caused by an anatomic anomaly (RR = 3.59, 95% CI 1.21–10.67, $p = 0.02$). Moreover, incisors presented 1.90 times higher relative risk of root development failure (RR = 1.90, 95% CI: 1.37–2.62, $p < 0.01$) and 1.98 times higher relative risk of incomplete apical closure (RR = 1.98, 95% CI: 1.34–3.13, $p = 0.02$) than premolars. A significant association was also detected for the presence of apical lesions. Immature teeth presenting apical lesions presented 2.55 times higher relative risk of root development failure than teeth without an apical lesion (RR = 2.55, 95% CI: 1.63–4.86, $p = 0.01$). Substantial heterogeneity was detected for gender ($I^2 = 53.11\%$). The subgroup analysis revealed that part of the heterogeneity was attributed to the study design (Figure S35). For other variables, no substantial heterogeneity was detected ($I^2 < 20.74\%$).

Exploratory meta-regression models were used to test the effects of age and follow-up duration on REP outcomes. As shown in Table 6, neither age nor follow-up duration demonstrated a statistically significant association with failure of clinical and radiographic healing, root development or apical closure (*p*-values > 0.10). Residual heterogeneity (I^2) ranged from 11% to 22% across models.

Six studies assessed pulp responsiveness to electric and cold tests after REP (Alagl et al. 2017; Bezgin et al. 2015; Chrepa et al. 2020; Elheeny and Tony 2024; Theekakul et al. 2024; Yang et al. 2023). The overall positive responsiveness ranges from 7.5% to 63.5% in the included studies. None of the studies presented significant associations between pre-operative factors and pulp sensitivity responsiveness rates.

Publication bias was assessed only in the model evaluating the association between the aetiology of pulp necrosis (trauma vs. caries) and REP failure, as it was the only model that included at least 10 studies. Visual inspection of the funnel plot (Figure S47) and Egger's linear regression test ($p = 0.29$) did not indicate potential publication bias.

3.5 | Certainty Assessment

The certainty of evidence for the effect of pre-operative factors on REP outcomes was rated as very low for all factors. The main reasons for this rating were the predominance of observational studies, substantial risk of bias identified in the included studies,

TABLE 5 | Meta-analysis of the association between pre-operative predictors and failure of root development and complete apical closure in regenerative endodontic procedures according to the different subgroups' characteristics.

	Root development			Complete apical closure		
Subgroup	**RR (95% CI)**	***p***	I^2	**RR (95% CI)**	***p***	I^2
Gender						
Male vs. female	0.70 (0.24–2.04)	0.51	20.74%	1.29 (0.81–2.06)	0.28	53.11%
Age						
< 10 years vs. 11–16 years	0.96 (0.18–5.04)	0.86	0%	—[a]		
Aetiology of pulp necrosis						
Trauma vs. caries	1.09 (0.49–2.40)	0.33	0%	1.29 (0.49–3.43)	0.61	16.44%
Anatomic anomaly vs. caries	0.59 (0.25–1.36)	0.21	0%	—[a]		
Trauma vs. anatomic anomaly	3.59 (1.21–10.67)	**0.02**	16.55%	—[a]		
Tooth type						
Incisors vs. premolars	1.90 (1.37–2.62)	**< 0.01**	0%	1.98 (1.34–3.13)	**0.02**	16.35%
Incisors vs. molars	2.13 (0.47–9.55)	0.32	0%	—[a]		
Clinical signs/symptoms						
Presence vs. absence	0.94 (0.46–1.93)	0.87	0%	—[a]		
Apical lesion						
Presence vs. absence	2.55 (1.63–4.86)	**0.01**	14.75%	—[a]		

Note: Values in bold indicate a statistically significant association ($p < 0.05$).
Abbreviations: CI, confidence interval; RR, risk ratio.
[a]No data available for grouping.

TABLE 6 | Meta-regression for the effect of age and follow-up duration (continuous variables) on primary outcome failure, root development and apical closure.

Outcome	No. of studies	β (95% CI)	*p*	I^2
Age				
Primary outcome failure	12	0.19 (−0.08 to 0.47)	0.15	22.59%
Root development	9	0.28 (−0.27 to 0.82)	0.28	
Apical closure	5	0.63 (−0.68 to 1.40)	0.12	14.99% 11.23%
Follow-up duration				
Primary outcome failure	14	0.21 (−0.11 to 0.44)	0.17	12.33%
Root development	10	0.37 (−0.31 to 1.09)	0.21	19.69%
Apical closure	9	0.44 (−0.52 to 1.22)	0.24	11.39%

Abbreviation: CI, confidence interval.

small effect sizes accompanied by wide confidence intervals in the meta-analysis and limited sample sizes across studies. Table S5 details the reviewers' judgments for each domain based on the GRADE approach.

4 | Discussion

REP has emerged as a contemporary treatment modality for immature permanent teeth with a necrotic dental pulp. Previous systematic reviews have indicated that the overall success rate of REP exceeds 80% (Sabeti et al. 2024; Yang et al. 2024). However, despite numerous reviews investigating REP outcomes from a one-sided approach focused on treatment protocols, the evidence on the pre-operative factors that may influence treatment outcomes is limited. The present review found that gender, age, aetiology of pulp necrosis, tooth type, stage of root development, pre-operative signs and symptoms and the presence of an apical lesion were not significantly associated with clinical and radiographic healing. However, teeth with pulp necrosis due to trauma, incisors and those presenting apical lesions exhibited a higher risk of root development failure.

The primary goal of REPs includes the resolution of clinical signs and symptoms of infection and radiographic evidence of bone healing (American Association of Endodontists 2021). The quantitative analysis showed that the investigated pre-operative factors were not significantly associated with clinical and radiographic healing. These findings suggest that REP success may be more dependent on the bioactive properties of the pulp-dentin complex and the treatment protocol rather than some demographic and clinical variables. Previous studies have demonstrated that the success of REPs primarily depends on effective infection control and the creation of a favourable environment for tissue regeneration, with the resolution of clinical signs and symptoms of infection and bone

healing generally being achievable (Duncan et al. 2019; Kim et al. 2018; Li et al. 2024; Wikström et al. 2022). Therefore, treatment failure is often attributed to inadequate disinfection protocols (Kim et al. 2018; Wikström et al. 2022; Zymovets et al. 2023). These results suggest that REP is clinically applicable across a broad spectrum of cases, reinforcing its viability as a treatment option regardless of individual patient characteristics if infection control is achieved.

The success of regenerative endodontics is not solely defined by the resolution of clinical signs and symptoms of infection and evidence of radiographic healing but also by its potential to promote continued root development (root canal walls thickening and/or root lengthening) and apical closure (American Association of Endodontists 2021). Continued root formation is particularly desirable in immature teeth, as it strengthens the thin and fragile root structure, reducing the risk of fractures and improving long-term tooth survival (Murray 2023). Although studies report high rates of root development following REP, root maturation remains unpredictable (Kim et al. 2018). This unpredictability is primarily attributed to disruptions in the interaction between Hertwig's epithelial root sheath (HERS) and mesenchymal stem cells in the apical follicle, which play a fundamental role in root formation by regulating stem cells differentiation into odontoblast-like cells responsible for root dentin deposition (Li et al. 2017). Therefore, any factor that compromises the integrity or function of HERS can negatively impact root development (Li et al. 2017).

The meta-analysis results indicate that pulp necrosis due to trauma significantly impairs root development following REPs, increasing the relative risk of root development failure by 3.59 times compared to pulp necrosis caused by anatomical anomalies. This effect is likely due to the high risk of mechanical disruption of HERS during traumatic injury, compromising its ability to regulate mesenchymal stem cell differentiation and root formation (Li et al. 2017). These findings align with previous studies indicating that root maturation after REP is less predictable in traumatised teeth, likely due to structural damage and the resulting biological impairment that compromises the regenerative potential of periradicular tissues (Cheng et al. 2022; Swaikat et al. 2023). Furthermore, the meta-analysis found no significant association between root development and the aetiology of pulp necrosis when comparing trauma versus caries. However, the smaller number of patients and studies included in this comparison may have influenced the total effect size. The meta-analysis also reveals that incisors had a higher relative risk of root development failure and were less likely to achieve apical closure compared to premolars. This difference appears to be primarily related to the aetiology of pulp necrosis, as incisors are more frequently exposed to direct trauma, increasing the possibility of severe damage to HERS and periradicular tissues and ultimately compromising root development and apical closure (Swaikat et al. 2023; Cheng et al. 2022).

The meta-analysis indicates that the presence of an apical lesion presented a 2.55 times higher relative risk of root development failure compared to teeth without apical pathology. This association may be explained by the severity and duration of apical periodontitis in immature necrotic permanent teeth, which appear to play a crucial role in determining the viability of HERS. Prolonged inflammation can cause irreversible damage to this essential structure, compromising its ability to regulate root development (Li et al. 2017). Chronic inflammation in the apical region can also disrupt the biological function of mesenchymal stem cells, impairing their differentiation and limiting their ability to contribute to root development (Li et al. 2017; Verma et al. 2017). Moreover, histological investigations have demonstrated that residual persistent bacteria, often associated with apical lesions, negatively impact the deposition of mineralized tissue (Reis-Prado et al. 2025; Verma et al. 2017; Zymovets et al. 2023). The presence of bacteria sustains an inflammatory microenvironment, triggering the continuous release of pro-inflammatory cytokines and disrupting the balance of M1/M2 macrophage phenotypes, which interfere with tissue repair and regeneration (Reis-Prado et al. 2025; Verma et al. 2017; Zymovets et al. 2023). These findings support the idea that successful root development following REP depends not only on the presence of viable stem cells and intact HERS but also on effective infection control.

Despite providing insights, the findings of the meta-analysis should be interpreted with caution due to certain limitations. High between-study heterogeneity, limited data available for subgroup comparisons and small sample sizes in some studies contribute to imprecision in effect estimates. Regarding heterogeneity, notable variability was observed in specific analyses, as reflected in the I^2 statistic and the wide confidence intervals. Although we attempted to explore sources of heterogeneity through subgroup and meta-regression analyses, these approaches were limited by insufficient data. Subgroup analysis was feasible only due to the study design, and meta-regression models could be applied solely to age and follow-up duration. Nonetheless, the observed heterogeneity likely reflects a complex interplay of methodological and clinical factors. In some analyses, part of the heterogeneity appeared to be associated with study design. Prospective studies typically employed more rigorous sampling methods and clearer reporting standards. In contrast, retrospective designs may have introduced selection bias or inconsistencies in documenting and analysing baseline characteristics. Furthermore, several studies reported pooled outcome data without stratification by critical clinical variables, hindering efforts to isolate the impact of specific prognostic factors. Clinical protocols also varied considerably among studies, including differences in case selection criteria and treatment protocols. For instance, six studies included samples composed of both children and individuals over 18 years old (Alfahadi et al. 2022; Botero et al. 2017; Bukhari et al. 2016; Chrepa et al. 2020; Linsuwanont et al. 2017; Jeeruphan et al. 2012). This heterogeneous age range may have compromised the validity of comparisons, as patients from different age groups are not directly comparable due to inherent anatomical and biological differences. Hence, this improper comparison may introduce bias in estimating the true effect of the intervention.

Additionally, many studies applied inclusion and exclusion criteria based on factors such as age, root development stage and tooth type, which may have reduced variability in the analysed populations and limited the ability to detect potential associations with treatment outcomes. These variations may, in turn, influence the overall efficacy of REPs and their associations with pre-operative factors, thereby further contributing to the observed heterogeneity. Finally, conducting sensitivity analyses

was not feasible, as the limited number of studies included in most models precluded a meaningful re-evaluation following the exclusion of high-risk studies. Additionally, a substantial proportion of the included studies (35%) were assessed as having a high risk of bias, further emphasising the necessity for cautious interpretation of the synthesised evidence.

In terms of clinical protocol, variations in irrigation or intracanal medication appear to have a lower impact on REP outcomes, considering that they are sufficiently effective in reducing bacterial load and eliminating clinical signs and symptoms of infection (Reis-Prado et al. 2025, Sellami et al. 2023). All included studies met these objectives before the second treatment session, which involved either blood clot induction or the use of autologous scaffolds. The influence of scaffold type on treatment outcomes remains inconclusive, with recent systematic reviews reporting conflicting results (Rahul et al. 2023; Sabeti et al. 2024; Yang et al. 2024). Rahul et al. (2023) and Yang et al. (2024) demonstrated that intracanal scaffolds used during REPs did not significantly differ in clinical and radiographic healing, root maturation or pulp sensibility. However, Sabeti et al. (2024) reported that PRP and PRF achieved the highest success rates in both primary and secondary outcomes up to 12 months compared to the traditional blood clot scaffold, although long-term follow-up data were limited. Therefore, heterogeneity in treatment protocols across studies must be recognised as a potential confounding factor, requiring caution when interpreting the results.

In this systematic review, the methodology of the included studies was critically evaluated based on study design and described in terms of the risk of bias. Among the observational studies, two (15.5%) were judged to have a high risk of bias, while six (46%) were considered to have a moderate risk of bias. In the selection domain, the main reason for downgrading was the exclusion of patients due to incomplete records in four studies, which may have compromised sample representativeness and potentially introduced selection bias. In the comparability domain, the primary reason for downgrading in eight studies was the inappropriate handling of pre-operative factors, such as the lack of stratification or regression analysis to assess their potential effects on outcomes, potentially compromising between-group comparability. Regarding the outcome domain, the main sources of bias in some studies were unclear descriptions of evaluator training or calibration for outcome assessment, failure to report dropout rates and high dropout rates without appropriate handling.

Among the randomised clinical trials, five out of seven (71.5%) were judged to have a high overall risk of bias. Regarding randomisation, three studies did not describe the randomisation process based on allocation concealment, leading to some concerns in this domain. Additionally, in some trials, randomisation was performed per patient, including more than one tooth from the same individual. This gave certain patients multiple opportunities to be assigned to different groups and potentially introduced bias in the allocation process. Another major reason for the high risk of bias was the absence of a registered protocol in five studies. The lack of a registered protocol raises concerns about potential deviations from the intended intervention and contributes to a high risk of bias in the reporting of selective outcomes. Minor limitations were identified in the outcome assessment, including the absence of blinding in two studies and a high dropout rate in one study, with no reported approach to handle the missing data.

The certainty of evidence for the effect of pre-operative predictors on REP outcomes was deemed very low based on the GRADE approach. Several factors contributed to this low grading, primarily potential limitations due to the nature of the included studies, risk of bias, and imprecision of the results. In observational studies, the initial evidence rating is low, whereas for randomised clinical trials, it started as high. However, the main reasons for downgrading certainty were shared between both study types, mainly due to methodological concerns identified in the risk of bias assessment. Only 38.5% of observational studies and 28.5% of randomised clinical trials were deemed as having a low risk of bias. Consequently, this domain was judged as very serious or serious across all pre-operative factors. Additionally, the small number of included studies and the limited sample size in some studies contributed to a lack of precision in the results, leading to wide confidence intervals and further downgrading the certainty of evidence due to imprecision.

The present review has limitations to discuss. The primary limitation is that the evidence emerges with very low certainty, primarily due to methodological concerns identified in the included studies. A significant proportion of studies exhibited a moderate to high risk of bias, which reduces confidence in the findings. Moreover, a predominant focus on treatment protocols rather than pre-operative factors was observed in randomised clinical trials. In most trials, pre-operative variables were analysed as secondary outcomes, limiting the depth of analysis and making the findings more exploratory than conclusive. Another key limitation was the inclusion of few studies in some meta-analyses, which led to imprecision in effect estimates. Limited sample sizes and wide confidence intervals reduced statistical power, making it difficult to draw significant conclusions. Publication bias was another concern, but it could not be fully assessed due to the small number of studies included in some analyses, as funnel plots and Egger's test are only considered reliable when at least 10 studies are included (Egger et al. 1997; Higgins et al. n.d.; Sterne et al. 2011). Heterogeneity in clinical protocols is another limitation. Studies varied in irrigation and intracanal medication protocols, scaffolds, follow-up duration and outcome assessment methods. This lack of standardisation increases uncertainty and limits the generalisability of results.

Despite its limitations, this review has important strengths. To the best of our knowledge, this is one of the first systematic reviews to provide both qualitative and quantitative analyses of the effect of pre-operative predictors on REP outcomes. By pooling data from multiple primary studies, the meta-analysis enhances the final sample size, increases statistical power and improves effect size estimates (Muka et al. 2020). This is particularly relevant in the context of REP research, where individual studies often have small sample sizes due to the inherent challenges of recruiting large cohorts. Additionally, subgroup analyses and meta-regression tests improve the precision of estimates while reducing methodological bias (Muka et al. 2020). Another key strength is the rigorous methodology applied throughout all stages of this review, particularly in assessing methodological

quality. To ensure a comprehensive evaluation, robust tools were employed for risk of bias assessment: RoB 2, endorsed by the Cochrane group, for randomised clinical trials, and the NOS for observational studies tool (Stang 2010; Sterne et al. 2019). Additionally, the certainty of evidence was evaluated using the GRADE approach, as recommended by the Cochrane Collaboration (Muka et al. 2020). The review was conducted and reported following PRISMA guidelines, ensuring transparency and methodological robustness. Furthermore, a comprehensive approach was adopted for study identification and data retrieval, incorporating multiple databases and grey literature with no restrictions on date or language. This approach minimised the risk of publication bias and increased the inclusion of relevant studies.

Based on the identified limitations and findings, several recommendations are proposed for future research. First, well-designed prospective cohort studies with large samples are recommended to evaluate the effect of pre-operative factors on REP outcomes over time. To ensure valid and reliable findings, these studies must standardise treatment protocols, minimising confounding variables and isolating the true impact of prognostic factors. Methodological rigour is also essential, including the precise classification of exposed and non-exposed groups, the implementation of blinding, and comprehensive evaluator training to enhance reliability and reproducibility. Additionally, long-term follow-up studies are crucial for assessing REP outcomes over time. For clinical trials investigating different treatment protocols, sample stratification based on pre-operative factors is strongly recommended. Specific factors, particularly those that influence root development, may impact treatment success. Stratification will enable a more nuanced understanding of these variables, thereby improving the interpretation of the findings. By addressing these methodological considerations, future research can produce more robust evidence, ultimately refining clinical decision-making and enhancing patient-centred outcomes in regenerative endodontics.

5 | Conclusion

Pre-operative factors were not significantly associated with clinical and radiographic healing in REPs. However, trauma-related pulp necrosis, tooth type (incisors) and the presence of apical lesions were associated with an increased risk of root development failure. These findings should be interpreted with caution due to between-study heterogeneity, the moderate to high risk of bias and the very low certainty of the evidence. Future high-quality studies with larger samples are needed to clarify the prognostic value of pre-operative factors in REPs.

Author Contributions

All authors have made significant contributions to the conception, design, analysis and/or interpretation of data for this manuscript. All the authors approved the final version of the manuscript.

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Disclosure

Protocol Registration: International Prospective Register of Systematic Reviews (PROSPERO) database (code CRD42024600746).

Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figures S1–S47:** iej70025-sup-0001-FigureS1-S47.pdf. **Tables S1–S5:** iej70025-sup-0002-TableS1-S5.docx.