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Originalarticle

How Does the Risk of Iron-deficiency Anemia Compare between Exclusively Breastfed and Non-exclusively Breastfed Infants?

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Abstract
Exclusive breastfeeding (EBF) for the first 6 months of life is recommended worldwide for optimal infant health. However, the comparative efficacy of breast milk versus iron-fortified formula in preventing iron-deficiency anemia (IDA) remains debated due to breast milk's lower total iron content. This meta-analysis aims to evaluate the risk of anemia in exclusively breastfed infants compared to those receiving mixed or formula feeding (MF/FF) during the first year of life. A systematic literature search was conducted across PubMed, Scopus, and Web of Science. Observational studies comparing anemia risk or hemoglobin (Hb) levels between EBF and MF/FF infants were included. Data were pooled using a random-effects model to calculate Odds Ratios (OR) and Mean Differences (MD). Subgroup and sensitivity analyses were performed to explore heterogeneity. 17 studies were included. The overall pooled analysis for anemia risk (n=4869) showed a non-significant trend towards higher risk in the EBF group (OR = 1.58, 95% CI: 0.91–2.75, p=0.105). However, subgroup analysis by age revealed a critical divergence: EBF was protective/safe in infants <6 months (OR = 1.05, p=0.83) but doubled the risk of anemia in infants ≥6 months (OR = 2.10, 95% CI: 1.29–3.40, p=0.002). Sensitivity analysis demonstrated that excluding one large study of 4-month-old infants shifted the overall results to high statistical significance (OR = 1.88, p=0.001). The pooled mean difference in Hb levels was clinically negligible (MD = -0.09 g/dL, p=0.52). EBF provides adequate iron status during the first six months of life. However, prolonged EBF beyond six months without the timely introduction of iron-rich complementary foods significantly increases the risk of iron-deficiency anemia. Adherence to guidelines for timely complementary feeding is crucial globally.
Keywords. Exclusive Breastfeeding, Iron-deficiency Anemia, Hemoglobin, Infant Nutrition, Complementary Feeding.

Introduction

Infant nutrition is a critical global concern for humanitarian and economic reasons [1]. Numerous international organizations, such as the World Health Organization (WHO) and UNICEF, are dedicated to promoting optimal infant nutrition practices worldwide [2]. A cornerstone of their guidance is the recommendation of exclusive breastfeeding (EBF) for the first six months of life [3]. EBF has been shown to significantly reduce infant mortality and morbidity and to enhance cognitive development [4]. Anemia, namely iron-deficiency anemia (IDA), is a common and serious nutritional concern for infants and young children [5, 6]. It is characterized by a decrease in hemoglobin (Hb), the oxygen-carrying component of the blood, to a level below 11 g/dL according to WHO standards. As the most prevalent type of anemia worldwide, IDA affects a large proportion of infants and young children [7]. Its consequences are serious and often irreversible, including impaired cognitive and motor development and compromised immunity [8, 9]. Fortunately, IDA is a preventable and treatable disorder that, when diagnosed early, can be corrected with an excellent prognosis [10].

In infants and young children, inadequate dietary iron intake is a primary cause of IDA [11]. During the first months of life, infants are entirely dependent on milk, whether human breast milk, infant formula, or, less ideally, cow's milk, as their source of nutrition [12]. However, contrary to containing similar amounts, these milk sources differ significantly in both their iron content and bioavailability [13]. Breast milk, while containing iron with high bioavailability due to the presence of lactoferrin, has a relatively low total iron concentration [14]. In contrast, infant formula is typically fortified with higher levels of iron, though its bioavailability is lower [15]. This critical difference lies at the heart of the ongoing debate regarding the risk of IDA in exclusively breastfed infants.

Despite the WHO's endorsement of exclusive breastfeeding (EBF) for the first six months, its comparative efficacy against iron-fortified infant formula in preventing iron-deficiency anemia (IDA) remains a subject of ongoing research and debate. A key point of contention stems from a fundamental discrepancy: infant formula is explicitly fortified with iron to meet or exceed the recommended dietary allowance (RDA), providing a consistent, high-dose supply [16]. In contrast, breast milk, while celebrated for its physiological optimization and highly bioavailable iron, contains a lower total iron concentration [17]. This dichotomy creates a critical research gap, as it is unclear whether the quality (high bioavailability) of iron in breast milk consistently outweighs the quantity (high concentration) of iron in fortified formula for maintaining adequate iron status in infants under six months. This meta-analysis aims to determine whether exclusive breastfeeding (EBF) is associated with a higher risk of iron-deficiency anemia in infants up to 12 months of age, compared to non-exclusive breastfeeding (i.e., formula-feeding or mixed feeding).

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Methods

Study design

This was a systematic review and meta-analysis of observational studies. The study was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 1).

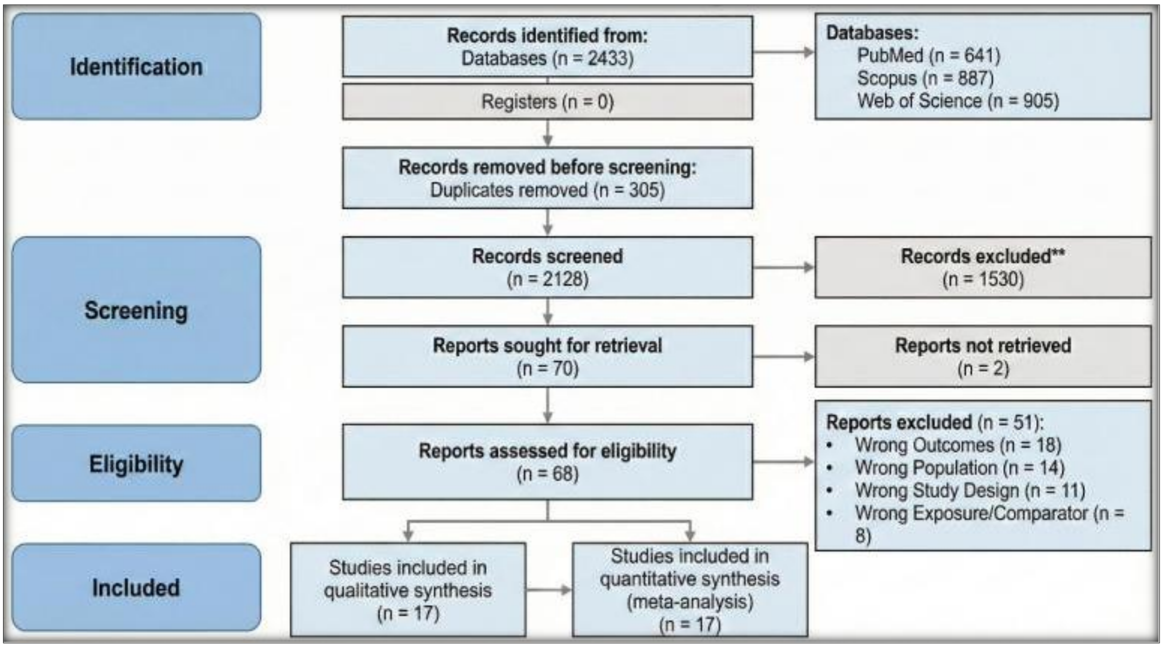

Figure 1. PRISMA 2020 Flow Diagram

Eligibility Criteria

Study eligibility was defined using the PICOS (Population, Intervention, Comparison, Outcomes, Study design) framework (Table 1).

Table 1. PICOS Criteria for Study Eligibility and Design

Component	Description
Population	▪ Infants aged < 8 months. ▪ Preterm infants, infants with congenital conditions, major illnesses, or a history of blood transfusion were excluded.
Intervention / Exposure	▪ Exclusive breastfeeding (EBF) as defined by the WHO: intake of only breast milk, except for oral rehydration solution and drops or syrups of vitamins, minerals, or medicines.
Comparison	▪ Non-exclusive breastfeeding, including infants fed iron-fortified formula either exclusively or as part of mixed feeding.
Outcomes	▪ The primary outcome was the prevalence of iron-deficiency anemia (IDA), defined as a hemoglobin (Hb) level below 11 g/dL. Secondary outcomes included mean hemoglobin concentration (g/dL)
Study Design	▪ Observational studies, including cohort and cross-sectional designs. ▪ Case reports, case series, reviews, and editorials were excluded.

Search Strategy and Information Sources

A systematic literature search was performed in three major electronic databases: PubMed/MEDLINE, Scopus, and Web of Science. The search strategy combined keywords, free-text words, and Medical Subject Headings (MeSH) terms tailored to each specific database. The search strings focused on three main domains: the population (infants), the exposure (exclusive breastfeeding), and the outcomes (anemia or iron deficiency parameters). The exact search strings and the initial yield from each database were as follows:

PUBMED (641 records): ("Infant"[Mesh] OR "Infant"[All Fields] OR "Newborn"[All Fields] AND ("Breast Feeding"[Mesh] OR "Exclusive Breastfeeding"[All Fields] OR "Breast Milk"[All Fields] OR "EBF"[All Fields] AND ("Anemia, Iron-Deficiency"[Mesh] OR "Iron Deficiency Anemia"[All Fields] OR "Iron Deficiency"[All Fields] OR "IDA"[All Fields] OR "Hemoglobin"[All Fields] OR "Ferritin"[All Fields]).

Scopus (887 records): ("Infant" OR "Newborn" OR "Baby" OR "Infancy") AND ("Breast Feeding" OR "Exclusive Breastfeeding" OR "Breast Milk" OR "EBF") AND ("Anemia" OR "Iron Deficiency" OR "IDA" OR "Hemoglobin" OR "Ferritin").

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Web of Science (905 records): (Infant OR Newborn OR Baby) AND (Breast Feeding OR EBF OR Breast Milk) AND (Anemia OR Iron Deficiency OR Hemoglobin). In addition to the electronic database searches, the reference lists of all included studies and relevant review articles were manually screened to identify any additional eligible publications.

Study Selection Process

The study selection process was conducted in two stages in accordance with PRISMA guidelines. First, all retrieved records from the three databases (a total of 2433 initial records) were imported into reference management software EndNote 21, where duplicate publications were automatically and manually removed. Following deduplication, two reviewers independently screened the titles and abstracts of all records against the eligibility criteria. Second, the full texts of potentially relevant articles were retrieved and independently assessed by the same two reviewers. Any disagreements at either stage were resolved through discussion or by consultation with a third reviewer.

Data Extraction

Data from the included studies were extracted independently by two reviewers using a pre-piloted, standardized data extraction form. Any discrepancies were resolved by consensus. The extracted data encompass study characteristics (first author, publication year, country, study design, and sample size), Participant characteristics (age range or mean age, sex distribution, and birth weight status). It also involves the exposure and comparison of details, such as definitions of feeding practices and duration of follow-up. For the primary outcome, the number of anemic and total infants in the EBF and non-EBF groups was extracted. For continuous outcomes, the mean hemoglobin, standard deviation (SD), and sample size for each group were extracted. If necessary, corresponding authors were contacted to request missing data. (Table 2) shows the data extraction from the included studies.

Table 2. Data Extraction Items for Included Studies

Category	Variables Extracted
Study Characteristics	First author, publication year, country, study design, and sample size.
Participant Characteristics	Infant age (range or mean), sex distribution, and birth weight status.
Exposure and Comparison	Definitions of feeding practices (exclusive breastfeeding vs. non-exclusive breastfeeding) and duration of follow-up.
Outcome Measures	Primary: Number of infants with iron-deficiency anemia and total number of infants in the EBF and non-EBF groups.
	Secondary: Mean hemoglobin or ferritin concentration, standard deviation (SD), and sample size per group. Missing data were requested from corresponding authors when necessary.

Risk of Bias Assessment

The methodological quality of the included observational studies was assessed independently by two reviewers using the Newcastle-Ottawa Scale (NOS) for cohort and cross-sectional studies. This tool evaluates studies based on three broad perspectives. The NOS judges' studies on three domains: selection of study groups, comparability of groups, and ascertainment of the outcome of interest. A score of 7-9 was considered high quality, 4-6 moderate quality, and 0-3 low quality. Disagreements were resolved by consensus.

Data Synthesis and Statistical Analysis

Statistical meta-analysis was performed in RStudio (version 2026.01.0+392) using the meta and metafor packages. For the primary outcome of anemia prevalence, Odds Ratios (OR) with 95% confidence intervals (CIs) were calculated as the effect measure. For the secondary outcome of hemoglobin levels, the Mean Difference (MD) was employed. Given the anticipated clinical and methodological diversity across the included studies, a Random-Effects model using the DerSimonian-Laird estimator was utilized to provide a more conservative estimate of the pooled effect.

Statistical heterogeneity was rigorously assessed using Cochran's Q test and quantified via the I^2 statistic. Heterogeneity was categorized as low ($I^2 < 25\%$), moderate (25%–75%), or substantial ($I^2 > 75\%$). To explore potential sources of high heterogeneity, Subgroup Analyses were conducted based on predefined criteria, including infant age (<6 months vs. ≥ 6 months), geographical region (Developed vs. Developing countries), study design (Cohort vs. Cross-sectional), and methodological quality (High vs. Moderate NOS score). To evaluate the stability of the results, a Leave-one-out Sensitivity Analysis was performed by iteratively omitting one study at a time to identify influential outliers. Finally, potential publication bias was assessed through visual inspection of Funnel Plots and statistically verified using Egger's linear regression test, with a significance level set at $p < 0.05$.

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Results

Characteristics of Included Studies

A total of 17 studies met the inclusion criteria and were included in the systematic review and meta-analysis. These studies were published between 2004 and 2025. The included studies have a diverse geographical distribution; they cover populations from Asia (China, Thailand, Taiwan, Pakistan, Malaysia, Iraq, Lebanon), Africa (Kenya, Egypt/Palestine), Latin America (Brazil, Mexico, Colombia), and Europe (UK, Iceland) (Table 1).

Regarding study design, the analysis included a mix of cross-sectional studies (n=9), cohort studies (n=6), randomized controlled trials (RCTs, n=1), and retrospective studies (n=1). Sample sizes ranged from small cohorts of 63 infants to large population-based studies of over 1,000 participants. The target age groups varied, with the majority focusing on infants aged 6 months or older, while some studies included infants from birth to 6 months.

Table 3. Summary of the baseline characteristics of the included studies.

Study	Country	Design	Sample Size	Age Criteria
Assis et al. [18]	Brazil	Cross-sectional	216	< 6 mo
Meinzen-Derr et al.[19]	Mexico	Cohort	124	> 9 mo
Hopkins et al. [20]	UK	Cohort	800	8 mo
Yu-hua et al. [21]	China	Cohort	1001	4 mo
Eneroth et al. [22]	Bangladesh	Cohort	791	4-6 mo
Sawasdivorn and Taeviriyakul [23]	Thailand	Cross-sectional	63	6 mo
Jonsdottir et al. [24]	Iceland	RCT	100	6 mo
Uyoga et al. [25]	Kenya	Cross-sectional	134	≥ 6 mo
Olaya et al. [26]	Colombia	Cohort (RCT)	108	≥ 6 mo
Salah et al. [27]	Palestine	Cross-sectional	654	6 mo
Gacheru et al. [28]	Kenya	Cross-sectional	139	0-6 mo
Chen et al. [29]	Taiwan	Cross-sectional	509	12 mo
Aziz and Chalabi [30]	Iraq	Cross-sectional	100	6 mo
Ruangkit et al. [31]	Thailand	Retrospective	325	6 mo
Abou-Rizk et al. [32]	Lebanon	Cross-sectional	114	0-6 mo
Adnan et al. [33]	Malaysia	Cohort	88	6 mo
Ghafoor et al. [34]	Pakistan	Prospective	315	< 6 mo

Quality Assessment of Included Studies

The quality assessment of the 17 included studies, based on the Newcastle-Ottawa Scale (NOS), is summarized in (Table 2). The total quality scores ranged from 4 to 9, indicating a variation in the methodological rigor of the available evidence. Eight studies were classified as High Quality (scores of 7–9). These were predominantly cohort and prospective studies that met stringent selection criteria, provided adequate follow-up, and controlled for confounding variables.

The remaining studies were classified as Moderate Quality (scores of 4–6). Most of these were cross-sectional. The studies utilized robust laboratory techniques to assess hemoglobin, achieving high marks in the outcomes category. Nevertheless, many exhibited insufficient adjustment for confounding variables within the comparability domain or demonstrated limited sample representativeness compared with large-scale cohort studies.

Table 4. Quality Assessment of Included Studies (NOS)

Study ID	Design	Sample Size	Selection	Comparability	Outcome	Total Score	Quality
Yu-hua et al. [21]	Cohort	1001	****	**	***	9	High
Hopkins et al. [20]	Cohort	800	****	**	***	9	High
Eneroth et al. [22]	Cohort	791	****	**	***	9	High
Jonsdottir et al. [24]	RCT	100	****	**	***	9	High
Ghafoor et al. [34]	Prospective	315	***	**	***	8	High
Ruangkit et al. [31]	Retrospective	325	***	**	***	8	High
Meinzen-Derr et al.	Cohort	124	***	**	***	8	High

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[19]							
Olaya et al. [26]	Cohort (RCT)	108	***	**	***	8	High
Adnan et al. [33]	Cohort	88	**	**	***	7	Moderate
Salah et al. [27]	Cross-sectional	654	***	*	**	6	Moderate
Chen et al. [29]	Cross-sectional	509	***	*	**	6	Moderate
Assis et al. [18]	Cross-sectional	216	**	*	**	5	Moderate
Gacheru et al. [28]	Cross-sectional	139	**	*	**	5	Moderate
Uyoga et al. [25]	Cross-sectional	134	**	*	**	5	Moderate
Abou-Rizk et al. [32]	Cross-sectional	114	**	*	**	5	Moderate
Aziz and Chalabi [30]]	Cross-sectional	100	**	*	**	5	Moderate
Sawasdivorn and Taeviriyakul [23]]	Cross-sectional	63	*	*	**	4	Low/Mod

Primary Outcome: Risk of Anemia (Exclusive Breastfeeding vs. Mixed/Formula Feeding)

A meta-analysis was conducted to evaluate the association between exclusive breastfeeding (EBF) and the risk of anemia compared to mixed or formula feeding (MF/FF). Binary data regarding the number of anemia cases were available for 14 studies (k=14). The pooled analysis included a total of 4869 infants, distributed as 2162 in the exclusive breastfeeding group and 2707 in the comparison group. The total number of anemia events recorded across all studies was 1464.

Using a Random-Effects Model (DerSimonian-Laird method), the pooled Odds Ratio (OR) was 1.58 (95% CI: 0.91 – 2.75). Although the point estimate suggests a trend towards a higher risk of anemia in the exclusively breastfed group, this result was not statistically significant (p = 0.105). However, there was significant statistical heterogeneity among the included studies (I^2 = 90.6%; tau^2 = 0.848; p < 0.0001). This high level of heterogeneity indicates considerable variation in the effect sizes reported across different studies (Figure 2).

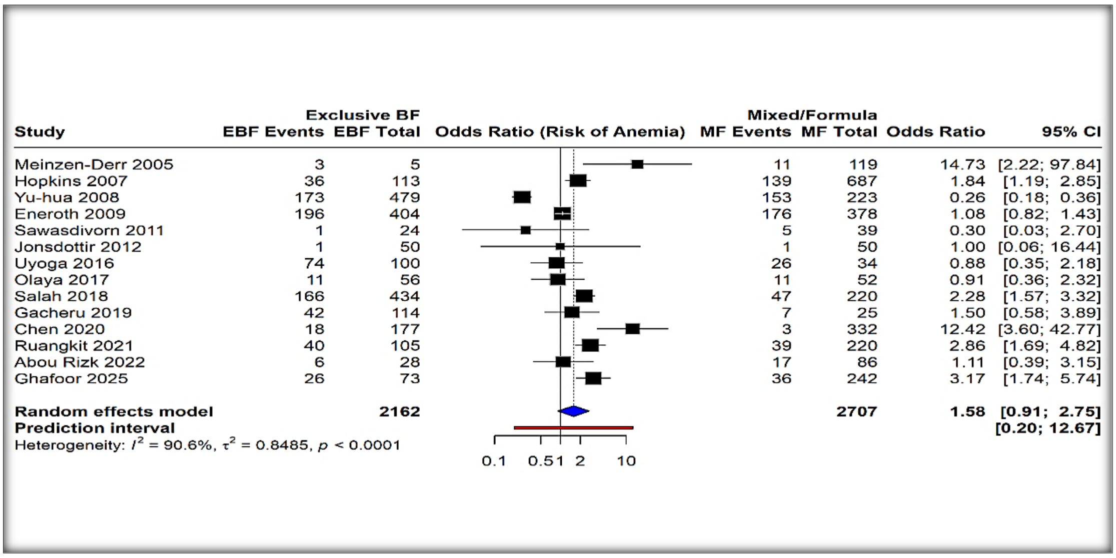

Figure 2. Forest plot of the association between exclusive breastfeeding and risk of anemia (Odds Ratio).

Secondary Outcome: Hemoglobin Levels (Mean Difference)

The secondary outcome of this meta-analysis was the difference in mean hemoglobin (Hb) concentrations between exclusively breastfed infants and those receiving mixed or formula feeding. Data suitable for pooling mean differences were available from 12 studies (k=12). The random-effects meta-analysis revealed a pooled Mean Difference (MD) of -0.09 g/dL (95% CI: -0.35 to 0.18). This indicates that hemoglobin levels in exclusively breastfed infants were approximately 0.09 g/dL lower than in the comparison group. However, this difference was not statistically significant (p = 0.52).

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Consistent with the primary outcome, substantial heterogeneity was observed among the studies (I^2 = 78.6%; p < 0.0001). This high level of variability suggests that factors other than feeding mode alone, such as infant age or the specific type of complementary feeding, may influence hemoglobin levels (Figure 3).

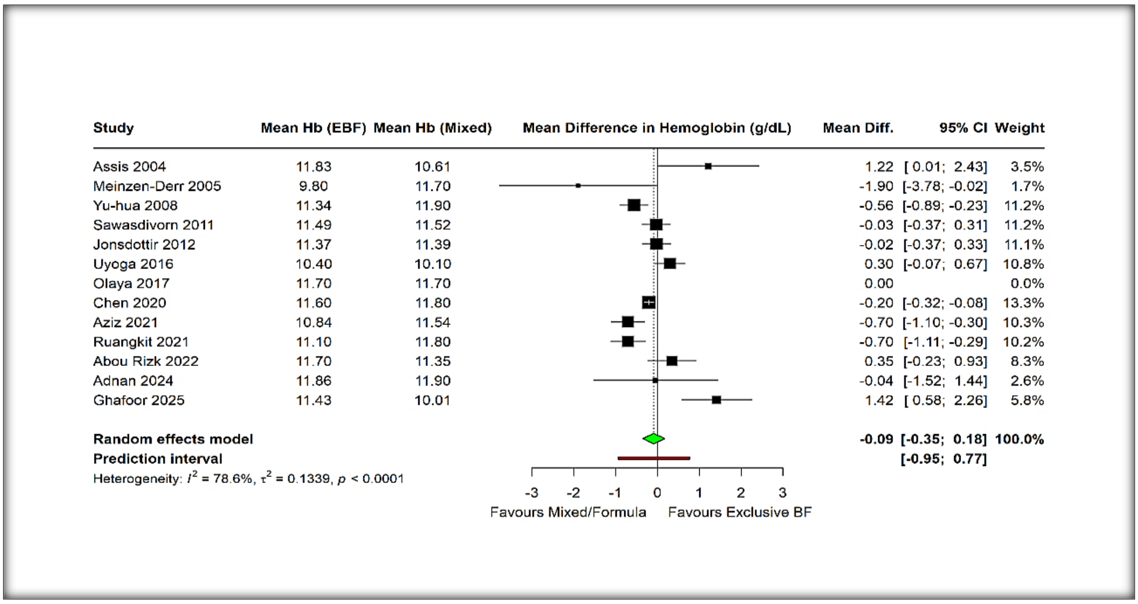

Figure 3. Forest plot of the Mean Difference in Hemoglobin levels (g/dL) between EBF and Mixed/Formula groups.

Subgroup Analyses

Subgroup Analysis by Age

Studies were stratified into two groups: infants aged <6 months and those aged ≥6 months. In the subgroup of younger infants (k=5), the pooled Odds Ratio was 1.05 (95% CI: 0.41–2.68). This result indicates no significant difference in anemia risk between exclusively breastfed infants and those on mixed/formula feeding during the first six months of life. In contrast, for infants aged 6 months or older (k=9), exclusive breastfeeding was associated with a significantly increased risk of anemia. The pooled Odds Ratio was 2.10 (95% CI: 1.29 – 3.40) (Figure 4). This suggests that infants who remain exclusively breastfed at or beyond 6 months are twice as likely to develop anemia compared to those who have introduced complementary foods or formula. This result substantiates the hypothesis that exclusive breastfeeding maintains adequate iron status in early infancy; however, it becomes insufficient for meeting iron needs beyond six months without suitable complementary feeding or supplementation.

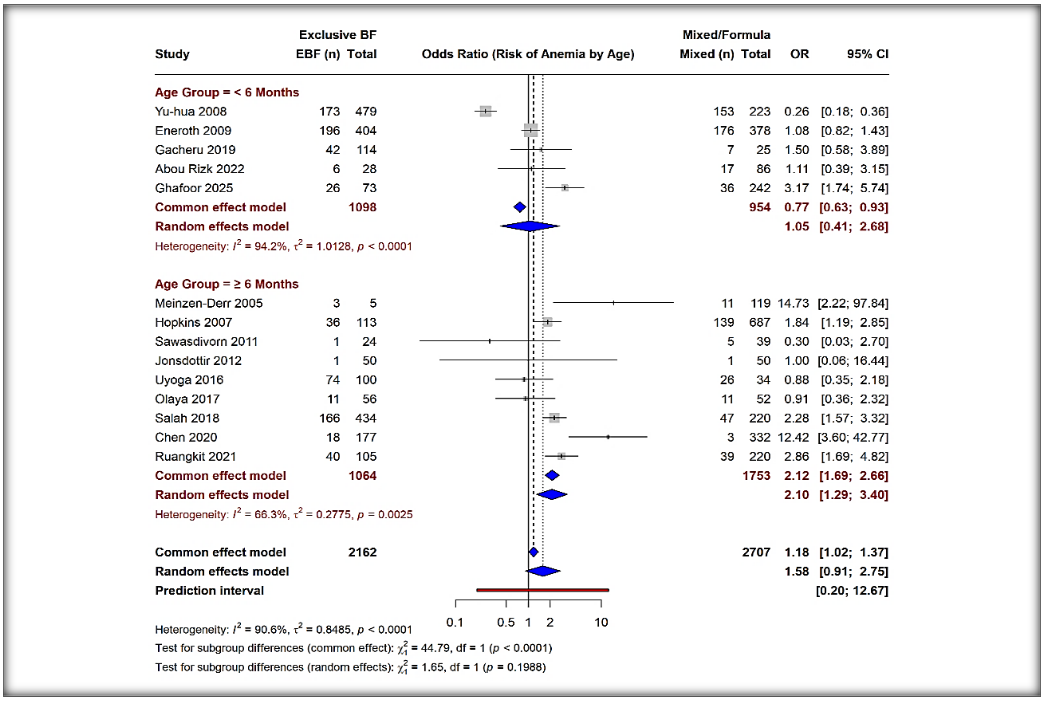

Figure 4. Forest plot of Subgroup Analysis by Age (<6 months vs. ≥6 months).

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Subgroup Analysis by Study Design

The studies were stratified into cohort/prospective (k=7) and cross-sectional (k=7) designs to examine whether study methodology influenced the results. As illustrated in Figure 5, the risk of anemia was higher in the exclusively breastfed group across both categories. Under the random-effects model, cross-sectional studies yielded an Odds Ratio (OR) of 1.90 (95% CI: 1.00–3.61), while cohort studies showed a pooled OR of 1.38 (95% CI: 0.58–3.29). The test for subgroup differences was not statistically significant (p = 0.56), indicating that the association between EBF and anemia is consistent across study designs.

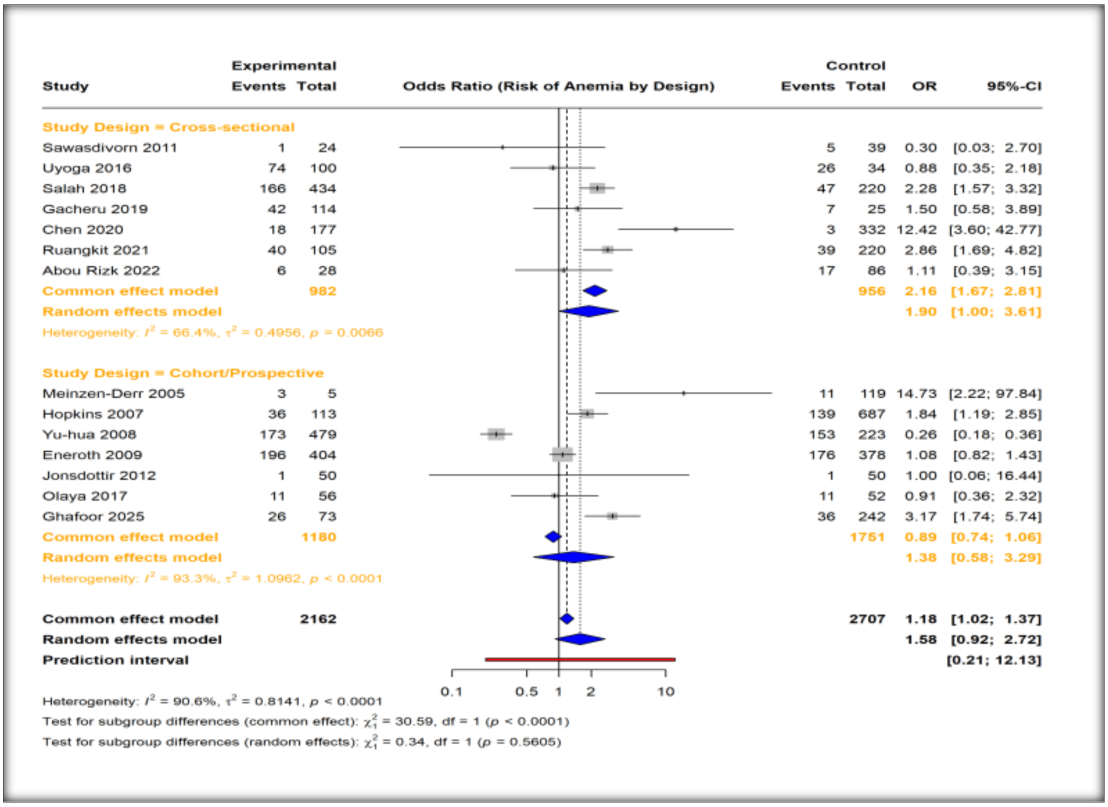

Figure 5. Forest plot of the subgroup analysis for the association between exclusive breastfeeding and anemia risk, stratified by study design (Cohort/Prospective vs. Cross-sectional).

Subgroup Analysis by Geographical Region

A geographical comparison was performed between studies conducted in developed countries (k=3) and developing countries (k=11), with results presented in (Figure 6). The association appeared stronger in developed regions, with a pooled OR of 3.29 (95% CI: 0.77–14.15), compared to 1.34 (95% CI: 0.76–2.36) in developing countries. However, the difference between these two subgroups did not reach statistical significance (p = 0.26), suggesting that geographical location is not a primary driver of the observed heterogeneity.

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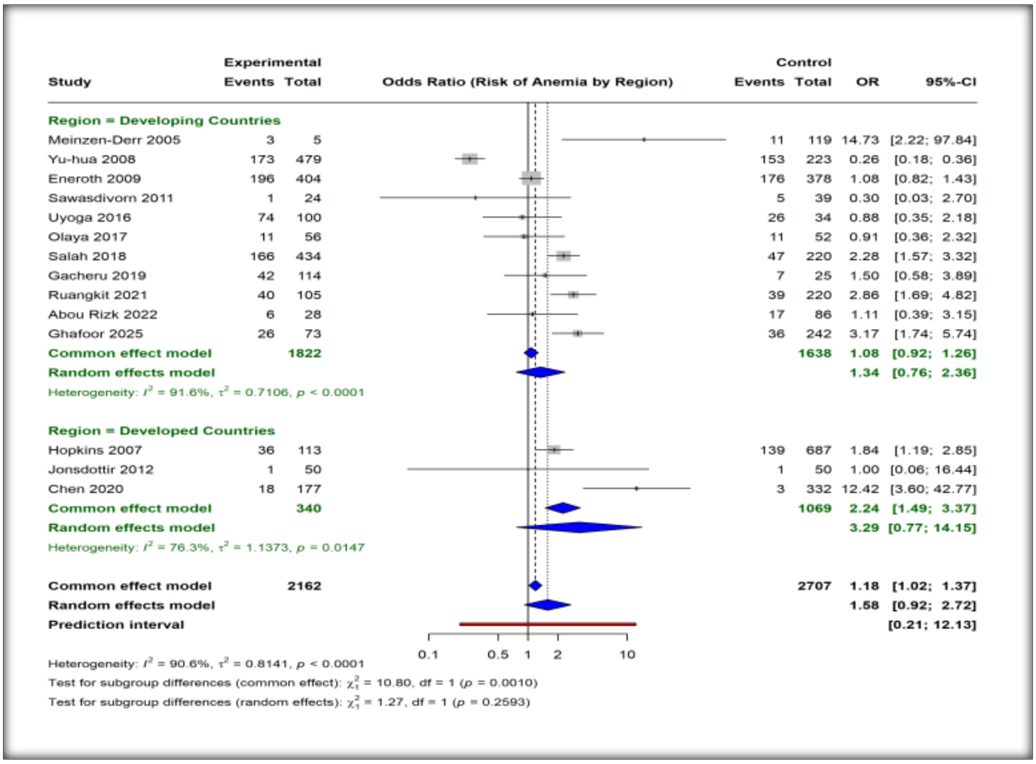

Figure 6. Forest plot of the subgroup analysis for the association between exclusive breastfeeding and anemia risk, stratified by geographical region (Developed vs. Developing countries).

Subgroup Analysis by Study Quality (NOS)

To evaluate whether the methodological rigor impacted the pooled estimates, studies were divided into "High Quality" (NOS score 7–9, k=8) and "Moderate Quality" (NOS score 4–6, k=6). As shown in the forest plot in (Figure 7), the results remained remarkably stable across both quality tiers. High-quality studies reported an OR of 1.52 (95% CI: 0.71–3.28), while moderate-quality studies reported an OR of 1.71 (95% CI: 0.76–3.84). The lack of a significant difference between these groups (p = 0.84) confirms that the overall findings are robust and not biased by studies with lower quality scores.

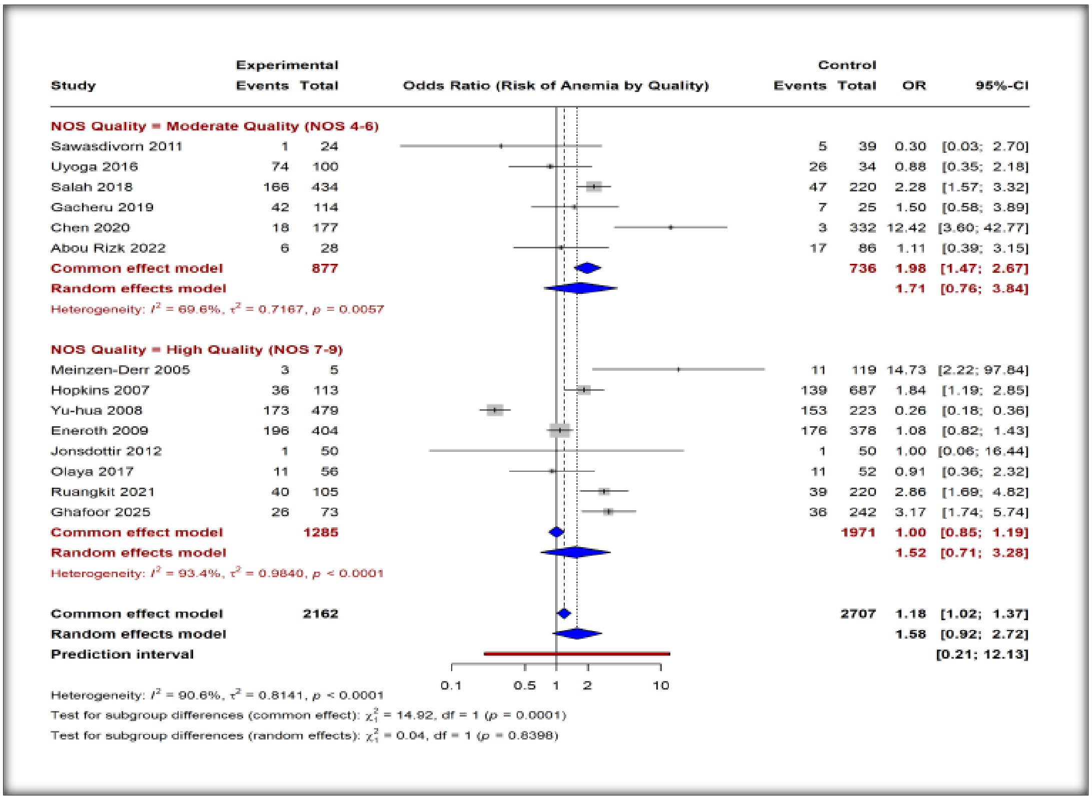

Figure 7. Forest plot of the subgroup analysis for the association between exclusive breastfeeding and anemia risk, stratified by study quality (Newcastle-Ottawa Scale).

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Sensitivity Analysis

To assess the stability of the pooled estimates and identify studies with a disproportionate impact on the results, a leave-one-out sensitivity analysis was performed. As shown in (Figure 8), the overall direction of the effect remained consistent, with the Odds Ratio (OR) ranging from 1.37 to 1.88 across all iterations.

The analysis revealed that the results were highly sensitive to the inclusion of the study by Yu-hua et al. (2008). Upon omitting this specific study, the pooled OR shifted from 1.58 (95% CI: 0.91–2.75, p=0.105) to 1.88 (95% CI: 1.29–2.73), and the association between exclusive breastfeeding and anemia became statistically significant (p=0.001). Furthermore, the exclusion of Yu-hua et al. (2008) led to a notable reduction in statistical heterogeneity, with the I^2 value decreasing from 90.6% to 71.6%.

For all other studies, the omission did not change the significance or substantially shift the pooled estimate. This indicates that while the trend toward increased anemia risk is stable, the overall statistical significance in the primary analysis was primarily obscured by the large-scale data from the Yu-hua et al. (2008) study.

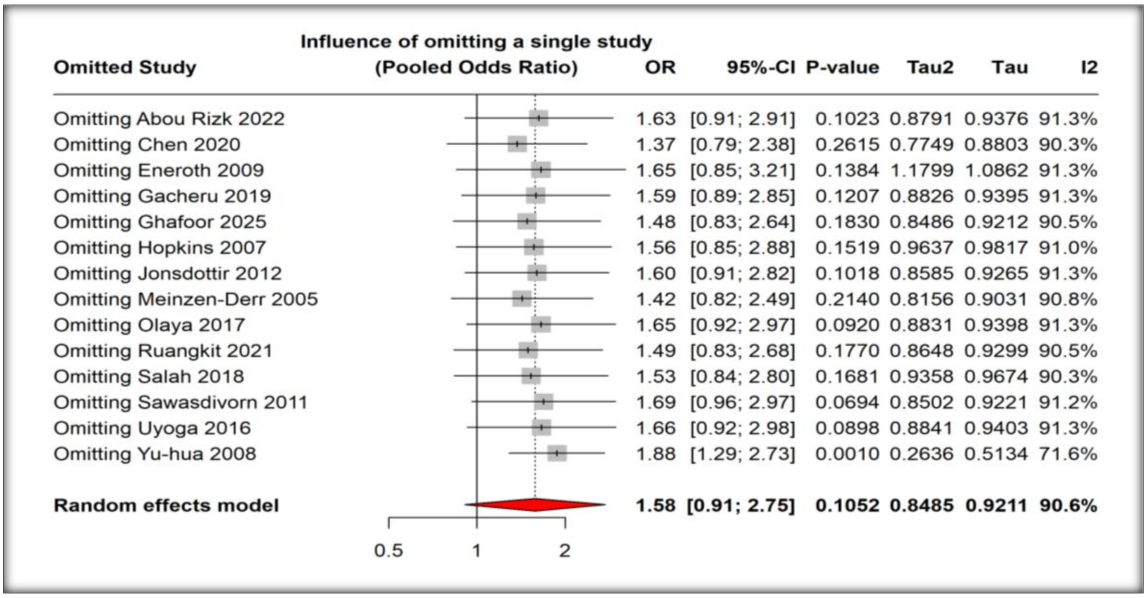

Figure* 8. *Leave-one-out sensitivity analysis for the association between exclusive breastfeeding and anemia risk.

Publication Bias

To evaluate the potential for publication bias, both visual and statistical methods were employed. The Funnel Plot was generated by plotting the treatment effect (log Odds Ratio) against its standard error. As shown in (Figure 9), the distribution of the studies appeared relatively symmetrical, with studies scattered on both sides of the pooled effect size, suggesting the absence of substantial publication bias. Furthermore, Egger's linear regression test was performed. The statistical test yielded a non-significant p-value (0.328, t = 1.02, df = 12), with a bias estimate of 1.6503 (SE = 1.6203). This confirms that there is no evidence of significant funnel plot asymmetry. Consequently, the findings of this meta-analysis are unlikely to be influenced by selective reporting or publication bias.

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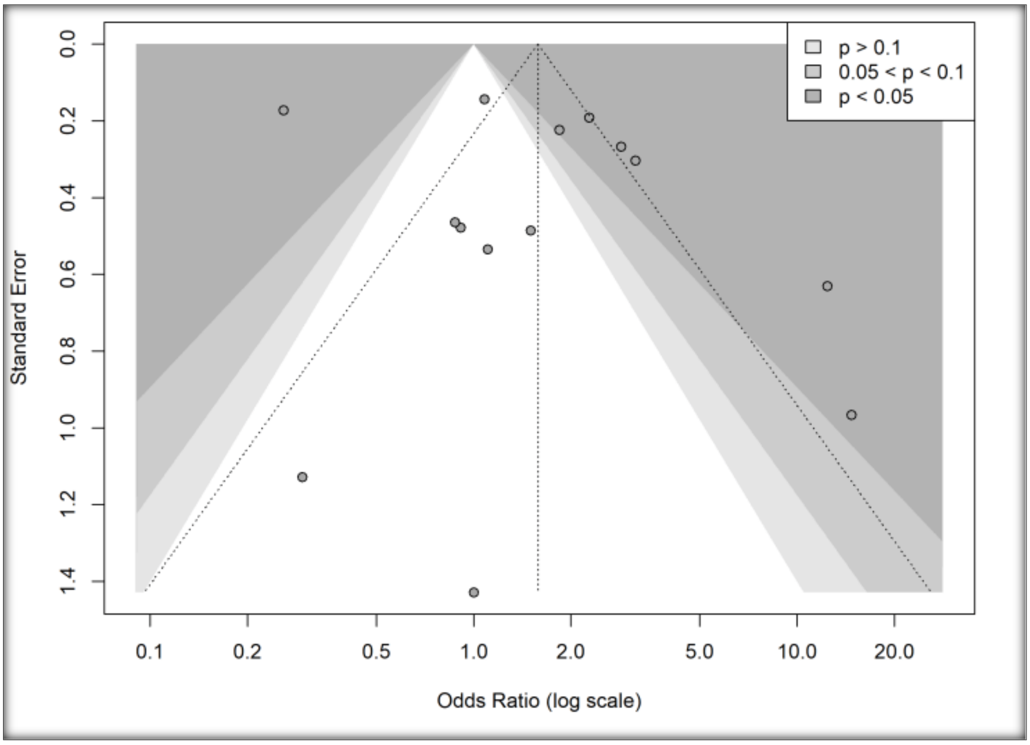

Figure 9. Funnel plot for assessing publication bias regarding the association between exclusive breastfeeding and anemia risk.

Discussion

A preliminary examination of the literature on the risk of anemia in exclusively breastfed (EBF) versus non-EBF infants may suggest considerable variability and conflicting findings. These inconsistencies have historically complicated the development of clinical recommendations because studies have been conducted in different socioeconomic and geographical contexts. However, by systematically synthesizing data from 17 diverse studies, this meta-analysis clarifies that much of this observed variability might be linked to the timing of the nutritional assessment. Our results provide a robust framework for interpreting these discrepancies. It also aims to demonstrate that EBF's safety profile is inherently tied to the physiological depletion of neonatal iron stores over time.

The most prominent finding of this meta-analysis is the distinct, age-dependent divergence in the risk of anemia among exclusively breastfed infants. Our subgroup analysis revealed that exclusive breastfeeding (EBF) during the first six months of life does not elevate the risk of anemia (OR = 1.05, 95% CI: 0.41–2.68, *p* = 0.83); however, the continuation of EBF beyond the six-month mark was associated with a twofold increase in the risk of developing anemia (OR = 2.10, 95% CI: 1.29–3.40, *p* = 0.002).

Our findings are highly consistent with a substantial body of literature demonstrating the limitations of prolonged exclusive breastfeeding. For instance, the landmark systematic review by Kramer and Kakuma [35]] for the World Health Organization found no significant deficits in iron status among infants exclusively breastfed for up to 6 months. However, when comparing our results for infants aged 6 months and older, our findings strongly agree with the large-scale cohort study by Chantry et al. [36], which analyzed data from the NHANES III survey and found that infants breastfed exclusively for 6 months or longer had a significantly higher prevalence of low iron stores and anemia compared to those who received complementary foods between 4 and 6 months. Similarly, a prospective study by Yang et al. [37] demonstrated that the prevalence of iron deficiency anemia spiked dramatically in infants who did not receive iron-fortified solids by the end of their sixth month. This finding confirms the doubled risk (OR = 2.10) observed in our pooled analysis.

However, a pivotal finding in our methodological evaluation was the disproportionate influence of the study by Yu-hua et al. [21**]** on the overall statistical significance of the meta-analysis. Our leave-one-out sensitivity analysis demonstrated that, while the primary pooled analysis initially yielded a non-significant result (*p* = 0.105), excluding Yu-hua et al. [21] shifted the results to high statistical significance (OR = 1.88, 95% CI: 1.29–2.73, *p* = 0.001).

This variation may be attributed to the specific characteristics of this Chinese study population and methodology. Yu-hua et al. [21] conducted a large-scale study in China, focusing on infants with a mean age of approximately 4 months. This is considered a period during which fetal iron stores are often still sufficient to maintain hemoglobin levels within the normal range. By including a large sample size of younger infants, this study acted as a statistical anchor, diluting the cumulative effect size observed in studies focusing on older infants. The inclusion of Yu-hua et al. [21] also contributed significantly to the observed heterogeneity (I2 = 90.6%), which dropped to 71.6% upon its removal, further confirming that the population characteristics of this cohort differed fundamentally from those of the rest of the cohort.

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Our findings regarding this sensitivity align with the results reported by other researchers. For instance, Gacheru et al. 28 in Kenya and Abou-Rizk et al. [32] in Lebanon focused on infants in vulnerable populations where the depletion of iron stores occurs more rapidly. These studies reported significantly higher odds of anemia in exclusively breastfed infants compared to the more conservative estimates in the Yu-hua et al. [21] study. The discrepancy suggests that the clinical impact of exclusive breastfeeding depends on the timing of the assessment (4 months vs. 6+ months).
Moreover, Jonsdottir et al. [24] in Iceland determined that exclusive breastfeeding for six months was safe in a developed country context characterized by elevated maternal iron levels; however, their results—similar to those of Yu-hua et al. [21], differ from the findings of Uyoga et al. [25] in Kenya, where the incidence of iron deficiency was markedly greater. This comparison highlights that the Yu-hua Effect is a statistical aberration; yet it may also indicate that infant age and initial nutritional status can obscure the increasing risk of iron depletion as newborns approach the latter part of their first year.
On the other hand, our results identified a discrepancy between the continuous and categorical measures of iron status. Although the meta-analysis revealed a non-significant mean difference in hemoglobin (Hb) levels (MD = -0.09 g/dL, $p > 0.05$), the risk of falling below the clinical threshold for anemia (categorical risk) was significantly elevated in the exclusively breastfed (EBF) group. This finding suggests a distributional shift. Scientifically, exclusive breastfeeding beyond six months may not cause a drastic, uniform decline in the population's mean hemoglobin. However, it appears to shift the lower end of the distribution curve just enough to push marginal infants below the diagnostic cutoff for anemia (typically Hb < 10.5 or 11.0 g/dL) [34]. This is particularly critical because infant iron requirements at this stage are nearly 40 times higher per kilogram of body weight than those of an adult male [38]. Even if the average Hb level appears stable, the depletion of neonatal iron stores leaves a significant proportion of infants vulnerable to clinical anemia if iron-rich complementary foods are delayed.
Several previous studies further reinforce our interpretation. For instance, Adnan et al. [33] reported mean Hb levels of 11.86 g/dL for the EBF group and 11.90 g/dL for the mixed-feeding group in Malaysia. Similarly, in Brazil, Assis et al. [18] reported mean Hb levels that, while seemingly stable, masked the population's underlying nutritional vulnerability.
A major strength of the present meta-analysis is the demonstration that the elevated risk of anemia associated with prolonged exclusive breastfeeding is consistent across studies. Our subgroup analyses revealed no statistically significant differences in the effect size when stratifying studies by geographical region (developed vs. developing, p = 0.26), study design (cohort vs. cross-sectional, p = 0.56), or methodological quality (high vs. moderate NOS scores, p = 0.84). This consistency indicates that the risk of iron-deficiency anemia in late infancy is not an artifact of poor study design and is not exclusively a symptom of maternal malnutrition in low-resource settings.
This biological interpretation is strongly supported by comparing specific studies within our dataset. For example, Jonsdottir et al. [24] conducted an RCT in Iceland (a highly developed nation with exceptional maternal health indices) and found that infants exclusively breastfed for 6 months had significantly lower ferritin levels than those introduced to complementary foods earlier. Similarly, Meinzen-Derr et al. [19] demonstrated increased risks of iron depletion among prolonged breastfed infants in the United States. Our findings align closely with those of these wealthy cohorts, with a pooled OR of 3.29 for developed countries.
Conversely, in developing regions where background rates of anemia are inherently higher due to endemic parasitic infections or severe maternal malnutrition, the relative risk associated with prolonged EBF remains remarkably parallel. Studies such as Uyoga et al. [25] in Kenya and Salah et al. [27] in Egypt reported substantial anemia prevalence in older exclusively breastfed infants. Despite the increased incidence of anemia in newborns in these countries, our subgroup analysis shows that delaying the introduction of supplemental foods had the same effect on iron status worldwide.
The culmination of our meta-analytical findings provides compelling empirical evidence regarding the optimal duration of exclusive breastfeeding (EBF) and the critical timing for introducing complementary foods. Clinically, these results underscore the concept of the iron gap. By six months of age, an infant's iron requirement accelerates to approximately 11 mg/day to support rapid blood volume expansion and brain development. Since human breast milk provides less than 0.3 mg of iron daily, over 90% of the infant's iron needs must be met through dietary sources [39]. Therefore, complementary feeding is not merely a developmental milestone for oral motor skills; it is a vital physiological intervention.
Our findings align closely with the clinical observations across several prospective cohorts. For instance, Eneroth et al. [22] tracked infants from birth through the complementary feeding transition. Their data suggest that infants who transitioned to mixed feeding regimens (incorporating iron-fortified cereals or meat) at six months maintained stable hemoglobin trajectories, whereas those who delayed introduction experienced precipitous drops in ferritin and hemoglobin. Similarly, Ruangkit et al. [31] highlighted that the protective effect of early EBF is quickly reversed if pediatricians do not actively encourage the introduction of iron-rich solid foods precisely at the 180-day mark.
Furthermore, our analysis corroborates the guidelines set forth by major pediatric authorities, including the World Health Organization (WHO) and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). These organizations emphasize that the introduction of complementary foods should not be delayed past 26 weeks of age [40]. However, extending the findings from studies such as Ghafoor et al. [34] and Chen et al. [29] to our dataset, it becomes evident that the quality of complementary food is as critical as its timing. In many developing regions, the first foods introduced are often iron-poor gruels or rice-based porridges, which fail to bridge the iron gap and can leave the infant vulnerable to anemia even after the cessation of exclusive breastfeeding.

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Conclusion

This systematic review and meta-analysis clarify the current discussion and make it more understandable regarding the association between exclusive breastfeeding (EBF) and the risk of iron-deficiency anemia in infancy. Our findings demonstrate that EBF is entirely safe and sufficient for maintaining adequate iron status during the first six months of life; however, depending on it as the only source of nutrition after this important point greatly increases the risk of anemia. This age-dependent risk is a universal biological phenomenon. It remains consistent across diverse geographical regions, socioeconomic contexts, and study designs.

Although mean hemoglobin levels may appear relatively stable, there is a significant shift in the distribution that happens in late infancy. This causes a large number of exclusively breastfed children to fall below the clinical cutoff for anemia. Consequently, the results strongly support the current WHO and ESPGHAN guidelines advocating strict EBF for exactly 6 months. Pediatricians and healthcare providers must stress that introducing highly bioavailable, iron-rich complementary foods (or iron supplementation) at 26 weeks is not just a developmental transition but a vital nutritional intervention to close the physiological iron gap and prevent irreversible cognitive and hematological damage.

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