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Vitamin C Levels in Pregnant Women and the Efficacy of Vitamin C Supplements in Preventing Premature Rupture of Membranes: A Systematic Review and Meta-Analysis

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Background: Premature rupture of membranes (PROM) is defined as the leakage of amniotic fluid before the onset of labor and delivery contractions. Some studies found that women who experienced PROM had significantly lower vitamin C blood levels than those who did not, while others found no significant differences. Previous systematic reviews and meta-analyses on the efficacy of vitamin C in the prevention of PROM had conflicting results.

Aims: Conduct a systematic review and meta-analysis to determine if there was a significant difference in vitamin C blood levels in women who had PROM versus the control group who did not and to determine if vitamin C supplements could help prevent it.

Study Design: Systematic review and meta-analysis.

Methods: We registered our protocol with PROSPERO (CRD42022371644). We searched PubMed/MEDLINE, Web of Science, and Scopus through February 15, 2024. Additionally, backward and forward citation searches were conducted. Studies were selected based on predetermined inclusion and exclusion criteria. Meta-Essentials: Workbooks for Meta-Analysis (version 1.5) was used for analysis.

Results: Twenty-five studies (26 reports) met all eligibility criteria, with 18 studies (18 reports) assessing vitamin C levels and seven studies (eight reports) evaluating efficacy. Women with PROM, whether preterm or term, had significantly lower vitamin C levels [Hedges' g, -1.48; 95% confidence interval (CI): -2.82, -0.14; $p = 0.020$; $I^2 = 94.08\%$) and specifically preterm PROM after removing the outlying study [Hedges' g, -1.29; 95% CI: -1.85, -0.73; $p < 0.001$; $I^2 = 87.35\%$). Vitamin C supplementation significantly reduced the risk of preterm or term PROM [risk ratio (RR), 0.57; 95% CI: 0.39, 0.81; $p < 0.001$; $I^2 = 12.17\%$), particularly for preterm PROM (RR, 0.67; 95% CI: 0.45, 0.99; $p = 0.001$; $I^2 = 0.00\%$). There were no significant differences in vitamin C levels between women with term PROM and controls, and there were no differences in the risk of developing term PROM between women taking vitamin C supplements and controls. Results were not robust in all sensitivity analyses.

Conclusion: Women with PROM, particularly those who developed it preterm, appear to have significantly lower vitamin C levels, and vitamin C supplementation appears to be effective in reducing the risk of PROM, particularly preterm PROM. More high-quality studies with low risk of bias, more homogenous, and larger samples are needed to confirm these findings.

INTRODUCTION

Premature (prelabor) rupture of membranes (PROM) is defined as amniotic fluid leakage occurring before the onset of labor and delivery contractions.[1,2] Preterm PROM (PPROM) is defined as PROM that develops before 37 weeks of gestation, whereas term PROM (TPROM) occurs at or after 37 weeks.[3] The incidence of PROM during pregnancy ranges from 4% to 10%.[4] The exact cause is unknown, but the pathophysiology appears to be multifactorial.[4] According to recent research, membrane rupture may be associated with increased oxidative stress and abnormal collagen formation and structure.[2]

Vitamin C is an essential hydrosoluble micronutrient that plays a role in antioxidant defense mechanisms and collagen synthesis.[5,6] Humans cannot synthesize it, so adequate dietary intake is required to maintain body stores.[5,6] Some studies found that vitamin C blood levels in women who experienced PROM were significantly lower than those who did not,[7-11] but other studies found no significant

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differences.[12-14] To the best of our knowledge, there have been no systematic reviews or meta-analyses that compare vitamin C blood levels in women with and without PROM. However, some older systematic reviews and meta-analyses have examined the effects of vitamin C supplementation in the prevention of PROM, with conflicting results. First, published in 2015 reported that vitamin C supplementation alone could lower the risk of both PPROM and TPROM.[6] However, two subsequent systematic reviews and meta-analyses (one conducted in 2017 and published as a congress abstract in 2018[15] and another published in 2022[16]) found that vitamin C was ineffective in reducing the risk of PPROM.

Keeping in mind all previously mentioned, we aimed to conduct a systematic review and meta-analysis to determine if there is a significant difference in vitamin C blood levels in women with PROM compared with a control group who did not and to determine if vitamin C supplements could help prevent it.

MATERIALS AND METHODS

This systematic review and meta-analysis is a subset of a larger systematic review and meta-analysis that has been registered in the International Prospective Register of Systematic Reviews PROSPERO (registration number: CRD42022371644). This article presents PROM results, whereas preterm birth results will be reported elsewhere. We adhered to the Preferred Reporting Items for Systematic Review and Meta-Analysis Statement.[17] Informed consent is not required because we used data from published clinical studies.

Eligibility criteria

Inclusion criteria for the vitamin C level section included any original clinical study that reported maternal peripheral blood, serum, or plasma vitamin C levels measured at any point during pregnancy or at/after delivery, with a study group of pregnant women who experienced PROM and a control group of pregnant women who did not experience PROM (i.e., had a normal uncomplicated pregnancy).

In the part of vitamin C supplementation efficacy, the inclusion criteria were randomized controlled clinical studies comparing the efficacy of vitamin C supplementation alone in the prevention of PROM in pregnant women with a control group that received placebo or no vitamin C supplementation.

Exclusion criteria included studies in which the information required for the calculation of combined effect sizes could not be extracted, calculated, or obtained, conference abstracts, and studies with no full text. Non-randomized clinical studies and studies evaluating the efficacy of vitamin C in combination with other supplements (if both groups received the same supplements, such study was not excluded) were specifically excluded from the part on vitamin C supplementation efficacy.

Information sources, search strategy, and selection process

Two authors independently searched three databases (PubMed/MEDLINE, Web of Science, and Scopus) without language or date restrictions until December 21, 2022, and then updated their search on February 15, 2024. Table 1 shows the complete search strategy. Backward and forward citation searches were conducted on reports that met the eligibility criteria. We conducted backward citation searches by examining their reference lists. We used Google Scholar to find reports that cited them (the most recent check was on February 15, 2024). Two authors independently assessed the eligibility of retrieved reports using their titles and abstracts. When these proved insufficient for assessment, we attempted to retrieve and assess their full text. We attempted to contact the authors of seven reports,[18-24] requesting clarification or information about

TABLE 1. Complete Search Strategy for Databases.

Database	Search strategy
PubMed/MEDLINE	(“vitamin c”[All Fields] OR (“ascorbic acid”[MeSH Terms] OR (“ascorbic”[All Fields] AND “acid”[All Fields]) OR “ascorbic acid”[All Fields])) AND (“premature birth”[MeSH Terms] OR (“premature”[All Fields] AND “birth”[All Fields]) OR “premature birth”[All Fields] OR “preterm”[All Fields] OR “preterms”[All Fields] OR “preterm birth”[All Fields] OR “preterm delivery”[All Fields] OR “preterm labour”[All Fields] OR “preterm labor”[All Fields] OR (“premature birth”[MeSH Terms] OR (“premature”[All Fields] AND “birth”[All Fields]) OR “premature birth”[All Fields] OR “premature”[All Fields] OR “prematurely”[All Fields] OR “prematures”[All Fields] OR “prematurities”[All Fields] OR “prematurity”[All Fields]) OR “premature rupture of membranes”[All Fields] OR “prelabour rupture of membranes”[All Fields] OR “prelabor rupture of membranes”[All Fields] OR ((“ruptur”[All Fields] OR “rupture”[MeSH Terms] OR “rupture”[All Fields] OR “ruptured”[All Fields] OR “ruptures”[All Fields] OR “rupturing”[All Fields]) AND “chorioamniotic”[All Fields] AND (“membranal”[All Fields] OR “membrane s”[All Fields] OR “membraneous”[All Fields] OR “membranes”[MeSH Terms] OR “membranes”[All Fields] OR “membrane”[All Fields] OR “membranous”[All Fields])) OR “PROM”[All Fields] OR “PPROM”[All Fields])
Web of Science	In Web of Science Core Collection, KCI-Korean Journal Database, and SciELO Citation Index: (TI=((“vitamin c”) OR (ascorbic acid)) AND TI=(preterm OR “preterm birth” OR “preterm delivery” OR “preterm labour” OR “preterm labor” OR premature OR “premature rupture of membranes” OR “prelabour rupture of membranes” OR “prelabor rupture of membranes” OR “rupture of chorioamniotic membranes” OR “PROM” OR “PPROM”)) OR (AB=((“vitamin c”) OR (ascorbic acid)) AND AB=(preterm OR “preterm birth” OR “preterm delivery” OR “preterm labour” OR “preterm labor” OR premature OR “premature rupture of membranes” OR “prelabour rupture of membranes” OR “prelabor rupture of membranes” OR “rupture of chorioamniotic membranes” OR “PROM” OR “PPROM”))
Scopus	TITLE-ABS ((“vitamin c”) OR (ascorbic AND acid)) AND TITLE-ABS (preterm OR “preterm birth” OR “preterm delivery” OR “preterm labour” OR “preterm labor” OR premature OR “premature rupture of membranes” OR “prelabour rupture of membranes” OR “prelabor rupture of membranes” OR “rupture of chorioamniotic membranes” OR “PROM” OR “PPROM”)

data that were unavailable in the full text reports we had retrieved. Reports were included if all authors agreed that the eligibility criteria were met. The first author resolved disagreements.

Data extraction

The data from the included studies were extracted independently by two authors. For all studies, we extracted the study ID, citation, country/region, sample characteristics (i.e., participant groups, sample sizes, age, main group characteristics), and relevant study findings/conclusions. In addition, for studies measuring vitamin C levels, we extracted the mean and standard deviation (SD) of vitamin C level, method of vitamin C level measurement, blood sample type, time of blood sample collection, gestational age, and percentage of smokers in each group. For studies evaluating vitamin C efficacy, we extracted inclusion criteria regarding gestational age and previous history of PROM, vitamin C supplementation information (e.g., dosage and timing of commencement/duration of supplementation), type of control (placebo or no vitamin C supplementation), information about blinding (concealment of group allocation), frequency of occurrence of outcomes (PROM, PPROM, or TPROM) in each group, and reported information about observed adverse effects associated with vitamin C supplementation. The first author created the final extraction table by collating two tables and double-checking the accuracy of the extracted data.

Methodological quality (risk of bias) assessment

We used the Methodological Index for Non-Randomized Studies (MINORS) tool[25] to evaluate the methodological quality (risk of bias) of vitamin C level measurements in the included studies. The MINORS tool contains 12 items.[25] The score for each item can range from 0 to 2: 0 (not reported), 1 (reported but inadequate), and 2 (reported and adequate).[25] For comparative studies, the global ideal score is 24.[25] Quality assessment was divided into four categories based on total MINORS score: very low (0-6), low (7-12), moderate (13-18), and high (19-24) quality.[26]

The Cochrane Risk of Bias 2 (RoB 2) tool[27] was used to evaluate the risk of bias in the included randomized studies evaluating the efficacy of vitamin C supplementation, whereas the Robvis web application was used to visualize the assessment.[28]

All authors individually evaluated the risk of bias in each study, and any differences were resolved through discussion.

Statistical analysis

We performed statistical analysis using Meta-Essentials: Workbooks for Meta-Analysis (version 1.5).[29]

To combine results from vitamin C level studies, we used a random effects model and estimated combined effect sizes using Hedges' g, with its 95% confidence interval (95% CI), prediction interval, and corresponding significance tests. We chose Hedges' g because it is less likely to generate bias in small samples. The analyses used mean and SD of vitamin C levels and the number of participants. Using number of patients, we converted standard errors to SDs as needed. In studies where data were presented over several periods, we used the most recent available follow-up values. For studies that presented data for two subgroups of PROM patients, we computed pooled means and SDs and used them in meta-analysis to prevent oversampling the control groups and to ensure that results were not affected by a single study contributing a disproportionate number of data points to the analysis.[30]

To combine data from studies on vitamin C efficacy, a random effects model with inverse variance weighting was used. The combined effects sizes were expressed as a risk ratio (RR) with 95% CI, prediction interval, and corresponding tests of significance. As much as possible, analyses for all efficacy outcomes were conducted on an intention-to-treat basis, which means that we attempted to include all participants randomly assigned to each group in the analyses. The denominators for all outcomes were calculated by subtracting the number of randomized participants from any participants who had missing outcomes. Analyses were conducted using the number of participants with the outcome of interest and the corresponding denominators.

Three main comparisons were performed: (a) PROM versus control, (b) PPROM versus control, and (c) TPROM versus control. In the first comparison, PROM referred to both preterm and term PROM, meaning PROM regardless of whether it was preterm or term. We considered the combined effect size significant if two conditions were met: (a) the associated 95% CI did not include 0 (for Hedges' g) or one (for RR) and (b) the associated two-tailed p value was <0.05. We used the random effects model in all analyses due to the clinical and methodological heterogeneity of the studies we included.

We evaluated statistical heterogeneity using Cochran's Q test (significant if $p < 0.10$)[30] and the I^2 statistic (significant if I^2 value $> 50\%$). To investigate sources of significant heterogeneity, we performed additional subgroup analyses on available data [for levels about region (continent), measurement method, blood sample type, MINORS quality category; for efficacy about region (continent), overall risk of bias] and moderator analysis using available relevant continuous variables (for levels in relation to mean age and percentage of smokers; for efficacy about total daily vitamin C dose).

We used sensitivity analysis to remove one study at a time and recalculate combined effect size estimates for the remaining studies. Additionally, post-hoc sensitivity analysis was performed after excluding the outlying study, which had a much larger effect size than the other studies in meta-analyses of vitamin C levels in PROM and PPROM versus control.

We used three methods to assess publication bias in meta-analyses with at least 10 studies (because with less they are not very reliable):[31] funnel plot with trim-and-fill analysis, Egger regression test, and Begg and Mazumdar rank correlation test. A p value of $<$ 0.05 was considered significant in the Egger regression test and Begg and Mazumdar rank correlation test.

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach for summarizing confidence in the effects of interventions was used to evaluate the certainty of evidence for efficacy outcomes.[32]

RESULTS

A PRISMA flow diagram with the results of the search and selection process is shown in Figure 1. Only one author from one study published in two reports[18,19] provided the requested information. The remaining authors did not respond, but one of their studies was included,[20] and the remaining were excluded.[21-24] Twenty-five studies (26 reports) met all eligibility criteria, with 18 studies (18 reports)[5,7-14,33-41] assessing vitamin C levels and seven studies (eight reports)[2,18-20,42-45] assessing efficacy.

The characteristics of the included studies are shown in Tables 2, 3. Two studies reported some adverse effects associated with vitamin C supplementation. In one study, one patient experienced stomach pain after taking vitamin C tablets,[18,19] whereas three patients in the vitamin C group reported pyrosis and nausea (it was unclear whether these were caused by vitamin C supplements).[42] In two studies, no adverse effects were observed.[2,43]

Assessment of quality (risk of bias) is shown in Table 2 (for studies assessing levels) and Figure 2 (for efficacy studies). MINORS quality scores ranged from 14 to 21 of 24, with 5 (27.8%) and 13 (72.2%) of 18 studies assessing vitamin C levels scoring high and moderate, respectively. According to the RoB 2 tool, the overall risk of bias for efficacy studies was rated as "high" and "some concerns" for three (42.9%) and four (57.1%) of the seven studies.

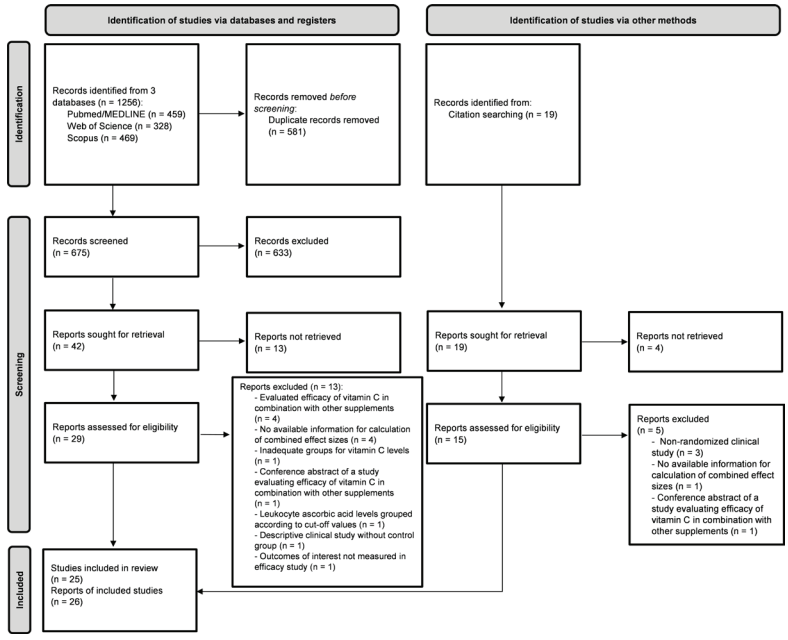

FIG. 1. Results of the search and selection process (PRISMA flow diagram).
PRISMA, Preferred Reporting Items for Systematic Review and Meta-Analysis.

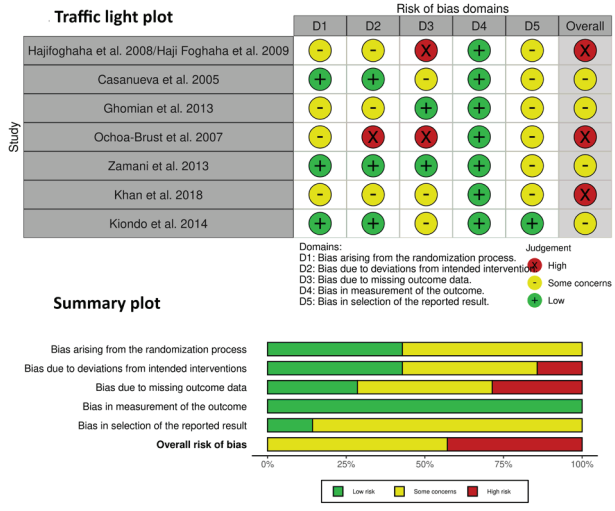

FIG. 2. Risk of bias assessment of efficacy studies according to RoB 2 tool.
RoB, Risk of Bias 2.

TABLE 2. Characteristics of Included Studies That Evaluated Vitamin C Levels.

	Study ID	Country	Blood sample type, measurement method, and time of drawing blood sample	Outcome	Number of participants		Mean age ± SD in years and mean gestational age ± SD in weeks		Mean ± SD of vitamin C levels		Conclusion	MINORS quality score and category
					Outcome	Control	Outcome	Control	Outcome	Control		
1	Eryürek et al.[41]	Türkiye	Blood, spectrophotometry (ascorbate + dehydroascorbate), immediately after the delivery	PROM PPROM TPROM	17 7 10	17 7 10	NR ± NR for all	NR ± NR for all	4.54 ± 1.30 µg/ml 4.50 ± 1.66 µg/ml 4.56 ± 1.08 µg/ml	5.39 ± 1.78 µg/ml 6.08 ± 1.75 µg/ml 4.91 ± 1.72 µg/ml	There were no significant differences between the groups.	14/24 moderate
2	Ali[9]	Iraq	Plasma, spectrophotometry, at enrolment before starting any medication (gestational age of 28-36 + 6 weeks)	PPROM	50	50	NR ± NR; 32.99 ± 0.38 (at birth)	NR ± NR; 38.20 ± 0.11 (at birth)	2.016 ± 0.15 mg/dl	5.04 ± 0.22 mg/dl	The level was significantly lower in the PPROM group (p = 0.0001).	17/24 moderate
3	Barrett et al.[12]	USA	Serum, colorimetric method, in the third trimester	PROM	11	23	24.4 ± NR; NR ± NR	26.4 ± NR; NR ± NR	56.78 ± 1.32 µmol/l	59.05 ± 1.82 µmol/l	No statistical difference was found between the PROM and control group (p = 0.779).	17/24 moderate
4	Darma et al.[7]	Indonesia	Serum, ELISA, NS	PROM	29	29	27.28 ± 3.67; 36.29 ± 4.44	28.52 ± 4.27; 38.87 ± 1.01	0.53 ± 0.09 mg/dl	0.58 ± 0.08 mg/dl	The level was significantly lower in the PROM group (p = 0.018).	20/24 high
5	Defrin and Rasyid[10]	Indonesia	Plasma, chromatography, NS	TPROM	72	72	29.00 ± 5.53; NR ± NR	29.47 ± 5.22; NR ± NR	60.07 ± 50.33 nmol/ml	97.56 ± 78.28 nmol/ml	The level was significantly lower in the TPROM group (p = 0.001).	15/24 moderate
6	Gupta et al.[11]	India	Serum, spectrophotometry, at enrolment at the same gestational age (28-36.6 weeks) before the administration of any medication	PPROM	50	50	24.36 ± 3.45; 34.00 ± 1.54 (at birth)	24.08 ± 3.33; 39.30 ± 1.76 (at birth)	0.60 ± 0.35 mg/dl	1.18 ± 0.43 mg/dl	The level was significantly lower in the PPROM group (p = 0.000).	21/24 high
7	Ibrahim et al.[33]	Egypt	Plasma, colorimetric method with ELISA, at recruitment (after 6-8 h of fasting)	PPROM	30	30	29.97 ± 4.86; 33.47 ± 2.87 (at recruitment)	30.67 ± 4.41; 33.47 ± 2.87 (at recruitment)	0.4 ± 0.3 mg/dl	0.6 ± 0.4 mg/dl	The level was significantly lower in the PPROM group (p = 0.033).	20/24 high

TABLE 2. Continued

	Study ID	Country	Blood sample type, measurement method, and time of drawing blood sample	Outcome	Number of participants		Mean age ± SD in years and mean gestational age ± SD in weeks		Mean ± SD of vitamin C levels		Conclusion	MINORS quality score and category
					Outcome	Control	Outcome	Control	Outcome	Control		
8	Ilhan et al.[34]	Türkiye	Plasma, HPLC, as reported in column for gestational age	PPROM	38	34	28.5 ± 5.9; 29.2 ± 3.2 (at PPROM and blood sample collection)	27.0 ± 4.0; 30.0 ± 3.5 (at blood sample collection)	7.29 ± 2.19 mg/l	13.85 ± 3.07 mg/l	The level was significantly lower in the PPROM group ($p = 0.0012$).	18/24 moderate
9	Ilhan et al.[35]	Türkiye	Plasma, HPLC, at the time of diagnosis of PPROM and routine examinations of controls matched for gestational age	PPROM	75	41	28.33 ± 5.64; 31.63 ± 4.00 (at delivery)	26.90 ± 4.10; 38.53 ± 1.28 (at delivery)	7.39 ± 2.37 µg/ml	13.83 ± 3.16 µg/ml	The level was significantly lower in the PPROM group ($p < 0.001$).	18/24 moderate
10	Kim et al.[36]	Korea	Plasma, HPLC, NS	PPROM	20	23	30.6 ± 0.89; 32.8 ± 3.13	31.2 ± 0.96; 32.7 ± 3.84	383.8 ± 212.43 nmol/ml	563.3 ± 353.93 nmol/ml	The level was significantly lower in the PPROM group ($p < 0.05$).	16/24 moderate
11	Lee et al.[37]	Korea	Plasma, HPLC, NS	PPROM	20	20	30.2 ± 0.89; 30.0 ± 3.13	30.5 ± 0.89; 31.7 ± 3.58	304.0 ± 134.61 nmol/ml	483.6 ± 309.47 nmol/ml	The level was significantly lower in the PPROM group ($p < 0.05$).	18/24 moderate
12	Net[8]	Indonesia	Serum, ELISA, NS	PROM	19	19	30.79 ± 6.713; 39.39 ± 10.16	27.21 ± 5.8108; 38.91 ± 7.71	1.53 ± 0.31 µg/ml	1.81 ± 0.25 µg/ml	The level was significantly lower in the PROM group ($p = 0.004$).	20/24 high
13	Osaikhuwuomwan et al.[38]	Nigeria	Plasma, spectrophotometry, at recruitment after 6-8 h of fasting (PPROM within 24 h, controls matched for gestational age)	PPROM	40	40	28.25 ± 3.74; 31.30 ± 3.23	28.55 ± 4.52; 31.30 ± 3.23	0.53 ± 0.05 mg/dl	0.58 ± 0.05 mg/dl	The level was significantly lower in the PPROM group ($p = 0.0001$).	20/24 high
14	Ponnaluri[39]	India	Plasma, spectrophotometry, NS (fasting samples)	PPROM	50	50	27.02 ± 3.59; 34.33 ± 3.06	27.83 ± 3.22; 40.07 ± 1.23	0.50 ± 0.30 mg/dl	1.12 ± 0.60 mg/dl	The level was significantly lower in the PPROM group ($p < 0.000$).	18/24 moderate
15	Rizka et al.[13]	Indonesia	Plasma, NR, NS	TPROM	26	26	NR ± NR; NR ± NR	NR ± NR; NR ± NR	0.731 ± 0.182 µg/dl	0.722 ± 0.169 µg/dl	There was no significant difference between groups ($p = 0.852$).	16/24 moderate

TABLE 2. Continued

	Study ID	Country	Blood sample type, measurement method, and time of drawing blood sample	Outcome	Number of participants		Mean age ± SD in years and mean gestational age ± SD in weeks		Mean ± SD of vitamin C levels		Conclusion	MINORS quality score and category
					Outcome	Control	Outcome	Control	Outcome	Control		
16	Saleem et al.[14]	Iraq	Serum, ELISA, NS	PPROM	30	38	25.55 ± 6.21; 30.78 ± 3.43	26.21 ± 6.71; 31.76 ± 2.54	15.25 ± 4.11 µg/ml	16.6 ± 4.83 µg/ml	There was no significant difference between groups ($p = 0.127$).	18/24 moderate
17	Sitepu et al.[40]	Indonesia	Plasma, HPLC, NS	PPROM	20	20	27.4 ± 5.6; NR ± NR	26.1 ± 5.4; NR ± NR	3.90 ± 1.61 mg/l	9.24 ± 2.31 mg/l	The level was significantly lower in the PPROM group ($p = 0.001$).	16/24 moderate
18	Tejero et al.[5]	Mexico	Plasma, spectrophotometry, followed up from the 16th week of gestation to 1 month after delivery, with evaluations every 4 weeks	PROM	15	26	NR ± NR; NR ± NR	NR ± NR; NR ± NR	16 weeks: 22.5 ± 6.6 20 weeks: 19.8 ± 3.9 24 weeks: 19.3 ± 5.8 28 weeks: 18.1 ± 5.0 32 weeks: 20.2 ± 4.6 36 weeks: 22.1 ± 3.9 Postpartum: 23.2 ± 5.4[a]	16 weeks: 21.0 ± 7.1 20 weeks: 18.6 ± 5.1 24 weeks: 18.4 ± 6.1 28 weeks: 20.9 ± 5.1 32 weeks: 20.2 ± 5.6 36 weeks: 19.4 ± 5.1 Postpartum: 27.0 ± 8.7[a]	Significantly lower level only at week 28 ($p < 0.05$), but not clear if in plasma (reported in Table 2) or leukocytes (reported in the text and the Abstract section).	18/24 moderate

ELISA, enzyme-linked immunosorbent assay; F, female; HPLC, high-performance liquid chromatography; MINORS, methodological index for non-randomized studies; NR, not reported; NS, not specified; *p*, statistical significance; PPROM, preterm premature (prelabor) rupture of membranes; PROM, premature (prelabor) rupture of membranes (both preterm and term, i.e., regardless of whether it was preterm or term, or not specified); SD, standard deviation; TPROM, term premature (prelabor) rupture of membranes, [a]no units were reported.

TABLE 3. An Overview of Included Studies Assessing the Efficacy of Vitamin C Supplementation in the Prevention of Premature Rupture of Membranes.

	Study ID	Country	Inclusion criteria regarding gestational age and if a previous history of PROM was required	Age of the participants in years (mean ± SD, unless otherwise noted)	Vitamin C dosage, commencement/duration of supplementation	Control, blinding	Frequency of outcome(s) in vitamin C group (n/N)	Frequency of outcome(s) in control group (n/N)	Study conclusions
1	Hajifoghaha et al.[19]; Foghaha et al.[18]	Iran	20 weeks, no	Vitamin C: 23.88 ± 4.62 Control: 24.00 ± 4.56	100 mg daily, from 20 to 36 weeks of gestation	Placebo, single-blind	PROM: 5/57 PPROM: 1/57 TPROM: 4/57	PROM: 22/60 PPROM: 4/60 TPROM: 18/60	The number of PROM ($p < 0.001$) and TPROM ($p = 0.001$) was significantly lower in the vitamin C group, but there was no significant difference in PPROM ($p = 0.5$).
2	Casanueva et al.[2]	Mexico	< 20 weeks, no (10.6% had a history of PROM)	Vitamin C: 27.5 ± 7.4 Control: 27.4 ± 7.7	100 mg daily, commenced after 20 weeks of gestation (duration not specified)	Placebo, double-blind	PROM: 4/52	PROM: 14/57	The incidence of PROM was significantly lower in the vitamin C group ($p = 0.018$).
3	Ghomian et al.[20]	Iran	14 weeks, history of at least one PPROM	Vitamin C: 29.8 ± NR Control: 29 ± NR	100 mg daily, from 14 to 37 weeks of gestation	Placebo, NR	PROM: 43/85 PPROM: 27/85 TPROM: 16/85[a]	PROM: 67/85 PPROM: 38/85 TPROM: 29/85[a]	The frequency of PPROM ($p = 0.001$) and TPROM ($p = 0.001$) was significantly lower in the vitamin C group.
4	Ochoa-Brust et al.[42]	Mexico	At least 12 weeks, no	Vitamin C: 22.3 ± 5.9 Control: 23.1 ± 5.5	100 mg daily, for 3 months	No vitamin C, single-blind	PROM: 0/55 PPROM: 0/55 TPROM: 0/55	PROM: 0/55 PPROM: 0/55 TPROM: 0/55	There was no premature membrane rupture in any of the groups.
5	Zamani et al.[43]	Iran	18 weeks, history of previous PROM and PPROM	Vitamin C: 24.90 ± 5.63 Control: 24.60 ± 5.53	500 mg daily (250 mg twice a day), from 18 to 28 weeks of gestation	Placebo, double-blind	PPROM: 2/30	PPROM: 5/30	There was no significant difference in PPROM ($p = 0.213$).
6	Khan et al.[44]	Pakistan	12-20 weeks, no	Vitamin C: 25.63 ± 4.92 Control: 25.37 ± 4.77	500 mg daily, started at 12-20 weeks of gestation (mean of 16 weeks, but duration was not specified)	No vitamin C, NR	PROM: 2/95	PROM: 3/96	There was no significant difference in PROM ($p = 0.856$).
7	Kiondo et al.[45]	Uganda	12-22 weeks, no	Age group (%) Vitamin C: ≤ 19 (19.5) 20-29 (53.0) 30-34 (17.0) ≥ 35 (10.5) Control: ≤ 19 (21.0) 20-29 (54.3) 30-34 (14.8) ≥ 35 (9.9)	1,000 mg daily, until delivery	Placebo, triple-blind	PROM: 15/415	PROM: 19/418	There was no significant difference in PROM ($p = 0.5$).

n, number of participants with the outcome; N, number of randomized participants minus any participants with missing outcomes (denominator); NR, not reported; PPROM, preterm premature (prelabor) rupture of membranes; PROM premature (prelabor) rupture of membranes (both preterm and term, i.e., regardless of whether it was preterm or term); SD, standard deviation; TPROM, term premature (prelabor) rupture of membranes, [a]Frequencies in Ghomian et al.[20] were reported for PPROM and PROM. We tried contacting authors for clarifications, but we did not receive a reply, so we considered frequencies reported for PROM as TPROM as did the authors of the previous Cochrane systematic review[6] (because the reported numbers for PROM were lower than PPROM), whereas the frequencies of PPROM and TPROM were summed to calculate the overall frequency of PROM in each group.

The combined effect size estimates for each main comparison, the results of sensitivity analyses, and the certainty of the evidence assessment are shown in Table 4. Figures 3, 4 show forest plots for main comparisons.

Women with PROM, whether preterm or term, had significantly lower vitamin C levels than controls, but the heterogeneity was significant. A subgroup of studies conducted on continents other than Asia[5,12,33,38] (I^2 = 29.06%; Hedges' g, -0.81; 95% CI: -1.15; -0.46) and studies in which levels were measured using ELISA[7,8,14] (I^2 = 25.89%; Hedges' g, -0.56; 95% CI: -0.93; -0.19) had acceptable and nonsignificant heterogeneity, whereas the combined effect size indicated that vitamin C levels were significantly lower in women

TABLE 4. Combined Effect Size Estimates for each Main Comparison, Results of Sensitivity Analyses, and Certainty of the Evidence Assessment.

Comparison	n	N	Combined effect size	Heterogeneity	Robust in SA	Certainty of the evidence (GRADE)
Vitamin C level						
(1) PROM vs. control	18	1220	g = -1.48 (95% CI: -2.82, -0.14; PI: -4.16, 1.20), Z = -2.32, p = 0.020*	Q = 287.08, p < 0.001, I^2 = 94.08%*	No	—
- SA: w/o Ilhan et al.[34]	17	1148	g = -1.41 (95% CI: -2.83, 0.00; PI: -4.11, 1.29), Z = -2.11, p = 0.034	Q = 264.82, p < 0.001, I^2 = 93.96%*	—	—
- SA: w/o Ilhan et al.[35]	17	1104	g = -1.41 (95% CI: -2.83, 0.00; PI: -4.09, 1.27), Z = -2.12, p = 0.034	Q = 254.61, p < 0.001, I^2 = 93.72%*	—	—
- SA: w/o Sitepu et al.[40]	17	1180	g = -1.41 (95% CI: -2.82, 0.01; PI: -4.11, 1.30), Z = -2.11, p = 0.035	Q = 272.62, p < 0.001, I^2 = 94.13%*	—	—
- Outlier SA: w/o Ali[9]	17	1120	g = -1.03 (95% CI: -1.43, -0.63; PI: -2.52, 0.45), Z = -5.47, p < 0.001*	Q = 116.67, p < 0.001, I^2 = 86.29%*	Yes	—
(2) PPROM vs. control	12	833	g = -2.06 (95% CI: -4.28, 0.16; PI: -5.73, 1.61), Z = -2.04, p = 0.041	Q = 243.03, p < 0.001, I^2 = 95.47%*	No	—
- SA (Outlier): w/o Ali[9]	11	733	g = -1.29 (95% CI: -1.85, -0.73; PI: -3.00, 0.42), Z = -5.16, p < 0.001*	Q = 79.04, p < 0.001, I^2 = 87.35%*	Yes	—
(3) TPROM vs. control	3	216	g = -0.30 (95% CI: -1.14, 0.54; PI: -1.70, 1.09), Z = -1.55, p = 0.120	Q = 3.82, p = 0.148, I^2 = 47.61%	Yes	—
Efficacy of vitamin C supplementation						
(1) PROM	7	1590	RR = 0.57 (95% CI: 0.39, 0.81; PI: 0.33, 0.96), Z = -3.82, p < 0.001*	Q = 6.83, p = 0.337, I^2 = 12.17%	Yes	⊕⊕⊕◯ Moderate[a]
(2) PPROM	4	457	RR = 0.67 (95% CI: 0.45, 0.99; PI: 0.45, 0.99), Z = -3.25, p = 0.001*	Q = 1.27, p = 0.737, I^2 = 0.00%	No	⊕⊕◯◯ Low[a,b]
- SA: w/o Zamani et al.[43]	3	397	RR = 0.69 (95% CI: 0.40, 1.18; PI: 0.40, 1.18), Z = -2.95, p = 0.003	Q = 0.82, p = 0.663, I^2 = 0.00%	/	/
- SA: w/o Ghomian et al.[20]	3	287	RR = 0.38 (95% CI: 0.13, 1.16; PI: 0.13, 1.16), Z = -3.73, p < 0.001	Q = 0.35, p = 0.839, I^2 = 0.00%	/	/
- SA: w/o Hajifoghaha et al.[19]; Foghaha et al.[18]	3	340	RR = 0.69 (95% CI: 0.45, 1.05; PI: 0.45, 1.05), Z = -3.77, p < 0.001	Q = 0.53, p = 0.769, I^2 = 0.00%	/	/
- SA: w/o Ochoa-Brust et al.[42]	3	347	RR = 0.67 (95% CI: 0.35, 1.27; PI: 0.35, 1.27), Z = -2.71, p = 0.007	Q = 1.22, p = 0.542, I^2 = 0.00%	/	/
(3) TPROM	3	397	RR = 0.44 (95% CI: 0.13, 1.49; PI: 0.10, 2.03), Z = -2.89, p = 0.004	Q = 2.29, p = 0.319, I^2 = 12.50%	Yes	⊕⊕◯◯ Low[a,c]

CI, confidence interval; g, Hedges' g; n, number of studies contributing to pooled estimate; N, number of included subjects; PI, prediction interval; PPROM, preterm premature (prelabor) rupture of membranes; PROM, premature (prelabor) rupture of membranes (both preterm and term, i.e., regardless of whether it was preterm or term); RR, risk ratio; SA, sensitivity analysis; TPROM, term premature (prelabor) rupture of membranes; w/o, without. GRADE Working Group grades of evidence. High quality: Further research is very unlikely to change our confidence in the estimate of effect. Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Very low quality: We are very uncertain about the estimate, [a]Downgraded one level for serious risk of bias: overall risk of bias in all included studies was rated as either "some concerns" or "high", [b]Downgraded one level for serious imprecision: low number of events, [c]Downgraded one level for serious imprecision: low number of events and wide confidence intervals, *Statistically significant (for combined effect size if both 95% CI did not include 0 [for Hedges' g] or one [for RR] and p < 0.05; for heterogeneity both p < 0.10 and I^2 > 50%).

with PROM. Sensitivity analysis revealed that removing data from three studies would have a significant impact on the results, resulting in a non-significant combined effect size (95% CI included 0). However, post hoc sensitivity analysis performed by excluding the outlying study by Ali,[9] which had a much larger effect size (-15.94) than other studies (from 0.05 to -2.63), revealed significantly lower levels in women with PROM, and these results were robust when the remaining studies were excluded one by one. The funnel plot (Figure 5) revealed no asymmetry in the distribution of effect sizes, considering that the observed combined effect size was the same as adjusted and there were no missing studies. The Begg and Mazumdar rank correlation test found no significant publication bias ($p = 0.161$), whereas the Egger regression test did ($p < 0.001$).

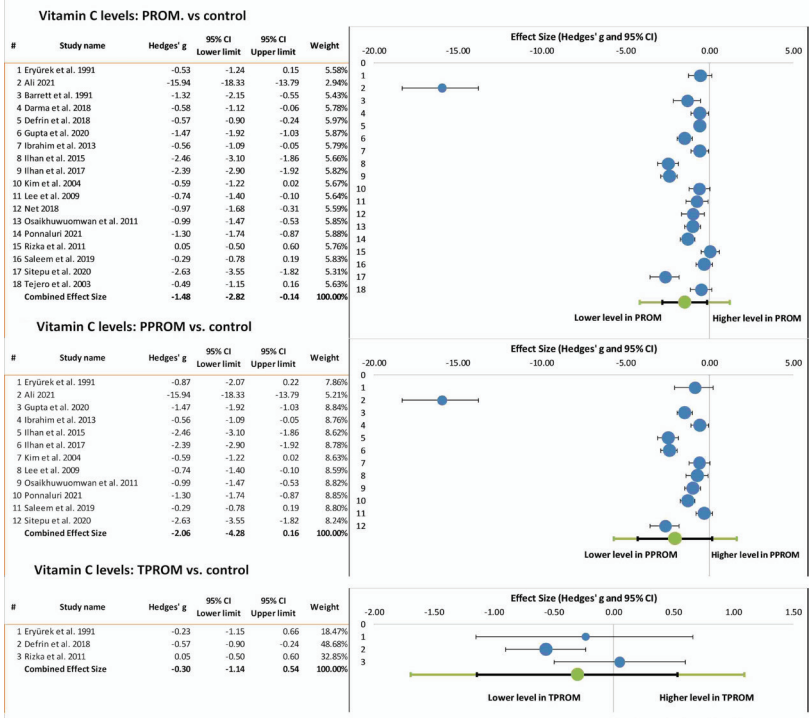

FIG. 3. Forest plots showing effect sizes of differences in vitamin C levels between women with PROM regardless of whether it was preterm or term, PPROM or TPROM and controls.

PROM, Premature rupture of membranes; PPROM, preterm premature rupture of membranes; TPROM, term premature rupture of membranes; CI, confidence interval.

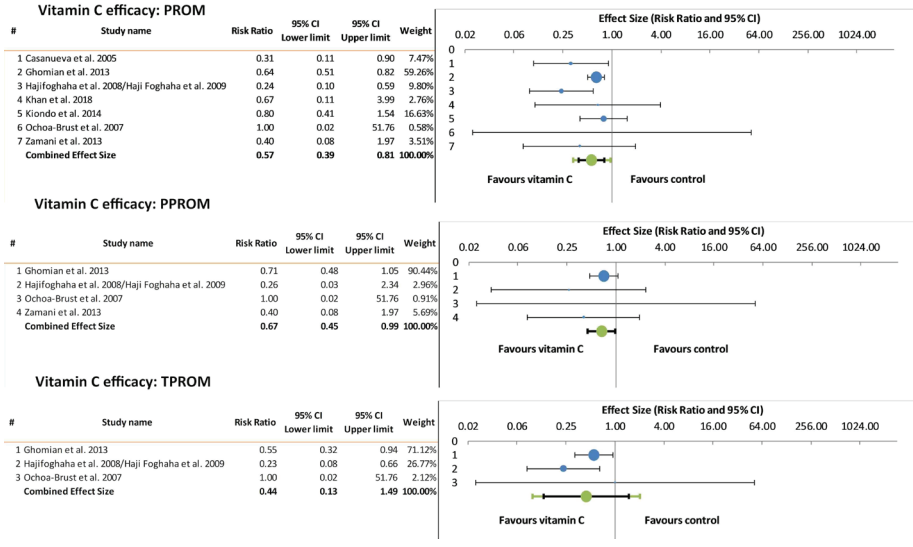

FIG. 4. Forest plots showing effect sizes of the efficacy of vitamin C supplementation in prevention of PROM regardless of whether it was preterm or term, PPROM and TPROM.

PROM, Premature rupture of membranes; PPROM, preterm premature rupture of membranes; TPROM, term premature rupture of membranes; CI, confidence interval.

Vitamin C supplementation was associated with a lower risk of PROM, regardless of whether it was preterm or term, and there was no significant heterogeneity, whereas sensitivity analysis revealed no significant changes after removing individual studies.

There was no significant difference in vitamin C levels between women with PPROM and controls, with a 95% CI including 0, but there was significant heterogeneity. The subgroup of studies conducted in Africa[33,38] (I^2 = 34.25%; Hedges' g, -0.79; 95% CI: -1.21; -0.36) had non-significant and tolerable levels of heterogeneity, whereas the combined effect size revealed that vitamin C levels were significantly lower in women with PPROM. Sensitivity analysis revealed that only removing data from the outlying study by Ali[9] would have a significant impact on the results, resulting in a significant combined effect size indicating significantly lower vitamin C levels in PPROM, whereas post hoc sensitivity analysis performed after removing the remaining studies one by one revealed that these results were robust. The funnel plot (Figure 5) indicated no asymmetry in the distribution of effect sizes, considering that the observed combined effect size was the same as adjusted and there were no missing studies. The Begg and Mazumdar rank correlation test did not detect significant publication bias (p = 0.337), whereas the Egger regression test did (p < 0.001).

Vitamin C supplementation was linked to a reduced risk of PPROM, with no significant heterogeneity. However, sensitivity analysis showed that removing data from any study resulted in a combined effect size that was not significant, as the 95% CI included one.

There were no differences in vitamin C levels between women with TPROM and controls nor between women who took vitamin C supplements and controls in risk of TPROM. There was no significant heterogeneity in both comparisons, and the results were robust in sensitivity analysis.

DISCUSSION

We found that women with PROM, whether preterm or term, had significantly lower vitamin C levels, but there were no differences in main comparisons of levels in PPROM and TPROM. The results in comparisons including PROM (whether preterm or term) or PPROM versus control were not robust in sensitivity analysis due to the outlier study,[9] and there was significant heterogeneity and evidence of publication bias detected by one of three tests. Outliers in meta-analysis can occur for a variety of reasons, including mistakes in data collection, analysis, or reporting in these studies.[46] Region and measurement methods were identified as potential sources of heterogeneity. Clear regional differences in vitamin C status and prevalence of deficiency have already been observed between low-, middle-, and high-income countries, most likely due to geographic, economic, social, and cultural factors.[47] Moreover, vitamin C can be measured in the blood using various methods, most of which have limitations and are susceptible to interference.[47] Additionally, vitamin C is oxidation-sensitive, so proper sample handling, processing, and storage before analysis is crucial for accurate measurement.[47] Furthermore, the majority of publication bias tests have weak-to-moderate agreement, so the results of a single test should be carefully interpreted.[46] It was previously observed that the Egger regression test identified publication bias in a greater number of meta-analyses, most likely due to its higher sensitivity or risk of false positives.[46]

Our results indicate that vitamin C supplementation may be effective in reducing the risk of PROM, particularly PPROM, but not TPROM. However, results for PPROM were not robust in sensitivity analysis, most likely due to the small number of studies included in the analyses (only three). One previous meta-analysis found that vitamin C supplementation alone was effective in reducing the risk of both PPROM and TPROM,[6] but the other two did not show it was effective in preventing PPROM.[15,16] These differences could be

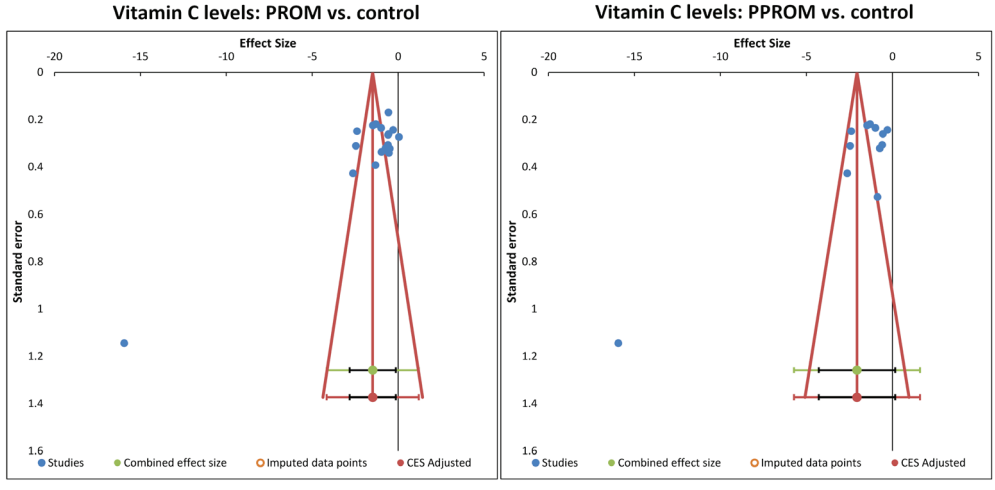

FIG. 5. Funnel plots with trim-and-fill analysis for comparisons of vitamin C levels between women with PROM regardless of whether it was preterm or term and controls and PPROM and controls.

PROM, Premature rupture of membranes; PPROM, preterm premature rupture of membranes.

attributed to differences in meta-analyses' inclusion and exclusion criteria for studies on PROM, PPROM, and TPROM and the inclusion of new studies in our meta-analysis.

Considering that membrane rupture has been linked to increased oxidative stress and abnormalities in collagen formation and structure, vitamin C may be effective in preventing PROM, particularly PPROM, due to its antioxidant properties and role in collagen synthesis.[43] Three studies found a significant effect on PROM/PPROM with a daily dose of 100 mg.[2,18-20] Higher doses of 500 or 1,000 mg per day resulted in insignificantly lower rates of PROM/PPROM.[43-45] So, 100 mg daily appears to be sufficient for reducing the risk of PROM, particularly PPROM. The recommended daily intake of vitamin C during pregnancy is 85 and 105 mg in the United States and the European Union, respectively, with 2,000 mg/day being considered the tolerable upper limit in pregnancy in the United States.[48] It should be noted that none of the efficacy studies included in our meta-analysis measured vitamin C levels before supplementation, so vitamin C supplementation was administered regardless of vitamin C level status. Although our meta-analysis indicated that vitamin C levels were significantly lower in women with PROM, particularly PPROM, after removing the outlying study, we are unable to conclude whether vitamin C supplementation is effective in all pregnant women or only those with low vitamin C levels because efficacy studies did not evaluate this. A related question is whether it is relevant if women have a history of PROM or preterm birth in previous pregnancies. Unfortunately, while some of the studies included women with a history of PROM or PPROM, this information was not reported in most of them, so we were unable to determine its relevance in our meta-analysis. More research is needed to clarify both issues.

This study has several limitations. First, our findings should be interpreted with caution due to the relatively small number of studies included in some analyses. Second, we found significant heterogeneity among the included studies. We assessed some factors that may be potential sources of heterogeneity, but some of them were unavailable in all studies. We were also unable to assess the effects of other relevant factors, such as gestational age and dietary vitamin C intake because they were either not reported in any of the included studies or were reported inconsistently and in a small number of studies. Third, we were unable to obtain the full text of some reports to determine whether they met our inclusion criteria, and the majority of the authors we contacted did not respond to our requests for data and clarifications.

In conclusion, women with PROM, particularly those diagnosed preterm, appear to have significantly lower vitamin C levels, and vitamin C supplementation appears to be effective in reducing the risk of PROM, particularly PPROM. However, considering the high heterogeneity and lack of robustness in some analyses, more high-quality studies with low risk of bias and more homogenous and larger samples are needed to confirm these findings.

Ethics Committee Approval: Since our article was a systematic review and meta-analysis of data extracted from published clinical studies, ethics committee approval is not applicable for our article.

Data Sharing Statement: The data that support the findings of this systematic review and meta-analysis are openly available in Figshare repository at https://doi.org/10.6084/m9.figshare.22096160.v1.

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