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Title page

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Lactoferrin or ferrous salts for iron deficiency anemia in pregnancy: a meta-analysis of randomized trials.

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Condensation

In iron deficiency anemia with pregnancy, bovine lactoferrin is just as good as ferrous sulfate at increasing hemoglobin with fewer gastrointestinal side effects.

Abstract

This systematic review and meta-analysis aimed to evaluate the efficacy of daily oral bovine lactoferrin versus daily oral ferrous iron preparations for treatment of iron deficiency anemia (IDA) during pregnancy. Searches were conducted on PubMed, ScienceDirect, ClinicalTrials.gov and CENTRAL databases from inception to February 2017, and the bibliographies of retrieved articles were screened. The PRISMA Statement was followed. Published English language randomized trials comparing lactoferrin with oral ferrous iron preparations in pregnant women with iron deficiency anemia were included. Quasi-randomized, non- randomized or studies including other known cause of anemia, gestational or pre-existent maternal diseases were excluded. Accordingly, 4 eligible trials (600 women) were analyzed. Primary outcome was change in hemoglobin level at 4 weeks of treatment. Secondary outcomes were; change in serum ferritin and iron, rates of gastrointestinal side effects, preterm birth, low birthweight, neonatal death and mean birthweight. Quality assessment was performed by the Cochrane risk of bias tool. Odds ratio and mean difference were used to integrate dichotomous and continuous outcomes respectively.

Pooled estimates for change in hemoglobin levels at four weeks favored daily oral lactoferrin over daily oral ferrous sulphate (mean difference 0.77; 95% confidence interval [CI] 0.04-1.55; *P*=0.04, 4 trials, 600 women). However, after subgroup analysis (degree of anemia),

no significant difference in hemoglobin levels were found between both groups in mild anemia (mean difference 0.80; 95% CI -0.21 to 1.82, 3 trials, 372 women), but a significant increase favoring lactoferrin was reported in moderate anemia (mean difference 0.68; 95% CI 0.53-0.83; $P<0.00001$, one trial, 228 women). Significantly less gastrointestinal side effects were reported with lactoferrin treatment. No significant differences existed with regard to other outcomes. In conclusion, for pregnant women with iron deficiency anemia, daily oral bovine lactoferrin is just as good as ferrous sulfate in improving hematological parameters with fewer gastrointestinal side effects. Thereby, lactoferrin should be the iron replacement agent of choice for treatment of IDA in pregnancy.

Key Words: Iron deficiency, iron deficiency anemia, pregnancy, lactoferrin and bovine lactoferrin

Introduction

Iron deficiency anemia (IDA) during pregnancy continues to remain one of the most important public health problems in both developed and developing countries. Globally, it affects 56.4 million pregnant women (41.8%). Higher prevalence rates are present in Africa, South East Asia and Eastern Mediterranean (57.1%, 48.2% and 44.2% respectively), meanwhile lower rates are in Europe and Americas (25.1% and 24.1% respectively) [1]. Hemoglobin (Hb) represents the most widely used indicator to assess anemia during pregnancy. Hb level <11g/dL has been utilized by the World Health Organization (WHO) to define IDA during pregnancy [1, 2]. Noteworthy, there are no current WHO recommendations on the use of different Hb cut-off points for anaemia by trimester [2]. A drop of Hb level by approximately 0.5 g/dL in the second trimester of pregnancy has been recognized. This drop could be explained by the physiological pregnancy associated hemodilution, i.e. an increased red blood cell mass by approximately 25% vs. the expanding plasma volume by approximately 50% [3,4]. In this regard, the US Centers for Disease Control and Prevention (CDC) define IDA during pregnancy when Hb level <11g/dL during the first and third trimesters and <10.5g/dL in the second trimester [5]. Serum ferritin is the best hematological parameter to evaluate iron stores and a concentration <15mcg/L is diagnostic of IDA in pregnancy [6]. IDA has a negative impact on maternal and fetal health. It affects not only the general wellbeing of the mother (i.e. fatigue, dyspnea, palpitations, headaches and irritability), but also results in increased maternal morbidity and mortality from pregnancy and childbirth. IDA increases the risk of preterm birth, fetal growth retardation, low birthweight and perinatal death [7-9].

Treatment of IDA in pregnancy still poses a challenge not only for clinicians, but also for patients. Food itself does not contain enough iron. Thereby, oral ferrous iron preparations such as ferrous fumarate, ferrous sulphate and ferrous gluconate have been recommended as the

first-line of iron supplementation. The recommended daily dose is 100-200 mg of elemental iron [6,10]. A rise in Hb concentration by approximately 2g/dL over 3-4 weeks is considered a satisfactory response to treatment.6 Unfortunately, gastrointestinal side effects such as epigastric discomfort, nausea, vomiting, diarrhea, constipation, abdominal colicky pain and dark stools are frequently associated with ferrous iron supplements and up to 30% of patients experience dose limiting side effects [6,11,12].

In a randomized controlled trial (RCT), Paesano et al. 2006 [13] reported oral bovine lactoferrin as a promising alternative to ferrous sulphate for treatment of IDA in pregnancy. Lactoferrin is a glycoprotein from transferrin family. It is presented in high concentrations in milk of humans and other mammals. It is also synthesized in most exocrine secretions and neutrophils in inflammation and infection sites. Lactoferrin has two times higher affinity for iron than serum transferrin. It reversibly chelates two Fe^{+3} ions per molecule [14]. Another potential mechanism is permitting iron export from tissues to the blood by interplaying with ferroportin and hepcidin which are key proteins of systemic iron homeostasis [15,16]. Unlike ferrous sulphate, lactoferrin was superior in that it did not provoke adverse gastrointestinal side effects [13].

To our knowledge, there has been no meta-analysis to provide definite evidence for the efficacy of bovine lactoferrin treatment for IDA during pregnancy. In that respect, and given that this is a clinically important area to address, this systematic review and meta-analysis was conducted to evaluate the efficacy of daily oral bovine lactoferrin vs. daily oral ferrous iron preparations for treatment of IDA during pregnancy on the basis of the available evidence so far in RCTs.

Materials and methods

Our systematic review was conducted using only RCTs. We conformed methodological approaches reported in the PRISMA Statement [17]. The clinical question posed was: in pregnant women with IDA, what is the effectiveness of oral bovine lactoferrin compared with oral ferrous iron preparations for improving hematological response (Hb level)?

Information sources and search strategy

The following electronic databases were searched: PubMed, ScienceDirect, ClinicalTrials.gov (each from inception to February 2017) and CENTRAL (Cochrane Central Register of Controlled Trials, Issue 2, 2017). To generate a subset of citations relevant to our research question, the following Medical Subject Headings (MeSH) and text words were used: "iron deficiency" OR "iron deficiency anemia" AND "pregnancy" OR "pregnant" AND "lactoferrin" OR "bovine lactoferrin". Adjustment of search terms was carried out for each database as necessary. The search was limited to articles conducted on human beings, females and published in English. The reference lists of retrieved publications were manually searched to identify any missing relevant publications. The database search details are described in Table S1.

Study selection

Two reviewers (H.A. and O.F.) independently screened the titles and abstracts of retrieved citations for relevance to our meta-analysis by using the following inclusion criteria: a) pregnant women with IDA, diagnosed in the second or third trimester according to the WHO [Hb <10.5g/dL during the second trimester and <11g/dL in the third trimester of pregnancy][2]; b) only RCTs which compared oral bovine lactoferrin with oral ferrous iron preparations and reported at least one of the following outcomes: Hb level, serum ferritin, serum iron after at least 4 weeks of treatment, rate of gastrointestinal side effects during the treatment period (epigastric discomfort, nausea, vomiting, diarrhea, constipation, abdominal

colicky pain and dark stools) or any of the following obstetric outcomes; preterm birth, mean birthweight, low birthweight and neonatal death were included.

Exclusion criteria were: any RCT in which Hb level for IDA was not prespecified before starting the treatment, quasi-RCTs, non-RCTs, other known cause (s) of anemia, gestational (such as hypertension, gestational diabetes) or pre-existent maternal diseases (such as thyroid dysfunctions, liver pathologies, nutritional diseases). Full texts were obtained by contacting the author when this could not be obtained online. In instances of any disagreement regarding study eligibility, it was resolved by consensus after discussion with the third reviewer (E.G.).

Data extraction

Two reviewers (H.A. and O.F.) independently extracted the data from each included study according to a data extraction form designed in accordance with the Cochrane Checklist of items [18]. This form included the following details; source, eligibility, methods, participants characteristics, interventions, outcomes, results in addition to any other important miscellaneous data. Primary outcome measure was change in Hb level (g/dL) after at least 4 weeks of treatment. Secondary outcome measures were; change in serum ferritin (mcg/L) and serum iron (mcg/dL) after at least 4 weeks of treatment, rates of gastrointestinal side effects (epigastric discomfort, nausea, vomiting, diarrhea, constipation, abdominal colicky pain and dark stools) and the following obstetric outcomes; preterm birth (less than 37 weeks of gestation), mean birthweight (kg), low birthweight (less than 2500 g) and neonatal death (within 28 days after delivery). The unit of analysis was per woman randomized according to the intention-to-treat (ITT) principle.

Assessment of risk of bias

The Cochrane Collaboration risk-of-bias tool was utilized to assess the methodological quality and risk of bias of included studies [18]. Each article was assessed according to seven

specific domains (random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective outcome reporting and other biases). These domains were evaluated and scored as high, low or unclear risk of bias. The GRADE approach was utilized for quality rating of a body of evidence into: high, moderate, low and very low [18]. Two reviewers (H.A. and O.F.) independently conducted the quality assessment. In case of disagreements, a consensus was reached after discussion with the third reviewer (E.G.).

Data synthesis

The data analysis was performed using RevMan software 5.1 of the Cochrane Collaboration. The fixed-effects model was used for pooling of results. Odds ratios (OR) with 95% confidence intervals (CI) were calculated for dichotomous outcomes. Meanwhile, the mean difference (MD) was used for continuous outcomes. If any heterogeneity existed (by the chi-squared test, with $P \leq 0.1$), random-effects model was employed. If I^2 statistic was $\geq 50\%$, exploration of causes of heterogeneity was performed by subgroup analysis. We tested the preferential effect of each study on overall result of our meta-analysis through performing multiple sensitivity analyses removing one study in each step.

Results

Study selection

The process of literature search and study selection is summarized in the PRISMA flow diagram (Figure 1). Of the 203 publications screened, 10 were identified as potentially eligible for inclusion. After examination of the full manuscripts, six articles were excluded for the following reasons: one study (ClinicalTrials.gov Identifier: NCT01221844) was a prospective non randomized trial and included pregnant women with IDA associated with

hereditary thrombophilia[19]; one RCT was a duplication of another RCT by the same authors[20]; one study was a prospective cohort non controlled trial[21]; one study was a prospective controlled non randomized trial and not addressing iron deficiency anemia in pregnancy [22] and two studies were review articles[16,23]. Finally, only four RCTs satisfied the selection criteria and were included in this review [13,24-26].

Study characteristics and risk of bias of included studies

Of the four included studies; three were performed in Italy [13,24,25] and one in Egypt [26]. The four included studies enrolled 600 participants (297 women received treatment with oral bovine lactoferrin and 303 were treated with oral ferrous sulphate). The sample size varied across the trials and ranged from 75 to 300 participants. All the four studies were published between 2006 and 2016 in peer- reviewed indexed journals with an impact factor. All trials were single center and two [13,25] were funded through industry and governmental grants. The baseline of all trials was comparable. The duration of treatment was 4 weeks in 3 trials [13,24,25] and 8 weeks in 1 trial [26]. The characteristics of the included studies are presented in Table 1. The risk of bias summary for included studies is demonstrated in Figure 2.

Synthesis of results

Pooled estimates for Hb levels at four weeks from the 4 RCTs using random effects model favored daily oral bovine lactoferrin over daily oral ferrous sulphate (MD 0.77; 95% CI 0.04-1.55; P=0.04, 4 RCTs, 600 women). However, significant heterogeneity across the studies were noticed (I^2=99%) (Figure 3). Accordingly, we performed subgroup analysis by the degree of anemia at the start of supplementation to explore possible causes (mild; Hb:10-10.9 g/dl; moderate; Hb: 7-9.9 g/dl [2]. In mild IDA, pooled analysis from the 3 RCTs [13,23,24] using random effects model found no significant difference in Hb levels at four weeks between both groups (MD 0.80; 95% CI -0.21 to 1.82; P=0.12, 3 RCTs, 372 women).

Noteworthy, two RCTs [13,25] had individual significant differences favoring oral lactoferrin treatment (MD 1.20; 95% CI 0.99-1.41 and MD 1.50; 95% CI 1.45-1.55, respectively). Meanwhile one RCT involving 100 women [24] found a significant smaller increase in the mean level of Hb at four weeks with oral ferrous sulphate compared with bovine lactoferrin (MD -0.30; 95% CI -0.52 to -0.08) (Figure 3 and Table 2). Only one study [26] on moderate IDA showed a significant increase in the mean level of Hb at four weeks with oral lactoferrin compared with ferrous sulphate (MD 0.68; 95% CI 0.53-0.83; P<0.00001, one RCT, 228 women).The subgroups were not significantly different (P=0.81, I^2=0%) (Figure 3). Noteworthy in the aforementioned study [26], this significant rise in the mean level of Hb was more pronounced at 8 weeks (MD 1.27; 95% CI 1.14-1.40; P< 0.00001). Multiple sensitivity analyses were carried out to test the preferential effect of each study on overall result of our meta-analysis by removing one study in each step. However, significant heterogeneity persisted (data not shown). The evidence was considered to be of low quality being downgraded two levels for some potential limitations of the included studies and high heterogeneity I^2> 80% (Figure 2 and Table 2).

In regard to the increase in mean serum ferritin, no significant difference was observed under lactoferrin at four weeks as compared to ferrous sulphate in 2 RCTs [24,25] using random effects model (MD 9.82; 95% CI -9.97 to 29.62; P=0.33, 2 RCTs, 163 women with mild IDA) but with significant heterogeneity across the studies (I^2=99%) (Table 2). Notably, one RCT involving 63 women had significant difference favoring oral lactoferrin treatment (MD 20.00; 95% CI 16.50-23.50) [25]. Meanwhile, the other RCT involving 100 women found a significant smaller increase in the mean level of ferritin at four weeks with oral ferrous sulphate compared with bovine lactoferrin (MD -0.20; 95% CI -0.38 to -0.02) [24]. The evidence was considered to be of low quality being downgraded two levels for some

potential limitation in one of the included studies and high heterogeneity I^2> 80% (Figure 2 and Table 2).

Combined data of 3 studies [13,24,25] (372 women with mild IDA) using a random effects model revealed a non significant increase in serum iron with oral lactoferrin at 4 weeks (MD 29.07; 95% CI -0.39 to 58.54, *P*=0.05) (Table 2). Heterogeneity between studies was high (I^2=99%). Notably, 2 RCTs [13,25] involving 270 women showed significant difference favoring oral lactoferrin treatment (MD 37.00; 33.50-40.50 and MD 53.00; 95% CI 45.55-60.45, respectively). Meanwhile, the other RCT[24] involving 100 women revealed no difference (MD -2.60; 95% CI -8.10 to 2.90). This evidence was considered of low quality being downgraded two levels for some potential limitations of the included studies and high heterogeneity I^2> 80% (Figure 2 and Table 2).

Gastrointestinal side effects were reported in two trials (328 women) [24,26]. Pooled analysis from both trials using fixed-effects model demonstrated an evidence of significantly fewer rates of epigastric discomfort (OR 0.11; 95% CI 0.05-0.22; *P*< 0.00001), vomiting (OR 0.32; 95% CI 0.15-0.67; *P*=0.002) and constipation (OR 0.22; 95% CI 0.12-0.40; *P*< 0.00001) in patients treated with lactoferrin in comparison with those treated with ferrous sulphate without significant heterogeneity across the studies (I^2=36%, I^2=0% and I^2=0% respectively). Abdominal colicky pain and dark stools was observed predominately in the oral ferrous sulphate group in one trial [26] (OR 0.21; 95% CI 0.12-0.39; *P*<0.00001 and OR 0.01; 95% CI 0.00-0.22; *P*=0.002, respectively, 1 RCT, 228 women with moderate IDA). No reported cases of diarrhea in either group in the aforementioned trial [26]. This evidence was considered of moderate quality being downgraded one level for some potential limitations of the included studies (Figure 2 and Table 2).

Only one study [26] (228 women with moderate IDA) looked at low birthweight and neonatal mortality with no reported cases of in either group (Table 2).

Comments

Main findings

Pooled analysis found a modest difference in Hb change at 4 weeks between the two treatment types (favoring lactoferrin); subgroup analysis found this effect to be limited to pregnant women with moderate IDA and not mild IDA. Oral bovine lactoferrin is just as good as ferrous sulfate in improving other hematological parameters (serum ferritin and iron levels) at 4 weeks in patients with mild IDA. Gastrointestinal side effects were less common in patients treated with lactoferrin. Evidence concerning obstetric outcomes is insufficient.

Noteworthy, improvement in hematological parameters with lactoferrin in pregnant women with IDA is not only related to its nature as a protein with a higher iron-binding affinity and specific receptors in intestinal cells (i.e. an efficient iron absorption mechanism in apical sites of enterocytes) [27-29], but also could be ascribed to its unique ability to promote cellular iron efflux from tissues to the blood [15,16]. Interestingly, the latter effect is achieved by modulating systemic iron regulatory proteins namely ferroportin and hepcidin [15,30,31]. Ferroportin, the only known cellular iron exporter from tissues into blood, has been displayed on basolateral membranes of enterocytes as well as in all cell types involved in iron export including hepatocytes, placental cells and macrophages. Ferroportin synthesis is down-regulated by inflammatory cytokines, such as Interleukin 6 (IL-6) [32,33]. Hepcidin, a peptide hormone secreted by the liver, inhibits the iron export into plasma via binding and degradation of ferroportin (hepcidin- ferroportin complex). Hepcidin production is suppressed by anaemia and hypoxia and is induced by iron loading and inflammation [30, 31, 34-38].

In one of the included RCTs, the significantly increased levels of Hb, serum ferritin and serum iron have been associated with significant reduction of serum IL-6 (from 34±8 into 12±1pg/ml) in pregnant women with mild IDA treated with bovine lactoferrin[25]. The authors pointed out that reduction of serum IL-6 contributed to up regulation of ferroportin and down regulation of hepcidin, thereby promoting iron export to the circulation resulting in improvement of hematological parameters. Meanwhile, a significant increase of serum IL-6 was found in the ferrous sulphate group (from 33±13 into 52±13pg/ml). The authors admitted this finding to result in hepcidin- ferroportin complex formation, thus hindering iron export from tissues to blood stream [25]. Recently, these Findings were supported by a study in which incubation of THP-1 monocytes/macrophages with lactoferrin was found to prevent the LPS-induced decrease of ferroportin by reducing secretion of IL-6 [39].

A plausible explanation for the significant reduction in gastrointestinal adverse effects observed with oral lactoferrin is the absence of excess free iron available in the gastrointestinal tract. Thereby, it avoids mucosal irritation and disturbance of bowel motility. This is totally unlike treatment with oral ferrous salts of which only about 20-30% is absorbed, while the majority is carried through the gut lumen inducing free radical mediated damage to the gut mucosa and alteration of bowel motility [40,41]. These gastrointestinal side effects are well known to affect he general wellbeing of women and therefore represent the main reason for low compliance with oral iron therapy [6,10,42-46].

Comparison with existing literature

A subgroup analysis in a Cochrane review [47] included only one study [24] looking at difference in Hb change at 4 weeks between lactoferrin and oral ferrous sulphate. In our meta-analysis, data from 3 RCTs [13,24,25] were included in addition to another study [26] published after the Cochrane review.

Strengths and limitations

The strengths of the current meta-analysis are that it provides, as far as is known for the first time, quantitative estimates of the effectiveness of oral bovine lactoferrin in improving IDA in pregnant women, through the evaluation of change in hemoglobin, serum ferritin and iron levels on the basis of the available evidence so far in RCTs. Subgroup analysis according to the degree of anemia (mild or moderate) was possible. The PRISMA statement was followed to assure a rigorous methodology. The eligible RCTs had strict inclusion and exclusion criteria and baseline characteristics of the patients were largely comparable. Thereby the patient population was representative. In addition, the Cochrane Collaboration guidelines for data extraction and quality assessment regarding a potential risk of different types of biases have been followed in all included studies. Hence, we believe that the chance of reviewer error and bias has been minimized.

On the other hand, this meta-analysis has several limitations. First, only 4 RCTs including 600 pregnant women with IDA are available in the literature. Another limitation is that in pregnant women with moderate IDA, there exists only one RCT [26] in which assessment of serum ferritin and iron levels had not been carried out. Third, the overall quality of the evidence was rated as moderate to low. The reasons for downgrading the evidence included unclear reporting of study methods in some trials (especially with respect to randomization, allocation concealment and blinding), high heterogeneity in hematological outcomes and there is lack of answer to some secondary outcomes, while others were included in only one or two studies (Table 2). Thereby, it could be argued that findings of this meta-analysis should be interpreted with caution. Another limitation is that funnel plot analysis to test for publication bias was not performed because of the relatively small number of included studies. Such analysis warrants the inclusion of 10 or more studies in the review

to be performed. Finally, this review included 4 studies in which Hb levels were assessed at four weeks and only one study had 8 weeks assessment [26]. Thereby more long term-effects of treatment remain to be evaluated.

Conclusions and Implications

For pregnant women with IDA, daily oral treatment with bovine lactoferrin is just as good as ferrous sulfate in increasing Hb and other hematological parameters with fewer gastrointestinal side effects. Considering the latter as a critical side effect causing lack of compliance of ferrous sulfate, lactoferrin should be the iron replacement agent of choice for treatment of IDA in pregnancy. The findings of our meta-analysis should fuel an in depth enthusiasm to obtain a high-quality evidence from adequately powered and blinded RCTs investigating different maternal, fetal, and neonatal outcomes of oral bovine lactoferrin treatment for pregnant women with IDA.

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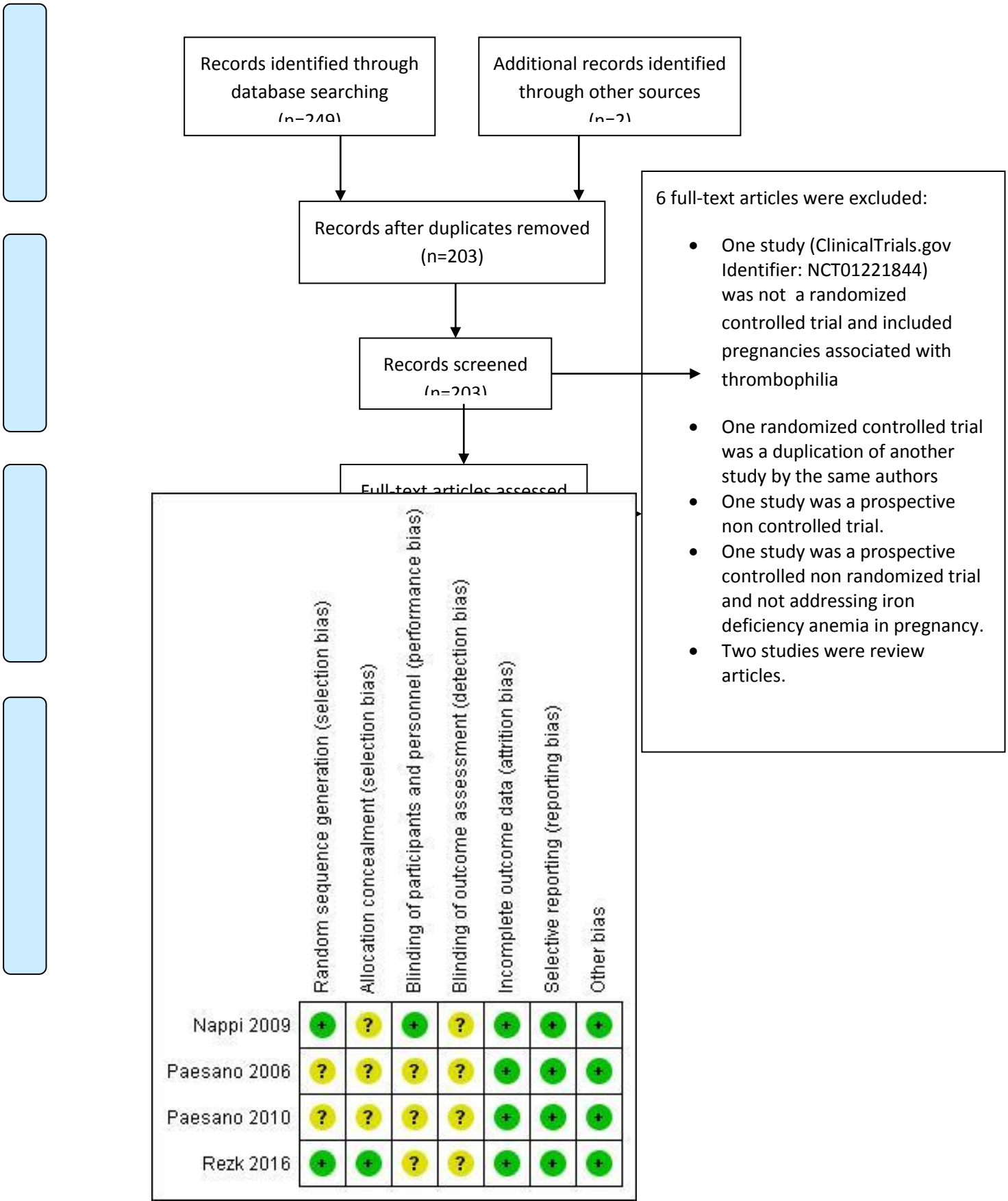
Figure and table legends

Figure 1. PRISMA flow diagram of the study selection.

Figure 2. Risk of bias summary for included studies

+=yes (low risk of bias); - =No (high risk of bias); ?= unclear risk of bias

Figure 3. Forest plot for hemoglobin levels at four weeks



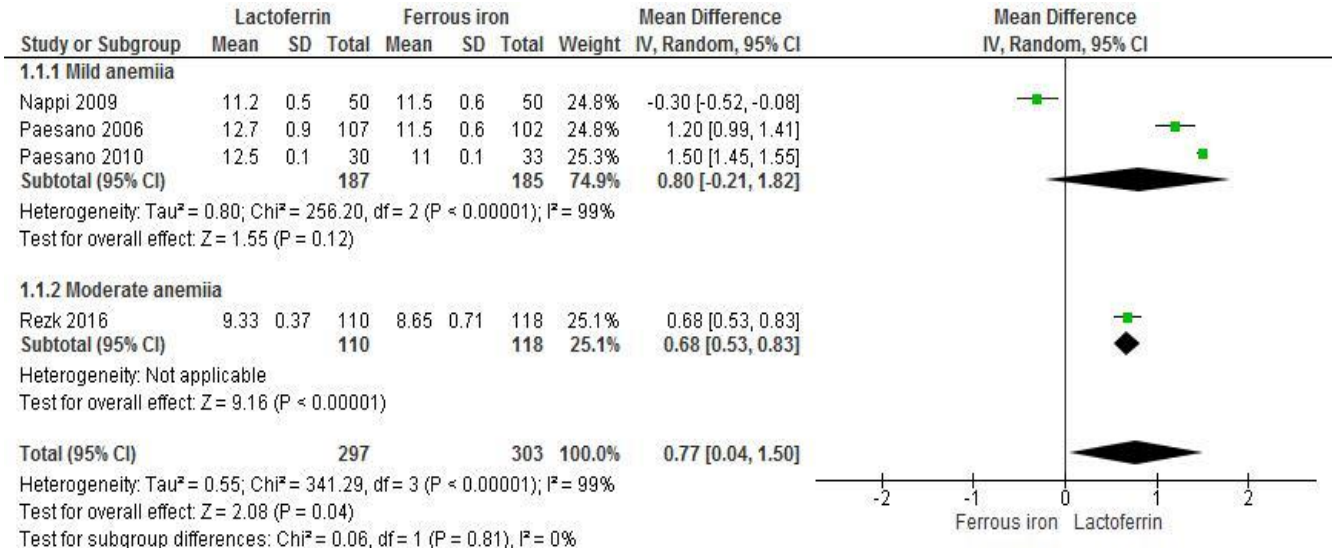


Table 1 Characteristics of included studies.

RCT	Country	Population	Mean age, parity, BMI and Hb level	Intervention	Comparison	Outcomes
Nappi 2009 [24]	Italy	100 pregnant women with mild IDA, gestational age >12weeks and <36 weeks	Mean age: lactoferrin = 27.3±2.7 years, ferrous sulphate = 26.0±5.4 years. Mean parity: lactoferrin = 2.0±1.0, ferrous sulphate = 1.5±1.0. Mean BMI: lactoferrin = 23.2±2.1 kg/m^2, ferrous sulphate = 23.6±1.9 kg/m^2. Mean Hb: 10.1±0.5 g/dL in each group	Bovine lactoferrin (N=50), oral dose of one capsule of 100 mg twice a day before meals for four weeks	Ferrous sulphate (N=50), daily oral dose of one tablet of 520 mg containing 100 mg elemental iron for four weeks	Hb, serum ferritin, serum iron and gastrointestinal adverse effects.
Paesano 2006 [13]	Italy	300 pregnant women with mild IDA, gestational age >12weeks -31 weeks	Not stated. The authors mentioned "baseline characteristics and laboratory measurements prior to therapy were similar in all arms"	Bovine lactoferrin (N=107), oral dose of one capsule of 100 mg (30% iron saturated) twice a day before meals for 30 days	-Ferrous sulphate (N=102), daily oral dose of one tablet of 520 mg containing 100 mg elemental iron for 30 days -Refusing treatment (N=91)	Hb, serum iron
Paesano 2010 [25]	Italy	75 pregnant women with mild IDA at third trimester.	Mean age, parity and BMI ; not stated. The authors mentioned " baseline characteristics prior to therapy were similar in all arms" Mean Hb:	Bovine lactoferrin (N=30), oral dose of one capsule of 100 mg (30% iron saturated) twice	-Ferrous sulphate (N=33), daily oral dose of one tablet of 520 mg containing 100 mg elemental	Hb, serum ferritin and serum iron

			lactoferrin = 10.0±0.5 g/dL, ferrous sulphate 10.0±0.7 g/dL	a day before meals for 30 days	iron for 30 days -Refusing treatment (N=12)	
Rezk 2016 [26]	Egypt	228 pregnant women with moderate IDA at second trimester.	Mean age: lactoferrin = 26.4±5.18 years, ferrous sulphate = 26.5±5.65 years. Mean parity: lactoferrin = 1.42±1.37, ferrous sulphate = 1.50±1.29, Mean BMI: lactoferrin = 21.86±1.94 kg/m^2, ferrous sulphate = 21.90±1.90 kg/m^2. Mean Hb: lactoferrin = 8.15±0.58 g/dL, ferrous sulphate 8.03±0.70 g/dL	Bovine lactoferrin (N=110), oral dose of one capsule of 250 mg daily for eight consecutive weeks	Ferrous sulphate (N=118), daily oral dose of one capsule of 150 mg of dried ferrous sulphate for eight consecutive weeks.	Hb after 1 month, 2 months and gastrointestinal adverse effects

BMI, body mass index; dL, deciliter; g, gram; Hb, Hemoglobin; IDA, iron deficiency anemia; Kg, kilogram; m^2, meter square; mg, milligram; N, number of cases; RCT, randomized controlled trial.

Table 2 Methods and results of the meta-analysis.

Outcome[#]	**No. of studies**	**No. of patients**	**Statistical method**	**Effect size**	**P-Value**	**Quality assessment**¶
Hb levels at four weeks (g/dL)	4	600	MD (IV, Random, 95% CI)	0.77(95% CI 0.04-1.55)	0.04*	Low†
Mild IDA	3	372	MD (IV, Random, 95% CI)	0.80(95% CI -0.21-1.82)	0.12	
Moderate IDA	1	228	MD (IV, Random, 95% CI)	0.68(95% CI 0.53-0.83)	< 0.00001*	
serum ferritin at four weeks (mcg/L)	2	163	MD (IV, Random, 95% CI)	9.82(95% CI -9.97 to 29.62)	0.33	Low†
serum iron at four weeks (mcg/dL)	3	372	MD (IV, Random, 95% CI)	29.0(95% CI -0.39 to 58.54)	0.05	Low†
Gastrointestinal side effects						
Epigastric discomfort	2	328	OR (M-H, Fixed, 95% CI)	0.11(95% CI 0.05-0.22)	< 0.00001*	Moderate‡
Vomiting	2	328	OR (M-H, Fixed, 95% CI)	0.32(95% CI 0.15-0.67)	0.002*	Moderate‡
Constipation	2	328	OR (M-H, Fixed, 95% CI)	0.22(95% CI 0.12-0.40)	< 0.00001*	Moderate‡
Abdominal colicky pain	1	228	OR (M-H, Fixed, 95% CI)	0.21(95% CI 0.12-0.39)	< 0.00001*	Moderate§
Dark stools	1	228	OR (M-H, Fixed, 95% CI)	0.01(95% CI 0.00-0.22)	0.002*	Moderate§
Diarrhea	1	228	OR (M-H, Fixed, 95% CI)	0.0 (95% CI 0.0, 0.0)		

Obstetric outcomes						
Preterm birth	0					
Mean birthweight	0					
Low birthweight	1	228	OR (M-H, Fixed, 95% CI)	0.0 (95% CI 0.0, 0.0)		
Neonatal mortality.	1	228	OR (M-H, Fixed, 95% CI)	0.0 (95% CI 0.0, 0.0)		

[#]Calculated per woman randomized.

[*]Statistically significant difference.

¶ According to GRADE approach (GRADE Working Group) (26): 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.

[†] Downgraded two levels for some potential limitations of the included studies and high heterogeneity I^2> 80%

[‡]Downgraded one level for some potential limitations of the included studies

[§] Downgraded one level being based on a single trial in which no data concerning blinding were provided.

CI, confidence interval; dL, deciliter; g, gram; Hb, Hemoglobin; IDA, iron deficiency anemia; IV, Inverse Variance; L, liter; mcg, microgram; MD, Mean Difference; M-H, Mantel-Haenszel; OR, Odds Ratio; No, number.