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Iron deficiency in pregnancy and perinatal outcome

A systematic literature review

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Preface

My interest in iron deficiency in pregnancy led me to contact Heidi Tiller, consultant in obstetrics and gynecology at UNN Tromsø, who accepted to be my supervisor. Since I was on maternity leave in Oslo, I contacted Marthe-Lise Næss-Andresen, a PhD student on a project concerning iron deficiency in pregnancy at UiO and asked her to be my co-supervisor.

The theme of this thesis was decided in collaboration with my supervisors. The project plan was written during the autumn 2019, while the literature search and writing of the thesis were carried out during spring and summer 2020. The Covid-19 pandemic prolonged the process.

I would like to thank my supervisors Heidi Tiller and Marthe-Lise Næss-Andresen for indispensable guidance with methods, theoretic framework, and academic writing. Thanks for continuing to help me with my thesis despite increased workload due to the Covid-19 pandemic.

I would like to address a special thanks to Vincent Virassamy, without his help with our little one it would not have been possible to write this thesis.

Eira Dahl Føyen,

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Abstract

Background: Iron deficiency (ID) is highly prevalent in pregnant women worldwide. While iron deficiency anemia (IDA) has been associated with preterm birth (PTB) and low birth weight (LBW), there is uncertainty about the possible effects of non-anemic ID on birth outcomes. Aim: The objective of this thesis was to investigate whether ID in pregnancy is an independent factor in contributing to LBW and PTB. Methods: A systematic search of the databases PubMed and Embase identified relevant articles published in the last ten years. Studies were considered eligible for inclusion if they addressed ID in pregnant women, using serum ferritin (SR) or soluble transferrin receptor (sTfR) as biomarkers, and possible associations with LBW or PTB. Studies were excluded if they were carried out in malaria endemic areas, included women with HIV or examined the effect of iron supplementation in pregnancy. Articles written in other languages than English were excluded. Titles and abstracts were screened for eligibility, and the articles considered relevant were read full text. Results: Of 694 individual articles identified from the database search, 15 were read full text and seven were included in the review. All included articles were observational studies. The results were inconsistent. Only one study reported significant correlation between first trimester maternal ID and lower birth weight. First trimester ID correlated with a higher risk of an LGA infant in another study. A third trimester study found that maternal ID was associated with a decreased risk of LWB and PTB. Conclusion: This literature has not found strong evidence of an isolated effect of maternal ID on LBW or PTB, but the possibility of effects cannot be dismissed based on the current evidence. The observational studies included have important differences in populations and study design, making comparisons problematic.

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Abbreviations

ID: Iron deficiency

IDA: Iron deficiency anemia

SF: Serum ferritin

sTfR: Soluble transferrin receptor

TBI: Total body iron

Hb: Hemoglobin

ST: Serum transferrin

TS: Transferrin saturation

LBW: Low birth weight

PTB: Preterm birth

SGA: Small for gestational age

LGA: Large for gestational age

IUGR: In utero growth restriction

Introduction

There is substantial evidence of an association between maternal anemia during pregnancy and adverse neonatal outcomes (1). Maternal anemia is associated with increased risk of miscarriage, stillbirth, prematurity, and LBW, in addition to increased risk of morbidity and mortality in the mother. Iron deficiency (ID) is the most common cause of anemia, and it has been estimated that half of all cases worldwide are due to ID (2). Other possible causes are parasitic infections, deficiencies in vitamin A, B_{12} or folic acid, and genetically inherited haemoglobinopathies (3). Worldwide, around 30% of women of reproductive age and around 40% of pregnant women are anemic (2). LBW is a major contributor to perinatal morbidity and mortality, and maternal anemia is one of many known factors that increase the risk of LBW. An estimated 15 to 20% of all births worldwide are LBW defined as birth weight <2500g (4). To increase the attention to and investments in combating anemia and LBW, the World Health Organization (WHO) has included a target of 50% reduction of anemia in women of reproductive age and a target of 30% reduction in LBW as two of its six Global Nutrition Targets for 2025 (2, 4).

There are inconclusive findings regarding the importance of the maternal iron status during pregnancy in the absence of anemia (1). An estimated 1-2 billion people worldwide are iron deficient without anemia (5). Non-anemic ID has gained attention as a possible disease in itself, not just a precursor to anemia (6). ID has been associated with fatigue, infant development and reduced birth weight in infants born to iron deficient mothers among other outcomes, but the research is not conclusive (5, 7). While some studies have associated a low serum ferritin (SF) with LBW and PTB, others show insignificant or contrary results. The aim of this systematic literature is to investigate whether ID in pregnancy is an independent factor in contributing to LBW and PTB.

Theory

Iron deficiency can lead to depleted iron stores, and severe ID can lead to iron deficiency anemia (IDA). Iron is not only needed for hemoglobin (Hb) production and oxygen transport but is also essential for enzymes involved in cellular energy metabolism. During pregnancy, the iron requirements increase due to the iron needs of the growing fetus, which poses pregnant women at a risk for developing ID and IDA (8). The fetus needs iron for organ development, and is dependent on maternal iron crossing through the placenta (9, 10). Iron is particularly important for fetal brain development and production of red blood cells and muscle cells (10). There is evidence of a negative effect on neurological outcomes in children with ID, and babies born to mothers with ID or IDA are at risk of developing iron deficiency 3-4 months after birth (10).

The Norwegian guidelines for maternal health were revised in 2018 to include a recommendation of measuring the SF in addition to hemoglobin (Hb) in women in their first trimester of pregnancy, and a recommendation of iron supplementation to all pregnant women with a SF <70 µg/L starting in pregnancy week 18-20 (11). The previous guidelines from 2005 recommended measuring hemoglobin (Hb) in the first trimester and in week 28 to determine whether there was a need for iron supplementation. The guidelines from 2005 have been criticized due to concerns that measuring the hemoglobin concentration does not identify pregnant women with ID, or those at risk of developing ID (12).

Considering the recent changes in guidelines for maternal health in Norway, it is of interest to investigate whether non-anemic ID (defined as a low SF concentration or a high soluble transferrin receptor concentration (sTfR)) is an independent risk factor for negative perinatal outcomes. The perinatal period is defined by WHO as the period between 22 completed weeks of gestation and 7 days after birth (13). The main adverse perinatal outcomes associated with anemia are LBW, PTB, perinatal mortality and maternal mortality (14). Some previous studies have pointed in the direction of an association between isolated ID and the outcomes LBW and PTB, but the results are not unequivocal (1).

Birth weight is defined as the birth weight weighed within few hours after birth. Several definitions of LBW exist. WHOs definition of a LBW is that of less than 2500 g regardless of gestational age (13). This definition includes LBW as a result of PTB, intrauterine growth

restriction (IUGR) or both. IUGR is usually defined as an estimated fetal weight <10th percentile and refers to the fetus that does not achieve its expected in utero growth potential. Small for gestational age (SGA) infant is usually defined as a birth weight that is less than the 10th percentile for gestational age. The term does not differentiate between a constitutionally small infant (due to factors as maternal height, weight and ethnicity) and an IUGR infant that does not reach its own growth potential (15).

There are many risk factors associated with LBW. Of most importance are socio-economic factors, medical risks before or during pregnancy and an unfavorable maternal lifestyle (16). Neonates born with a LBW have a higher risk of neonatal mortality and morbidity compared with neonates with normal birthweight. LBW is also associated with several long-term health consequences including neurologic disability, impaired language development, and increased risk of several chronic diseases in adulthood (17).

PTB is defined by WHO as birth of neonates before completed 37 weeks of gestation (13). Based on gestational age, PTB can be subclassified into three categories: extremely preterm (<28 weeks), very preterm (28 - <32 weeks) and moderate preterm (32 - <37 weeks) (18). Risk factors associated with PTB include infection, stress, low maternal nutritional status, uteroplacental ischemia or hemorrhage and uterine overdistention, but the casual pathway is often not possible to establish (19). PTB is associated with increased infant mortality and neonatal morbidity, and various health problems in childhood such as cerebral palsy, motor delay, visual and hearing impairment, lower IQs, and behavior problems (19). PTB has also been shown to increase the risk of non-communicable diseases such as asthma, high blood pressure and cardiovascular disease (18). Around 11% of births worldwide are PTBs, with rates ranging from around 9% in high-income countries to around 12% in low-income countries (18).

Hemoglobin concentration (Hb) is a widely used biomarker for anemia (8). The WHO definition of anemia is an Hb<11g/dl (13). IDA is anemia caused by depleted iron stores. The WHO definition of depleted iron stores for women is SF<15µg/l (13). The gold standard for determining iron stores is bone marrow examination, where lack of stainable iron is diagnostic for depleted iron stores (20). Since this is an invasive and resource-demanding procedure, the use of serum biomarkers to identify ID is more common. The most used

biomarker for ID is a low SF. Iron is stored in the body in the form of the molecule ferritin which is released to the plasma in small amounts. Since the concentration of SF is positively correlated with the total amount of stored iron in the body, SF can be used to assess the size of the body iron stores (21).

There are some factors that may complicate the interpretation of the SF and hemoglobin level in pregnant women. While a low SF concentration is pathognomonic for ID, the SF can be elevated in a state of inflammation or infection.(21) The physiological hemodilution due to plasma volume expansion normally occurring during pregnancy leads to a reduction in SF and Hb (7, 22). The plasma volume increases by ~50% while erythrocyte mass only increases by ~25%, thereby causing hemodilution(23). An inadequate plasma volume expansion in pregnancy can complicate the interpretation of SF and Hb (1). Conditions that are shown to impact the plasma volume expansion in humans or animals include preeclampsia, chronic hypertension, idiopathic fetal growth restriction, ID, food restriction and low protein intake (22). Interpersonal variation in the degree of hemodilution further complicates the interpretation of biochemical data in pregnant women (23).

When iron is transported in the circulation, it is mainly bound to the molecule transferrin. Serum transferrin (ST) and transferrin saturation (TS) are widely used in the diagnosis of ID, but there is lack of evidence of their usefulness (20). Total iron binding capacity (TIBC) is a biomarker which is closely related to serum transferrin. It represents the capacity of protein in plasma to bind iron, and transferrin is the most important iron-binding protein in plasma (20).

Another less used biomarker for ID is a high serum concentration of soluble transferrin receptor (sTfR), a biomarker that might be better than SF for assessing ID in pregnancy (24). Transferrin receptor is used by cells, particularly erythroid precursors in the bone marrow, to obtain iron from the circulation by binding transferrin, and the expression of TfR is upregulated when the cells needs more iron. A soluble version of TfR (sTfR) circulates in the serum and reflects the total amount of TfR in the body, which raises when iron stores are depleted and iron-deficient erythropoiesis occurs (25). SF is a good biomarker of indicating depleted iron stores, but does not indicate how severe the depletion is as it progresses (21). This is where the sTfR can be useful as a measure of the deficit in functional iron beyond the initial iron store depletion, especially in the case of pregnant women that often have SF

concentrations in the lower range (24). The concentration of sTfR is not affected by inflammation as is SF, but there are other conditions than ID that can cause an increased sTfR, for example thalassemia and pernicious anemia (25). A new method of measuring the body iron quantitatively uses transferrin receptor/serum ferritin (R/F ratio) and an equation to calculate total body iron (TBI) (20, 26).

Investigating the recent literature regarding whether ID in pregnancy is associated with PTB and LBW using the biomarkers SF and sTfR may lead to a better understanding of the clinical importance of isolated ID during pregnancy. The aim of this study was to perform a systematic literature review to assess current knowledge on maternal ID and impact on perinatal outcomes.

Methods

We performed a systematic literature review searching the databases PubMed and Embase to identify relevant articles concerning ID in pregnancy and perinatal outcome. The search strings were developed in collaboration with the librarian service at the University of Oslo. Since many of the studies concerning ID in pregnancy have anemia in pregnancy as their focus, we included “anemia” in the search string. In addition, we added “supplement” to make sure that the search included articles concerning ID and iron supplementation in pregnancy in case there were not enough articles specifically on ID in pregnancy and potential correlations with perinatal outcome. The main outcomes of interest were LBW and PTB. We also included SGA, IUGR and fetal growth retardation in the search strings since they are related terms. LGA was added to include articles with LGA as an outcome since we noticed that some articles described a correlation between ID and LGA. SGA is defined as a birth weight less than the 10^{th} percentile and LGA as birth weight above the 90^{th} percentile, unless otherwise stated throughout this thesis.

The search string used in Pubmed:

("Anemia, Iron-Deficiency"[Mesh] OR (iron[ti] AND (anemi*[tiab] OR anaemi*[tiab] OR deficien*[tiab] OR supplement*[tiab]))) AND ("Pregnant Women"[Mesh] OR "Pregnancy"[Mesh] OR maternal[tiab] OR pregnant[tiab] OR pregnancy[tiab] OR prenatal[tiab]) AND ("Pregnancy Outcome"[Mesh] OR "Birth Weight"[Mesh] OR "Infant, Low Birth Weight"[Mesh] OR "Premature Birth"[Mesh] OR "Infant, Premature"[Mesh] OR preterm[tiab] OR prematur*[tiab] OR birthweight*[tiab] OR lbw[tiab] OR sga[tiab] OR lga[tiab] OR "small for gestational age"[tiab] OR "large for gestational age"[tiab] OR "Fetal Growth Retardation"[Mesh] OR IUGR[tiab]) Filters: 10 years.

The search string used in Embase:

1. exp iron deficiency anemia/
2. exp iron deficiency/
3. exp iron therapy/
4. 1 or 2 or 3
5. exp pregnant woman/
6. maternal.ab,ti.
7. pregnan*.ab,ti.
8. prenatal.ab,ti.

9. 5 or 6 or 7 or 8
10. exp pregnancy outcome/
11. exp birth weight/
12. exp prematurity/
13. small for date infant/
14. exp large for gestational age/
15. exp intrauterine growth retardation/
16. IUGR.ab,ti.
17. 10 or 11 or 12 or 13 or 14 or 15 or 16
18. 4 and 9 and 17
19. limit 18 to last 10 years

Studies were considered eligible for inclusion if they addressed ID in pregnant women, using serum ferritin (SR) or soluble transferrin receptor (sTfR) as biomarkers, and possible associations with LBW or PTB. Articles written in other languages than English were excluded. Studies carried out in malaria endemic areas were excluded, as well as studies including women with HIV. Studies examining the effects of iron supplementation in pregnancy were excluded. Studies with missing full text articles were not included.

After removing duplicates, all titles and abstracts were screened for relevant studies by me. Articles were excluded based on title when it was clear that the study examined a not relevant topic. Otherwise, the abstract was read. I then read the full text version of the relevant articles with available full text and the articles that did not fit with the inclusion criteria were excluded. In cases of doubt, the articles were discussed with the supervisors before inclusion or exclusion.

Results

The initial database search resulted in 694 individual articles. After screening the titles and abstracts, 18 articles seemed relevant and were chosen to read full text. Of those, 15 full text articles were found and read. Seven articles were finally included based on the inclusion criteria. Figure 1 shows a flowchart of the work process from the database search to the included articles. Table 1 gives a detailed overview of the study designs and relevant results.

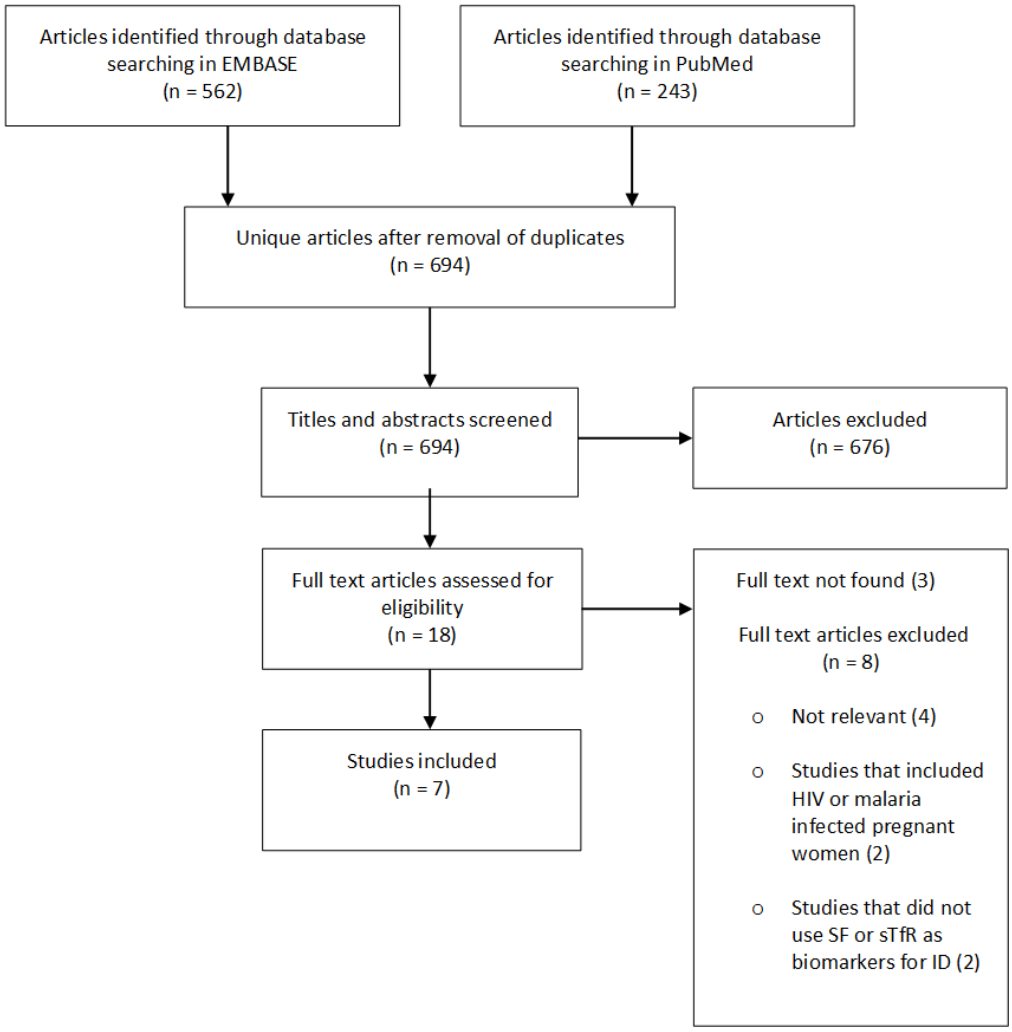

Figure 1 Flow chart; The work process from the database search to the included articles

1.1 Summary of each article included in the review:

Before discussing the overall results, a summary of each of the seven studies is presented.

Article 1: Khambalia et al. describe a population of 4420 pregnant women who had their data from a Down syndrome screening program analyzed by Pathology North in New Wales, Australia (27). The aim of the study was to describe the prevalence and determinants of first trimester ID using SF, sTfR and TBI, and associations with pregnancy and birth outcome. A total number of 122 women were excluded from the study based on twin pregnancy, medical abortion, infant with a major congenital anomaly or an undetectable ferritin and sTfR concentration. The article does not specify whether the participants took iron supplements during pregnancy. Data on hemoglobin levels were not included in the study. The analysis was adjusted for maternal age, gestational age at blood test, body weight, parity, smoking during pregnancy, private versus public health care, low socioeconomic status, and CRP levels. The prevalence of first trimester ID was 19.6% defined by SF. When women with inflammation were excluded, the prevalence of ID by SF decreased to 18.8%. An association was found between ID in the first trimester defined by SF and TBI with LGA in univariate analysis, but only ID measured by TBI was associated with LGA in multivariate analysis (AOR 1.38; 95% CI 1.03, 1.85). Other variables that remained associated with LGA in the model for ID defined by TBI and LGA were increased maternal weight (AOR 2.75: 95% CI 2.17, 3.48), multiparity (AOR 1.95; 95% CI 1.53, 2.48) and smoking during pregnancy (AOR 0.36; 95% CI 0.18, 0.72). No association between maternal ID and PTB or SGA infants was found.

Article 2: A study by Alwan et al. included 362 pregnant women who gave birth ≥ 34 gestational weeks at the Leeds Teaching Hospitals Trust Maternity in Leeds, UK (28). The purpose was to examine the relationship between first trimester iron status, as assessed by serum ferritin, transferrin receptor and their ratio, with size at birth and PTB. Number of women excluded and exclusion criteria applied are not described. Only 121 women (34%) took iron supplements during pregnancy. The prevalence of first trimester depleted iron stores was 23%. The variables included in the multivariable analysis were maternal age, smoking, gestational diabetes, pre-eclampsia, and area deprivation score. Maternal iron depletion was found to be associated with a higher risk of having an SGA baby (AOR 2.2, 9.5% CI 1.1, 41; p=0.02). Adjustment for iron supplements did not affect the association, but the addition of

Hb to the model made the association insignificant. The relationship between maternal ID and a higher risk of an SGA infant was interpreted by the authors to be mediated by Hb. Maternal sTfR was marginally associated with higher risk of SGA (AOR 1.1 for every nmol/l increase in sTfR (95% CI 1.0, 1.1 p=0.04). No association between SGA and maternal sTfR:SF ratio was found. No association between maternal iron status measured by SF, sTfR or log sTfR:SF ratio with preterm or gestational age was found.

Article 3: Bencaiova and Breymann investigated the relation between hemoglobin and iron status examined in second trimester with pregnancy outcome in a population of 382 iron supplemented, pregnant women recruited from the University Hospital of Zurich, Switzerland (29). Excluded women are not described. The women were divided into four groups based on their hemoglobin and ferritin status; women with IDA (group 1), depleted iron stores without anemia (group 2), anemia for other reasons (group 3) and women with normal status (group 4). Only univariate logistic regression analysis was performed. Groups 1, 2 and 3 were analyzed versus women with normal status (group 4). No significant association was found between second trimester iron deficiency or mild anemia and birth weight, IUGR or PTB. 32.2% of the women had depleted iron stores without anemia, however CRP was not measured. A difference in hemoglobin before delivery was found between women with depleted iron stores and normal women (p=0.005).

Article 4: Finkelstein et al. describe a population of 366 pregnant women who were enrolled in an RCT of vitamin B_{12} supplementation in Bangalore, India (30). The aim of the study was to examine the prevalence of anemia, ID, and inflammation during pregnancy and their associations with adverse pregnancy and infant outcome. Data on pregnancy outcome was available for 258 of the women. Excluded women are not accounted for in the article. The prevalence of ID was 48%. All the participants took iron and folic acid supplements starting at their first antenatal visit, while half of the participants received B12 supplements and the other half received placebo. No significant association between maternal ID and birth weight, gestation age or PTB was found. However, maternal serum ferritin concentrations were associated with increased infant length at birth (β(SE): 0.44 (0.20) cm, p = 0.03). Maternal IDA in the first trimester was associated with lower birth weight, increased risk of LBW, lower gestational age at delivery and 3.46 times higher risk of preterm delivery in multivariate analyses. Maternal serum ferritin concentrations and ID were associated with maternal

hemoglobin concentrations in linear regression analysis. The models were adjusted for gestational age, vitamin B12 intervention, maternal BMI, socioeconomic status, educational level, and inflammation.

Article 5: A Chinese study by Yuan et al. included 11,581 pregnant women that delivered in Changzhou Maternity and Child Health Care Hospital (31). The purpose of the study was to examine the prevalence of ID in the third trimester using SF, ST and their ratio and the relationship with birth outcomes. No information about iron supplementation was specified in the article, nor how many women were excluded and why. The prevalence of third trimester ID was 51.8%. When women with inflammation were excluded from analysis, the prevalence of ID increased to 54.3%. ID in the third trimester as defined by SF was found to be associated with decreased risk of PTB, LBW or SGA, and increased risk of macrosomia in women without inflammation in multivariable analysis. Similar associations were found when analyzing the data from women with and without inflammation were combined, and by using the definitions ST and ST/SF for ID. LGA was in addition found to be associated with ID measured by SF in the analysis of the data from all of the participants. ID was only found to be associated with LGA in women without inflammation when measured by ST and ST/SF ratio. The odds ratios for PTB, LBW, SGA and macrosomia were adjusted for maternal age, BMI, gravidity, parity, inflammation, and hemoglobin. The analyses of PTB were also adjusted for several maternal complications as well as blood pressure and infant sex. However, hemoglobin was not included in these analyses.

Article 6: A study by Srour et al. that aimed to investigate the prevalence of anemia and ID among pregnant women and their association with pregnancy outcome in Palestine, included 300 pregnant women who had a first trimester appointment at one of eleven maternal centers included in study (32). Only 163 infants had their birth weight included in the study and 122 had their gestational age included. The article does not give any information about excluded women. They found a correlation between first trimester ID and frequency of LBW ($p=0.001$) and frequency of PTB ($p=0.003$) in univariate analyses (32). The prevalence of ID in the study was 52%. Iron supplementation was an exclusion criterion at the enrolment of the study, but the article does not specify whether the anemic and iron deficient participants were treated with iron during the study. A difference was observed between maternal Hb and birth weight ($p=0.0009$) and gestational age ($p=0.0012$) when the women were divided in three

different Hb tertile groups. Serum ferritin was correlated with Hb (p=0.001). A correlation between maternal BMI and ID was observed (p=0.017). No significant correlation was observed between BMI and birth weight (pearson's correlation coefficient 0.061) or gestational age (pearson's correlation coefficient -0.016). Multivariable analysis was not performed.

Article 7: A total of 205 iron supplemented pregnant women who had their first antenatal visit at the University Hospital Sant Joan de Reus in Catalunya, Spain were included in the study by Ribot et al. (33). The aim was to assess the relationship of first trimester iron stores with birthweight in non-anemic pregnant women. The number of excluded women was 95. A multivariable analysis adjusting for first trimester ID, maternal age, BMI at first visit, parity, gestational age, gender, tobacco habit, socioeconomic status of the family and mean iron supplementation during the pregnancy was used to assess associations between ID and birth weight. An interesting result was that women with ID in the first trimester had infants with a birth weight of ~148g less than the infants from women that did not have ID in the first trimester. The difference in birthweight increased to ~192g when the model was adjusted for initial Hb and TS<16%. The prevalence of ID in the study was 20.0%. TS was correlated with SF (p<0.001) but Hb was not correlated with the SF (p=0.440) in univariate analysis. Anemic women were however excluded from the study, which might explain the lack of correlation. Initial BMI and gestation length were positively correlated to birth weight (-30.0g and +139.8g respectively, and smoking habit was negatively correlated with birth weight (-177.3g). Gestation length was not significantly correlated with depleted iron stores in univariate analysis (p=0.412).

1.2 Summary of results regarding birth weight:

The results regarding birth weight were not coherent. Of the seven articles included in the literature review, five articles found an association between maternal ID and birth weight. Alwan et al. found that maternal ID in the first trimester was associated with a higher risk of having a SGA newborn (28). However, the association seemed to be mediated through low hemoglobin. Ribot et al. found that women with ID in the first trimester had newborns with lower birth weight than women without ID, even after adjusting for Hb levels. Srour et al.

found an association between maternal ID in the first trimester and a higher frequency of LBW, but multivariate analysis was not performed (32). Yuan et al. found that third trimester ID gave a decreased risk of having a SGA or LBW newborn (31).

Khambalia et al. and Yuan et al. found an association between maternal ID and a higher risk of having a LGA newborn, with blood samples analyzed during the first trimester and third trimester respectively (27, 31). Interestingly, the association was no longer significant after adjusting for maternal inflammation in the work by Yuan et al. However, Yuan et al. also found an association between maternal ID and a higher risk of having a macrosomic newborn which was still present after adjusting for maternal inflammation. In sum, there was no evidence to support the idea that ID during pregnancy poses an isolated increased risk of LBW. Scarce evidence suggests that maternal ID measured in the third trimester is associated with lower birth weight, and that maternal ID measured in the third trimester may contribute to increased birth weight.

1.3 Summary of results regarding preterm birth (PTB):

Two studies report associations between maternal ID and PTB but the results point in opposite directions: Srour et al. found a higher frequency of PTB in the group of women with ID in the first trimester than the group with normal iron status, but only univariate analysis was performed (32). Yuan et al. found that maternal ID in the third trimester gave a decreased risk of PTB (31). Bencaiova and Breymann, and Finkelstein et al. found no association between maternal ID and birth weight, SGA, IUGR, length of gestation or PTB (29, 30). Finkelstein et al. did however find that increasing maternal SF correlated with increasing birth length when adjusted for confounders including inflammation, and that IDA was associated with higher risk of LBW and PTB. In sum, there was no substantial evidence to support the idea that ID during pregnancy poses an isolated increased risk.

Discussion

In this thesis I have carried out a systematic literature search in the databases PubMed and Embase with the aim of investigating whether ID in pregnancy is an independent risk factor in contributing to LBW and/or PTB. The strength of the study is the use of a systematic search strategy. One limitation is that only literature from the last 10 years has been included. It is possible that there was more focus on ID in pregnancy prior to 2009. Another limitation is that only one person screened the search result and read, reviewed, and included articles. Ideally, it should have been done by two persons independently.

We initially intended to perform quantitative assessments of the possible impacts of ID on birth weight and PTB, but due to large heterogeneity in the studies included this was not possible to do.

Only one of the seven studies, the study in Article 7, found that maternal ID in the first trimester was associated with a lower birth weight when adjusted for confounding factors including Hb and iron supplementation. Interestingly, two of the studies found opposite results: Article 1 found that maternal ID defined by TBI in the first trimester was associated with a higher risk of having a LGA newborn and Article 5 found that third trimester maternal ID defined by SF gave a higher risk of having a macrosomic newborn after adjusting for confounding factors including inflammation. However, Article 1 did not include data on Hb in their study and neither of the studies that found correlations with LGA or macrosomia included data on iron supplementation.

Only Article 5 found an association between maternal ID and PTB after adjustment for confounding factors. They found that third trimester maternal ID gave a decreased risk of PTB. Article 6 found that first trimester maternal ID correlated with LBW and PTB, but the analyses were not adjusted for confounding factors.

There is not much literature during the last ten years that focuses on maternal ID without anemia and associations with birth outcomes. The focus in articles regarding iron in pregnancy is mostly on anemia or iron supplementation. Some studies find that low SF levels do not predict pregnancy outcome, however the blood samples were not taken in the first trimester (34, 35). A study by Lao et al. published in 2000 found that ferritin concentrations in

the third trimester were inversely correlated with infant gestational age and birth weight in a population described as non-anemic, but they did not include data on inflammation and the definition of anemia was Hb<10g/l (36). It is difficult to separate the effects of low SF from low Hb in the existing literature as results are often not focused on an isolated effect of ID.

All of the seven studies included in this thesis are observational studies, which make them vulnerable to known and unknown confounders. Not all of the studies include data on Hb, iron supplementation or inflammation, and the ones that are univariate have not adjusted for important factors such as gestational age and maternal BMI. Furthermore, the blood samples have been taken in different trimesters, making comparison of results difficult. Sample sizes vary from around 300 to over 11 000 participants. A too small sample may lack statistical power, while a large sample may magnify biases (37). The studies' inclusion and exclusion criteria are comparable, however only one study have excluded anemic women. The studies are carried out in seven different countries and represent populations of different ethnicities and living standards, which can complicate comparison of results. The prevalence of ID in the populations vary from 20% to 59% measured by SF, but the studies used different cut-off values for ID including 12, 15 and 20µg/l.

Important confounders were considered to a different degree in the studies reviewed. Article 1 lacked data on Hb. An increasing Hb has been shown to be correlated with higher birth weight (3), so a possible correlation between maternal ID and a higher risk of LGA infant cannot be given much importance when Hb has not been included in the analysis. In addition, they found that increased maternal weight correlated with a higher risk of an LGA infant. Maternal obesity is associated with higher birth weight (16). It is also associated with increased risk of ID, possibly due to increased plasma volume, consumption of nutrient-poor food and chronic inflammation due to adiposity (38). The authors mention obesity as a possible contributor to the higher risk of an LGA infant in ID women in their study. They did not find that CRP measured in the first trimester was correlated with an LGA infant, but it is possible that inflammation occurred at a later stage in pregnancy.

The two studies that found a correlation between maternal ID with LGA or macrosomia lacked data on iron supplementation (27, 31). Article 5 also found that third trimester ID gave a decreased risk of LBW and PTB. Iron supplementation has been found to give a significant

reduction in maternal ID, IDA and risk of LBW in one systematic review (3), while another did not find a significant reduction (39). However, neither of the mentioned reviews found a reduction in risk of PTB by iron supplementation. Both mention substantial heterogeneity of results, which might be due to different study designs and populations. The possibility that iron supplementation has affected the results in ID women in the direction of a higher birth weight cannot be dismissed, but iron supplementation cannot explain the protective effect of third trimester ID on the risk of PTB based on the current evidence of the effects of iron supplementation.

Other important confounding factors that are associated with birth weight is the gestational age and smoking in pregnancy. The only study that found a correlation between ID and birth weight in non-anemic women when correlated for the all the above-mentioned confounders was the study in Article 7. However, the authors mention several known risk factors for lower birth weight that were not included in the analysis and might have affected the results, such as stress, strenuous work or genetics of the mother (33).

The results of the studies included are inconsistent, and the majority of studies did not consider important confounding factors. The articles included had observational designs and therefore require caution when interpreting the results. When important confounding factors were adjusted for, only one article found an association of first trimester maternal ID and a lower birth weight, and another found that third trimester maternal ID was associated with a lower risk of PTB and LBW. The two studies suggest a difference in effect according to which trimester the ID occurred. Hb has been found to show a stronger association with adverse birth outcomes when measured in the first trimester than in the second and third trimester (1). Perhaps maternal ID affects birth weight differently based on which trimester the ID occurs. To get more clarity around the effects of maternal ID in pregnancy, future studies should be prospective, include data on iron status from all three trimesters and consider important confounding factors for LBW and PTB.

Conclusion

Based on the results from this systematic literature search we cannot conclude whether ID during pregnancy poses an independent risk factor for LBW or PTB. However, two of the studies indicate that maternal ID is independently associated with birth weight, with first trimester ID causing a decrease in birth weight and third trimester ID causing an increase. Considering that ID is a common deficiency among pregnant women, an isolated effect of maternal ID on birth weight can have implications for maternal health guidelines. Well-designed prospective studies are needed clarify this.

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Table 1: Summary of study designs and results

Articles	Country	Design	Selection/exclusion	Definitions	Sample	Iron supplements	Results
Article 1: Khambalia et al. (2015)	Australia	*Type of study:* Retrospective observational study. Exposure assessed prospectively. *Time period:* January to October 2017 *Aim*: Describe the prevalence and determinants of first trimester ID and associations with pregnancy and birth outcomes	*Selection:* Random sample of pregnant women who attended first trimester Down syndrome screening in New South Wales, Australia *Exclusion:* Twin pregnancies, medical abortion, major congenital anomalies or an undetectable ferritin and sTfR concentration.	*ID:* SF<12µg/l TfR≥21.0nmol/l TBI<0mg/kg *Iron replete:* SF>70µg/l *Inflammation:* CRP>0.5mg/dl	*All women:* N=4420 Serum ferritin: N=3795 Transferrin receptor: N= 4406 Total body iron: N=3781 *Women with CRP≤0.5mg/dl* N=1390 Serum ferritin: N=1293 Transferrin receptor: N=1385 Total body iron: N=1288	No data	*All women* ID by SF: 742/3795 (19.6%) ID by SF+SGA: 46/742 (6.6%) ID by SF+LGA 90/742 (13.0%). ID by sTfR: 676/4406 (15.3%) ID by sTfR+SGA 53/676 (8.3%) ID by sTfR+LGA 74/676 (11.5%) ID by TBI: 594/3781 (15.7%) ID by TBI+SGA: 35/594 (6.3%) ID by TBI+LGA: 79/594 (14.1%) *Women with CRP<0.5mg/dl:* ID by SF: 18.8% ID by sTfR: 10.9% ID by TBI: 12.7% ID by TBI was associated with LGA in multivariate analysis. (AOR 1.38; 95% CI 1.03, 1.85) No association between maternal ID and preterm birth or SGA infants.

Article 2: Alwan et al. (2015)	Leeds, UK	*Type of study:* Retrospective observational study. Exposure assessed prospectively. *Time period:* February 2012 to January 2013 *Aim*: Examine the association between maternal Fe status during the first trimester of pregnancy, as assessed by serum ferritin, transferrin receptor and their ratio, with size at birth and preterm birth.	*Inclusion:* Women ≥ 18 years who gave birth to live offspring at the Leeds teaching Hospitals Trust maternity unit ≥34 weeks *Exclusion:* Stillbirth or neonatal death, serious maternal illness and babies with concurrent CVD	*ID:* SF<15µg/l *sTfR:sF ratio:* (µg/l:µg/l)	*All women:* N=362 *Women with SF data:* N=348 *Infants with customized weight centiles:* N=358 *Included in the multivariable analysis:* N=341	Extracted from the mother and the baby's clinical records.	Dep Fe: 79/348 (23%) Dep Fe+SGA: 25% No Dep Fe+SGA: 14% Total SGA: 64/358 (18%) Total LGA: 20/358 (6%) Total LBW: 40/362 Total PTB: 33/362 Maternal ID in the first trimester associated with higher risk of SGA (AOR 2.2, 9.5% CI 1.1, 41; p=0.02) in a multivariable analysis The association was not altered by maternal iron supplement in a sensitivity analysis (AOR 2.3, 95% CI, 4.5; p=0.02) When early pregnancy Hb was included in the model, the association between maternal iron depletion and a higher risk

							of a SGA baby was no longer significant (AOR 1.6, 95% CI 0.8, 3.2; p=0.2). Maternal sTfR was associated in the multivariable model with higher risk of SGA (AOR 1.1 (95% CI 1.0, 1.1 p=0.04) No association between maternal ID measured by SF, sTfR or log sTfR:SF ratio with preterm or gestational age.
Article 3: Bencaiova, Breymann (2014)	Switzerland	*Type of study:* Prospective observational study. *Time period:* No information *Aim*: To investigate the relation between hemoglobin and iron status examined in second trimester and pregnancy outcome.	*Inclusion*: Singleton pregnancies 16-20 weeks *Exclusion:* Chronic renal disease and malignancies and having a blood transfusion at least 3 months before enrolment	*Depleted iron stores:* SF<20µg/l *Anemia:* Hb<11.0g/dL *IDA:* Hb < 11.0 g/dL and SF ≤15µg/l *Depleted iron stores without anemia:* Hb>11.0g/dL and SF<20µg/l *Macrosomia:* birth weight above the sex-specific 95th percentile of	N=382 *Group 1* Women with iron deficiency anemia (Hb<11.0g/dL and SF≤15µg) N=25/382 *Group 2* Women with depleted iron stores without anemia: (Hb>11.0g/dL and SF<20µg/l) N=123/382	Women with hemoglobin (Hb) between 10.0 and 11.0 g/dL received oral iron supplementation. Women with hemoglobin <10.0 g/dL received intravenous iron	*Group 1* LBW: 2/25 (8.0%) IUGR: 0/25 (0.0%) Macrosomia: 4/25 (16.0%) Preterm delivery: 2/25 (8.0%) *Group 2* LBW: 7/123(5.7%) IUGR: 7/123(5.7%) Macrosomia: 14/123(11.4%) Preterm delivery: 7/123(5.7%) *Group 3* LBW: 3/45 (6.7%) IUGR: 3/45 (6.7%) Macrosomia: 0/45 (0.0%) Preterm delivery: 2/45 (4.4%) *Group 4* LBW: 19/189 (10.1%) IUGR: 12/189 (6.3%)

				weights for gestational age	*Group 3* Women with anemia for other reasons (Hb<11.0 g/dL and SF>15µg/l) N=45/382 *Group 4* Women with normal status (control group) N=189/382		Macrosomia: 15/189 (7.9%) Preterm delivery:18/189(9.5%) *All women:* LBW: 31/382 (8.1%) IUGR: 22/382 (5.8%) Macrosomia: 33/382 (8.6%) Preterm delivery:29/382 (7.6%) No significant association between second trimester iron deficiency or mild anemia and birth weight, IUGR or preterm birth
Article 4: Finkelstein et al. (2019)	Bangalore, India	*Type of study:* Prospective observational study *Time period:* *N*o information *Aim:* Determine the prevalence of anemia, ID, IDA, and inflammation in pregnant women; and examine the associations between maternal biomarkers of iron status with the risks of adverse pregnancy and infant outcomes.	*Inclusion:* Healthy pregnant women ≥18 years, ≤14 weeks of gestation with singletons, enrolled in a RCT study of vitamin B_{12} supplementation. *Exclusion:* HIV infection, hepatitis B, or syphilis. Serious preexisting medical condition, previous caesarean delivery, daily vitamin supplements other than iron and folic acid.	*ID:* SF<15µg/l *Iron insufficiency:* SF<30µg/l *Anemia:* Hb < 11.0 g/dl during the first trimester, Hb < 10.5 g/dl during the second trimester, Hb < 11.0 g/dl during the third trimester *Inflammation:* CRP>5.0mg/l, or AGP>1.0g/l	Total sample: N=366 *Maternal biomarkers available for all women in early in pregnancy:* N=360 *Data for pregnancy outcomes:* N=258 *Infant blood measures:* N=73	All women received daily iron (60mg) and folic acid (500mg) supplementation, beginning at their first prenatal visit. Half of the women received daily vitamin B12 supplementation (50µg)	ID: 48% ID after adjusting for inflammation: 59% IDA 30% LBW: 38/258 (15.1%) PTB: 33/258 (12.8%) PTB+LBW: 15/258 (6.0%) SGA: 89/258 (33.9%) Women with anemia at enrolment had infants with lower birth weight (β(SE): −166.8 (61.1) g; p=0.006) and 2.15 times higher risk of low birth weight (<2500 g; RR: 2.15, 95%CI: 1.20–3.84, p = 0.01), compared with the

				Anemia of inflammation: Hb<11.0 g/dl, SF>*15*.0µg/l, plus CRP>5.0mg/l or AGP>1.0g/l		and half receives placebo.	women who were not anemic, in multivariate analyses Maternal serum ferritin concentrations (β(SE): 0.79 (0.08) µg/L, p < 0.0001) and iron deficiency (β(SE):−1.19 (0.16), p < 0.0001) were associated with maternal hemoglobin concentrations in linear regression analysis. No significant association between maternal iron deficiency and birth weight, gestation age or preterm birth.
Article 5: Yuan et al. (2019)	China	*Type of study:* Retrospective observational study. Exposure assessed prospectively. *Time period:* participants delivered between April 2016 and march 2017. *Aim:* To explore the relationship between maternal iron status in the third trimester and adverse birth outcomes in chinese pregnant women, while taking into account systemic	*Inclusion:* Women > 18 years pregnant 28–41 weeks with singleton pregnancy and live birth *Exclusion:* multiple pregnancy, missing integrated and clear medical records, preexisting illnesses before getting pregnant: diabetes mellitus (type 1 or 2), chronic hypertension, thyroid diseases, chronic heart, liver and kidney diseases, immune rheumatic disease or thyroid diseases and	*ID:* *SF*<12µg/l or ST<4g/L or High ratio of ST/SF (log 10 transfom > 5.52) *Macrosomia:* >4000g	*All women*: N=11,581 *Women without inflammation:* N=8591	No data	*Women without inflammation:* ID by SF: 54.27% ID by ST: 56.23% ID by ST/SF: 56.29% *All infants:* LBW: 519 (4.48%) Macrosomia: 853 (7.37%) SGA: 1024 (8.84%) LGA: 1793 (15.48%) *ID by SF in women without inflammation:* Decreased risk of PTB (AOR=0.71, 95% CI, 0.57, 0.88) Decreased risk of LBW (AOR=0.58, 95% CI 0.40, 0.84)

		inflammation as measured by high sensitivity C-reactive protein (hsCRP) and anemia as measured by hemoglobin.	syphilis prior to pregnancy, cigarette smokers and alcohol drinkers in pregnancy.				Decreased risk of SGA (AOR=0.71, 95% CI, 0.59, 0.85 Increased risk of macrosomia (AOR=1.29, 95% CI, 1.06, 1.59) *ID by SF in all women:* Similar results as for women without inflammation, in addition to increased risk of LGA infant (AOR=1.17, 95% CI, 1.03, 1.33).
Article 6: Srour et al. (2018)	Palestine	*Type of study:* Prospective observational study Including 11 maternal and child healthcare (MCHC) centers. *Time period:* March to October 2015 *Aim:* To investigate the prevalence of anemia and iron deficiency among pregnant women in their first trimester and its association with pregnancy outcome	*Inclusion criteria:* Healthy *Exclusion criteria:* Iron supplementation, previous pregnancy complications, and pregravid chronic diseases.	*Anemia:* Hb < 11.0 g/dl Severe for Hb < 70 g/L, Moderate for Hb 70-99 g/L Mild for Hb 100-109 g/L *IDA:* Hb < 110 g/L and serum ferritin < 15 ng/mL *ID:* SF<15µg/l HbT1: Hb<110 g/L HbT2: Hb 110-120 g/L	Pregnant women: N=300 Newborns: N=163 (birth weight) N=122 (gestational age)	None were on iron supplements No information regarding treatment of anemic and ID women.	ID: 156/300 (52%) Anemia: 77/300 (27,5%) ID+LBW: 30/156 (18.4%) ID+PTB: 14/156 (11.5%) First trimester ID correlated with frequency of low birth weight (p=0.001) and preterm birth (p=0.003) in univariate analysis. Multivariable analysis was not performed. Increasing Hb concentration associate with increasing ferritin concentration. (p=0.001) Increasing Hb concentration associated with increasing birth weight (p=0.009), birth height (p=0.022), and head

				HbT3: Hb>120 g/L			circumference of newborns (p=0.17) as well as with gestational age (p=0.012).
Article 7: Ribot et al. (2012)	Spain	*Type of study:* Prospective observational study *Time period:* Pregnant women recruited in the time period 2005-2008 *Aim:* Investigate IBW (infant birth weight) in relation to maternal iron stores (depleted or non-depleted), based on SF levels early in pregnancy in non-anemic pregnant women receiving antenatal moderate iron supplementation.	*Inclusion criteria:* Caucasian, healthy women≥18 years at 8–12 weeks *Exclusion criteria:* chronic illness or a possible inflammation with high SF (>62µg/l) and low transferrin saturation (TS<16%), twin or triplet pregnancies. The analysis only included women without anemia, who were iron supplemented starting < week 30, and compiled with the scheduled visits, had blood drawn at each visit and gave birth at the study hospital.	*Depleted iron stores:* SF<12µg/l *Iron deficiency:* SF<12µg/l and TS<16% Anemia: Hb<110g/l in the first and third trimester Hb<105g/l in the second trimester *IDA:*anemia andID at the same time *Haemoconcentration:* Hb>130g/l in the second and third trimesters. *Fetal macrosomia:* <4000g	N=205 *Non-depleted iron stores (week 8-12 of gestation):* N=164 *Depleted iron stores (week 8-12 of gestation):* N=41	Daily antenatal iron supplements (48mg on average) were started on 17 (range: 16-18) weeks. 78% daily, 16% on most days, 6% on an average of 2 days/week The pregnant women took different iron supplements containing distinct iron compounds. No significant	ID: 41/205 (20.0%) LBW: 14/205 (6.8%) Macrosomia: 5/205 (2.4%) Maternal ID gave IBW of ~148 g (95% CI: -296, -0.5) less than newborns from mothers without ID. Adjusting for initial Hb and TS <16% increased the difference to ~192 g (95% CI: -363, -21) less than newborns from mothers with initially non-depleted iron stores. No significant association was found between maternal ID and PTB or gestation length.

						difference between the two groups with respect to weekly frequency of iron supplements or the quantity.	

Referance: "Maternal iron status in early pregnancy and birth outcomes: insights from the Baby's Vascular health and Iron in pregnancy study" NA Alwan, JE Cade, HJ Mcardle, DC Greenwood, HE Hayes, NAB Simpson

Design: Cohort

Grade - quality: Low

Aim of study	Methods and materials	Results	Discussion/comments/check list
Examine the association between maternal Fe status during the first trimester of pregnancy, as assessed by serum ferritin, transferrin receptor and their ratio, with size at birth and preterm birth. **Conclusion** The present study shows that depleted Fe stores in early pregnancy are associated with higher risk of SGA. There was no evidence of association between maternal FE depletion and preterm birth. **Country** The United Kingdom **Year** 2015	**Recruitment:** 362 women and their infants were recruited from a teaching hospital. **Inclusion/exclusion criteria:** • Inclusion criteria: Women aged 18 years or over who gave birth to live offspring at the Leeds teaching Hospitals Trust maternity unit at a gestational age of 34 weeks or over and had sufficient proficiency in English to understand what is involved in study participation, give consent and complete a written questionnaire. • Exclusion criteria: Stillbirth or neonatal death, serious maternal illness and babies with concurrent CVD (such as patent ductus arteriosus). **Statistical methods:** Univariable analysis: independent sample *t* test, one-way ANOVA or Mann-Whitney test for continuous variables, and X^2 test for categorical variables. Multivariable linear regression: customised birth centile and birth weight centile and gestational age as the main outcomes, and indicators of maternal Fe status as predictors. A P-value < 0.05 was considered statistically significant. **Adjustment for important confounding factors:** The variables included in the multivariate analysis were maternal age, smoking, gestational diabetes, pre-eclampsia, and area deprivation score. Iron supplementation was adjusted for in a sensitivity analysis for association between first trimester iron depletion and a higher risk of a SGA baby. Not adjusted for inflammation.	**Main findings:** First trimester maternal iron depletion associated with a higher risk of a SGA baby (AOR 2.2, 9.5% CI 1.1, 41; p=0.02) in a multivariable analysis. Adjustment for iron supplementation did not change the association (AOR 2.3, 95% CI, 4.5; p=0.02), but including early pregnancy Hb in the analysis did make the association insignificant (AOR 1.6, 95% CI 0.8, 3.2; p=0.2). Maternal sTfR was marginally associated in the multivariable model with higher risk of SGA (AOR 1.1 for every nmol/l increase in sTfR (95% CI 1.0, 1.1 p=0.04)	**Check list:** • Purpose clearly formulated? **Yes** • Are the groups recruited from the same section of population? **Yes** • Were the exposed individuals representative for a defined section of the population? **Yes** • Were exposure and outcome measured equally and reliably in the groups? **Yes** • Was the study prospective? **No, but exposure was assessed prospectively.** • Were enough people followed up in the cohort? **Yes** • Is analysis of subjects lost to follow up performed? • Has important confounding factors been adjusted for? **Not adjusted for inflammation** • Can the results be generalized to a larger population? **Yes** **Strengths:** The authors mention no measurement bias of gestational age and birth weight, adjustment of the main study outcome for matnernal weight, height, ethnicity, parity, baby's sex and gestational age, and an area-based measure of deprivation. Maternal Hb and Fe supplements ascertained objectively from medical records. **Weaknesses:** The authors mention the lack of calibration of sTfR assays against international reference standards which hinders comparability across studies.

Referance: "Anaemia and iron deficiency in pregnancy and adverse perinatal outcomes in Southern India" JL Finkelstein, AV Kurpad, B Bose, T Thomas, K Srinivasan, C Duggan			**Design: Cohort** Grade - quality: Low
Aim of study	**Methods and materials**	**Results**	**Discussion/comments/check list**
Determine the prevalence of anaemia, iron deficiency, iron deficiency anaemia (IDA), and inflammation in pregnant women; and examine the associations between maternal biomarkers of iron status with the risks of adverse pregnancy and infant outcomes. **Conclusion** The prevalence of anaemia and iron deficiency was high early in pregnancy and associated with increased risk of adverse pregnancy and infant outcomes. A comprehensive approach to prevent anaemia is needed in women of reproductive age, to enhance haematological status and improve maternal and child health outcomes. **Country** India **Year** 2019	**Recruitment:** All participants (n=366) were enrolled in a randomised, double-blind, placebo-controlled trial of vitamin B_{12} supplementation. The women were recruited to the RCT from Hoshalli Referral Hospital in Bangalore, India. **Inclusion/exclusion criteria:** • Inclusion criteria: Pregnant women at least 18 years of age, <=14 weeks of gestation at enrolment, healthy and carrying a single fetus. • Exclusion criteria: Known medical complications, including HIV infection, hepatitis B, or syphilis. Women with serious preexisting medical condition (defined by the need for regular medication us), or who had a previous caesarean delivery, or who were taking daily vitamin supplements in addition to iron and folic acid were also excluded. **Statistical methods:** Serum ferritin concentrations were adjusted for inflammation, considering Thurnham and BRINDA. Linear and binomial regression models were used to examine the associations of maternal biomarkers of iron status at enrolment (i.e., ≤14 weeks) with pregnancy and infant outcomes, for continuous and categorical outcomes, respectively, including birth weight (continuous), low birth weight (categorical), gestational age at birth (continuous), preterm delivery (categorical), SGA (categorical), and infant WHO z-scores (continuous). Associations between maternal iron status biomarkers and perinatal outcomes were examined independently in separate models. All models included an adjustment for the intervention regimen and gestational age at sample collection, to account for the study design and timing of sample collection. A P-value < 0.05 was considered statistically significant. **Adjustment for important confounding factors:** Final model adjusted for gestational age, vitamin B_{12} intervention, maternal BMI, standard of living index, educational level, and CRP	**Main results:** No significant association between maternal iron deficiency and birth weight, gestation age or preterm birth. ID: 48% ID: after adjusting for inflammation: 59% IDA: 30% LBW: 38/258 (15.1%) PTB: 33/258 (12.8%) PTB+LBW: 15/258 (6.0%) SGA: 89/258 (33.9%) Women with anemia at enrolment had infants with lower birth weight (β(SE): −166.8 (61.1) g; p=0.006) and 2.15 times higher risk of low birth weight (<2500 g; RR: 2.15, 95%CI: 1.20–3.84, p = 0.01), compared with the women who were not anemic, in multivariate analyses **Other findings:** Maternal serum ferritin concentrations (β(SE): 0.79 (0.08) µg/L, p < 0.0001) and iron deficiency (β(SE):−1.19 (0.16), p < 0.0001) were associated with maternal hemoglobin concentrations in linear regression analysis.	**Check list:** • Purpose clearly formulated? **Yes** • Are the groups recruited from the same section of population? **Yes** • Were the exposed individuals representative for a defined section of the population? **Yes** • Were exposure and outcome measured equally and reliably in the groups? **Yes** • Was the study prospective? **Yes** • Were enough people followed up in the cohort? **Birth outcome data available 258 out of 360 included in the analysis.** • Is analysis of subjects lost to follow up performed? **No information** • Was the follow-up long enough to show results: **Yes** • Has important confounding factors been adjusted for? **Yes,** • Can the results be generalized to a larger population? **Yes** **Strengths:** Adjustment for confounding factors. **Weaknesses:** The authors mention that data for birth outcomes were only available for a smaller subset of the total sample, and sTfR and TBI was not assessed. Furthermore, the findings are observational and do not inform causal interpretation of findings.

Referance: "Prevalence of anemia and iron deficiency among Palestinian pregnant women and its association with pregnancy outcome" MA Srour, SS Aqel, KM Srour, KR Younis, F Samarah			**Design: Cohort** Grade - quality: Low
Aim of study	**Methods and materials**	**Results**	**Discussion/comments/check list**
To investigate the prevalence of anemia and iron deficiency among pregnant women and its association with pregnancy outcome in Hebron Governorate in southern Palestine. **Conclusion** Iron deficiency is a moderate public health problem among the study subjects. Maternal Hb and serum ferritin significantly affect pregnancy outcomes. **Country** Palestine **Year** 2018	**Recruitment:** 300 pregnant women presenting at one of 11 maternal and child healtcare centers (MCHC) during their first trimester were asked to participate. They were contacted immediately after delivery and asked to inclyde their newborns in the study. **Inclusion/exclusion criteria:** • Inclusion criteria: Healthy • Exclusion criteria: Iron supplementation, previous pregnancy complications, and pregravid chronic diseases. **Statistical methods:** Statistical tests include independent sample t-test, one-way ANOVA, and Chi-Square tests. A P-value < 0.05 was considered statistically significant. **Adjustment for important confounding factors:** Multivariable analysis not performed.	**Main results:** ID: 156/300 (52%) Anemia: 77/300 (27,5%) ID+LBW: 30/156 (18.4%) ID+PTB: 14/156 (11.5%) First trimester ID correlated with frequency of low birth weight (p=0.001) and preterm birth (p=0.003) in univariate analysis. **Other findings:** Increasing Hb concentration associate with increasing ferritin concentration. (p=0.001) Increasing Hb concentration associated with increasing birth weight (p=0.009), birth height (p=0.022), and head circumference of newborns (p=0.17) as well as with gestational age (p=0.012).	**Check list:** • Purpose clearly formulated? **Yes** • Are the groups recruited form the same section of population? **Yes** • Are the groups comparable in relation to important background factors? **Yes** • Were the exposed individuals representative for a defined section of the population? **Yes** • Were exposure and outcome measured equally and reliably in the groups? **Yes** • Is the one considering the results blinded by group affiliation? **No information** • Was the study prospective? **Yes** • Were enough people followed up in the cohort? **Yes** • Is analysis of subjects lost to follow up performed? **No subjects lost to follow up.** • Was the follow-up long enough to show results: **Yes** • Has important confounding factors been adjusted for? **No** • Can the results be generalized to a larger population? **No** **Strengths:** **Weaknesses:** The authors mention a lack of data from second and third trimester blood samples.

Referance: "Mild anemia and pregnancy outcome in a Swiss collective" G Bencaiova, C Breymann			**Design: Cohort** Grade - quality: Low
Aim of study	**Methods and materials**	**Results**	**Discussion/comments/check list**
To investigate the relation between hemoglobin and iron status examined in second trimester and pregnancy outcome. **Conclusion** Mild anemia and depleted iron stores detected early in pregnancy were not associated with adverse maternal and perinatal outcomes in iron supplemented women. **Country** Switzerland **Year** 2014	**Recruitment:** 382 pregnant women in their first trimester were asked for consent to participate in the study conducted at the Department of Obstetrics at the University Hosptal of Zurich. **Inclusion/exclusion criteria:** • Inclusion criteria: Women in pregnancy week between 16 and 20, and with singleton pregnancy. • Exclusion criteria: Chronic renal disease and malignancies and having a blood transfusion at least 3 months before enrolment in the study. **Statistical methods:** Univariate regression analysis was performed to compute odds ratios with 95% CI of women in groups 1, 2, and 3 versus nonanemic women (group 4) for well-known adverse maternal and perinatal outcomes. No correction for multiple testing was performed when comparing single groups with nonanemic women. Multivariable analysis not performed. A P-value < 0.05 was considered statistically significant. **Adjustment for important confounding factors:** Results from linear regression analysis not adjusted for confounding factors.	**Main findings:** No significant association was found between second trimester iron deficiency or mild anemia and birth weight, IUGR or preterm birth **Other findings:** There was a significant difference of hemoglobin concentration before delivery between women with depleted iron stores and normal women (p=0.005) Women with iron deficiency anemia (group 1), depleted iron stores without anemia (group 2) and anemia for other reasons (group 3) came more often from former Yugoslavia and developing countries than women in group 4 (p=0.001).	**Check list:** • Purpose clearly formulated? **Yes** • Are the groups recruited form the same section of population? **Yes** • Were the exposed individuals representative for a defined section of the population? **Yes** • Were exposure and outcome measured equally and reliably in the groups? **Yes** • Is the one considering the results blinded by group affiliation? **No information** • Was the study prospective? **Yes** • Were enough people followed up in the cohort? **Yes** • Is analysis of subjects lost to follow up performed? **No women lost to follow up** • Has important confounding factors been adjusted for? **No, only univariate analysis performed** **Strengths:** The authors do not mention strengths of the study. **Weaknesses** The authors mention absence of CRP determination as a limitation of the study, as well as a lack of a comparison of the results with an untreated group of anemic women.

Referance: "Iron deficiency in early pregnancy using ferritin and soluble transferrin receptor concentrations are associated with pregnancy and birth outcomes" AZ Khambalia, CE Collins, CL Roberts, KL Powell, V Tasevski, N Nassar			**Design: Cohort** Grade - quality: Low
Aim of study	**Methods and materials**	**Results**	**Discussion/comments/check list**
Describe the prevalence and determinants of first trimester ID and associations with pregnancy and birth outcomes **Conclusion** Nearly one in five Australian women begin pregnancy with ID. Further investigation of excess maternal weight and inflammation in the relationships between ID and GDM and LGA is needed. **Country** Australia **Year** 2015	**Recruitment:** Random sample of 4420 pregnant women and their infants from New South Wales. See inclusion criteria. **Inclusion/exclusion criteria:** • Inclusion criteria: Random sample of pregnant women who attended first trimester Down syndrome screening in New South Wales, Australia and had their results analysed by Pathology North. • Exclusion criteria: Women with twin pregnancy, medical abortion, infant with a major congenital anomaly or an undetectable ferritin and sTfR concentration. **Statistical methods:** Univariate analysis: Chi-squared test (X^2) and Fischer's exact test to examine the association between maternal characteristics and pregnancy and birth outcome with each of the three definitions of ID, usingSF, sTfR and TBI. Multivariate logistic regression to account for confounders. A P-value < 0.05 was considered statistically significant. **Adjustment for important confounding factors:** The variables included in the multivariate analysis were maternal age, smoking, gestational diabetes, pre-eclampsia, and area deprivation score.	**Main findings:** No significant association between maternal iron deficiency and preterm birth or SGA infants was found. ID by TBI significantly associated with LGA in multivariate analysis. (AOR 1.38; 95% CI 1.03, 1.85) All women ID by SF: 742/3795 (19.6%) ID by SF+SGA: 46/742 (6.6%) ID by SF+LGA 90/742 (13.0%). ID by sTfR: 676/4406 (15.3%) ID by sTfR+SGA 53/676 (8.3%) ID by sTfR+LGA 74/676 (11.5%) ID by TBI: 594/3781 (15.7%) ID by TBI+SGA: 35/594 (6.3%) ID by TBI+LGA: 79/594 (14.1%) Women with CRP<0.5mg/dl: ID by SF: 18.8% ID by sTfR: 10.9% ID by TBI: 12.7% **Other findings:** Women with ID based on SF and TBI concentrations were more likely to be younger, multiparous and socioeconomically disadvantaged. For women with ID using sTfR, multiparity and low SES were also risk factors. They were also more likely to be heavier and have high CRP concentrations.	**Check list:** • Purpose clearly formulated? **Yes** • Are the groups recruited form the same section of population? **Yes** • Were the exposed individuals representative for a defined section of the population? **Yes** • Were exposure and outcome measured equally and reliably in the groups? **Yes** • Was the study prospective? **No, but the exposure was assessed prospectively.** • Were enough people followed up in the cohort? **Yes** • Is analysis of subjects lost to follow up performed? **No information.** • Was the follow-up long enough to show results: **Yes** • Has important confounding factors been adjusted for? **Not Hb, iron supplementation or third trimester inflammation.** • Can the results be generalized to a larger population? **Yes** **Strengths:** The auhtors mention large population-based cohort design with thorough measurement, high acertainment and reporting of first trimester iron status and pregnancy and birth examination of a combination of biomarkers of ID, which provide information on different stages of ID. **Weaknesses:** The authors mention lack of data on iron supplement use, maternal diet, maternal insulin and glucose concentrations and infant iron status. Lack of data on maternal anemia and lack of assay standardization for sTfR were described as notable limitations of the study.

Referance: "Iron deficiency in late pregnancy and its associations with birth outcomes in Chinese pregnant women: a retrospective cohort study" X Yuan, H Hu, M Zhang, W Long, J Liu, J Jiang, B Yu

Design: Cohort

Grade - quality: Low

Aim of study	Methods and materials	Results	Discussion/comments/check list
To explore the relationship between maternal iron status in the third trimester and adverse birth outcomes in chinese pregnant women, while taking into account systemic inflammation as measured by high sensitivity C-reactive protein (hsCRP) and anemia as measured by hemoglobin. **Conclusion** ID in the third trimester of pregnancy is frequent in Chinese women. The findings suggest that the ratio of ST/SF measured in late pregnancy could be useful as a significant predictor of unfavorable birth outcomes. **Country** China **Year** 2019	**Recruitment:** A total of 11.581 pregnant women who delivered in Changzhou Maternity and Child Health Care Hospital between April 2016 and March 2017 were recruited to the study. **Inclusion/exclusion criteria:** • Inclusion criteria: Women > 18 years pregnant 28–41 weeks with singleton pregnancy and live birth • Exclusion criteria: Twin or triplet pregnancy, missing integrated and clear medical records, preexisting illnesses before getting pregnant: diabetes mellitus (type 1 or 2), chronic hypertension, thyroid diseases, chronic heart, liver and kidney diseases, immune rheumatic disease or thyroid diseases and syphilis prior to pregnancy, cigarette smokers and alcohol drinkers in pregnancy. **Statistical methods:** General linear analysis was applied to examine the association of serum biomarkers for ID with fetal growth (gestational age, birth length and weight). After adjusting for maternalage, BMI, gravidity, parity, hemoglobin level, logistic regression analysis was used to explore the associations between maternal ID and pregnancy outcomes. A P-value < 0.05 was considered statistically significant. **Adjustment for important confounding factors:** OR and 95% CI for PTB were additionally corrected for gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, preeclampsia, pregnancy-induced hypertension, systolic and diastolic blood pressure and infant sex. The values for SGA, LGA, LBW and macrosomia were further adjusted for gestational age at delivery.	**Main results:** Women without inflammation: ID by SF: 54.27% ID by ST: 56.23% ID by ST/SF: 56.29% All infants: LBW: 519 (4.48%) Macrosomia: 853 (7.37%) SGA: 1024 (8.84%) LGA: 1793 (15.48%) ID by SF in women without inflammation: Decreased risk of PTB (AOR=0.71, 95% CI, 0.57, 0.88) Decreased risk of LBW (AOR=0.58, 95% CI 0.40, 0.84) Decreased risk of SGA (AOR=0.71, 95% CI, 0.59, 0.85 Increased risk of macrosomia (AOR=1.29, 95% CI, 1.06, 1.59) ID by SF in all women: Similar results as for women without inflammation, in addition to increased risk of LGA infant (AOR=1.17, 95% CI, 1.03, 1.33).	**Check list:** • Purpose clearly formulated? **Yes** • Are the groups recruited form the same section of population? **Yes** • Are the groups comparable in relation to important background factors? **Yes** • Were the exposed individuals representative for a defined section of the population? **Yes** • Were exposure and outcome measured equally and reliably in the groups? **Yes** • Is the one considering the results blinded by group affiliation? **No information** • Was the study prospective? **No, but exposure assessed prospectively.** • Were enough people followed up in the cohort? **Yes** • Is analysis of subjects lost to follow up performed? **No information** • Has important confounding factors been adjusted for? **Yes, with the exeption of iron supplementation.** • Can the results be generalized to a larger population? **Yes** **Strengths:** The authors mention sufficient sample size, adjustment of the confounders Hb, maternal BMI and hsCRP, prospective documentation of the ID biomarkers and birth outcomes and inclution of both rural and urban populations. **Weaknesses:** The authors mention a lack of data on maternal ID from the first and second trimester, and detailed maternal characteristics including pre-pregnancy BMI, weight gain during pregnancy, socio-economic level and iron supplementation.

Referance: "Depleted iron stores without anemia early in pregnancy carries increased risk of lower birthweight even when supplemented daily with moderate iron" B Ribot, N Aranda, F Viteri, C Hernández-Martínez, J Canals, V Arija

Design: Cohort

Grade - quality: Low

Aim of study	Methods and materials	Results	Discussion/comments/check list
Assess the relationship of first trimester maternal iron stores with birthweight, in non-anemic pregnant women. **Conclusion** Non-anemic pregnant women with depeted iron stoes have children who are smaller than those drom women who begin pregnancy with non-depleted iron stores, even when borth groups of women receive a moderate daily iron supplementation. **Country** Spain **Year** 2012	**Recruitment:** A total of 300 pregnant volunteers were recruited during a three-year period. All were attending their first visit for antenatal care at the Unit of Obstetrics and Gynaecology of the Hospital Universitari Sant Joan de Reus, Catalunya, Spain. **Inclusion/exclusion criteria:** • Inclusion criteria: Caucasian, healthy pregnant woman at 8–12 weeks of gestation and <18 years of age • Exclusion criteria: chronic illness or a possible inflammation with high SF (>62µg/l) and low transferrin saturation (TS<16%), twin or triplet pregnancies. The analysis only included women without anemia, who were iron supplemented starting < week 30, and compiled with the scheduled visits, had blood drawn at each visit and gave birth at the study hospital. **Statistical methods:** Univariate analysis: Student t-test to compare means and the Chi-squared test (X^2) to compare proportions. Two multiple linear regression models were applied, one with SF as the main measure of iron status and the other with SF adjusted for TS<16% (no,yes) at the start of pregnancy. A P-value < 0.05 was considered statistically significant. **Adjustment for important confounding factors:** The variables included in the multivariable analysis were first trimester ID, maternal age, BMI at first visit, parity, gestational age, gender, tobacco habit, socioeconomic status of the family and mean iron supplementation.	**Main findings:** Maternal ID gave IBW of ~148 g (95% CI: -296, -0.5) less than newborns from mothers without ID. Adjusting for initial Hb and TS <16% increased the difference to ~192 g (95% CI: -363, -21) less than newborns from mothers with initially non-depleted iron stores. ID: 41/205 (20.0%) LBW: 14/205 (6.8%) Macrosomia: 5/205 (2.4%) No significant association was found between maternal ID and PTB or gestation length.	**Check list:** • Purpose clearly formulated? **Yes** • Are the groups recruited form the same section of population? **Yes** • Are the groups comparable in relation to important background factors? **Yes** • Were the exposed individuals representative for a defined section of the population? **Yes** • Were exposure and outcome measured equally and reliably in the groups? **Yes** • Was the study prospective? **Yes** • Were enough people followed up in the cohort? **Yes** • Is analysis of subjects lost to follow up performed? **No women lost to follow up** • Was the follow-up long enough to show results: **Yes** • Has important confounding factors been adjusted for? **Yes** • Can the results be generalized to a larger population? **Yes** **Strengths:** Exclusion of anemic women and adjustment for important confounding factors. **Weaknesses:** The authors mention variables that were not included and might have affected the birth weight: stress, strenuous work, genetics of the mother or some other factors related to birthweigth.