First Trimester Iron Status and Obstetric and Perinatal Outcomes

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Research Article

Keywords: Iron status, iron deficiency, iron deficiency anemia, hemoglobin, ferritin, pregnancy

Posted Date: November 8th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1048128/v1>

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Abstract

Purpose: To assess the following in singleton pregnant women: 1) associations between first trimester iron deficiency and obstetric and perinatal outcomes, 2) overall first trimester iron status and 3) post-treatment iron status after intensified iron supplementation.

Methods: Prospective cohort study with linkage of first trimester hemoglobin and plasma ferritin with obstetric and perinatal data from a hospital database. Blood sample data were obtained at a Danish University Hospital. The cohort was divided into groups according to ferritin and hemoglobin: (1) iron deficient anemic (ferritin <30 ng/mL and Hb <110 g/L), (2) iron deficient non-anemic (ferritin <30 ng/mL and Hb $\geq$ 110 g/L), and (3) iron replete non-anemic (ferritin 30–200 ng/mL and Hb $\geq$ 110 g/L). Obstetric and perinatal outcomes in each iron deficient group were compared to the iron replete non-anemic group using multivariable logistic regression. The effect of 2–8 weeks intensified iron supplementation on hemoglobin and ferritin were assessed by pairwise comparisons.

Results: The cohort comprised 5,763 singleton pregnant women, of which 14.2% had non-anemic iron deficiency, and 1.2% had iron deficiency anemia. Compared to iron replete non-anemic women, iron deficient anemic women had a higher risk of gestational diabetes (aOR 3.8, 95% CI 1.4–9.0), and iron deficient non-anemic women had a higher risk of stillbirth (aOR 4.0, 95% CI 1.0–14.3). In group 1 and 2, 78.7% and 67.8% remained iron deficient after intensified iron supplementation.

Conclusion: First trimester iron deficiency may be associated with gestational diabetes and stillbirth. First trimester iron deficiency was present in 15.4% and often persisted despite 2–8 weeks intensified iron supplementation.

Introduction

Iron deficiency and iron deficiency anemia in pregnancy are common health problems. According to the World Health Organization (WHO), about 38% of pregnant women worldwide are anemic, whereas iron deficiency affects around 40%. Iron deficiency is the most common pathological cause of anemia in pregnancy, appraised to account for 50% of all cases[1, 2].

Iron deficiency occurs when the level of stored body iron becomes depleted. As iron is prioritized for erythropoiesis, iron deficiency anemia is the end-stage result of iron deficiency[3]. Iron is critical for oxygen delivery and enzyme functions involved in fetal organogenesis during pregnancy[4], and iron requirements increase as gestation proceeds[5]. Both low and elevated hemoglobin (Hb) and iron status in pregnancy have been associated with an increased risk of adverse obstetric and perinatal outcomes, and vulnerability to abnormal concentrations appears to depend on the gestational age at which they occur[6]. The association between low Hb and adverse outcomes is most evident when Hb is measured in early pregnancy[6], and anemia, including iron deficiency anemia in early pregnancy, has been associated with increased risk of preterm delivery (PTB) and low birthweight (LBW)[7, 8]. While low Hb is a valid and widely available diagnostic marker for anemia, it cannot detect the stages of iron deficiency that precede anemia[4, 9]. Hence, the combination of Hb and ferritin reflects the iron status spectrum more specifically than Hb alone. However, adverse effects of non-anemic iron deficiency in pregnancy are less explored than those of iron deficiency anemia[6].

In Denmark, the National Health Authority recommends routine supplementation with 40–50 mg iron from 10 weeks' gestation[10, 11]. In 2016, the Danish Society of Obstetrics and Gynaecology (DSOG) stated that neither routine iron supplementation nor routine screening with individualized iron dosing is supported by direct evidence[12]. However, DSOG further stated that screening is preferable from an expert point of view, as routine supplementation is more likely to lead to both under- and overtreatment with unnecessary and potentially harmful side effects[12]. Therefore, an individualized screening program for first trimester iron deficiency and anemia was implemented at Copenhagen University Hospital Hvidovre, and we designed a study in parallel to explore the screening-related data.

The overall first trimester Hb and iron status in women in Denmark is currently unknown and whether the associations of hematological markers and adverse obstetric and perinatal outcomes found in other populations also exist in women in Denmark remains unexplored. Therefore, the primary aim of this study was to investigate the associations between both anemic and non-anemic iron deficiency and obstetric and perinatal outcomes. Further objectives included to determine first trimester Hb and iron status in pregnant women and assess how these markers change in iron deficient women who receive intensified iron supplementation.

Materials And Methods

Study overview

Prospective cohort study based on blood samples from a first trimester (before 14 weeks' gestation) iron deficiency and anemia screening program in pregnant women, collected from June 2017 through May 2018 at a single tertiary University hospital in Denmark (Department of Obstetrics and Gynecology, Copenhagen University Hospital Hvidovre). Blood sample data were collected prospectively and subsequently cross-linked to demographic, obstetric, and perinatal data from a high-validity hospital-based clinical database – the Obstetric Database. The database has been described in detail by Brixval et al. and is a valid source for assessing the prevalence of obstetric interventions and outcomes[13]. Data were cross-linked using the unique personal identification code allocated to all citizens in Denmark at birth.

The anemia and iron deficiency screening program

The individualized screening program was implemented locally at Copenhagen University Hospital Hvidovre and adapted to routine antenatal care between 2017 and 2020. The practice was implemented in accordance with the guidelines developed by DSOG (2016)[12] as an alternative to the Danish standard of

routine prophylactic iron supplementation (Danish National Health Authority recommendation: 40–50 mg elemental iron starting from 10 weeks' gestation[10, 11]). The screening was performed together with the routine screening for trisomy 21 (double test), which approximately 94% of all pregnant women in Denmark attend[14]. The screening included Hb and plasma ferritin measurements from a venous blood sample, as guidance for an individualized iron dosing regimen. Based on the screening results, women were recommended daily iron supplementation dosed as described in Figure 1. Women with ferritin concentrations <30 ng/mL were recommended to intensify iron supplementation and have Hb and ferritin measurements repeated after four weeks.

Population

The cohort comprised all women with a singleton pregnancy who fulfilled the following criteria: screened for anemia and iron deficiency before 14 weeks' gestation from June 2017 through May 2018 after referral to standard antenatal care at Copenhagen University Hospital Hvidovre with their data included in the Obstetric Database (Fig. 2). If a woman had more than one screening sample in the same pregnancy, only the screening results from the first date were included. Further, if a woman had more than one pregnancy during the study period, only the first pregnancy was included.

The cohort was further categorized into groups to investigate the associations between iron deficiency and obstetric and perinatal outcomes (Fig. 2). Before grouping, we excluded women with non-iron deficient anemia (Hb <110 g/L and ferritin $\geq$ 30 ng/mL) (n=91) and women with high ferritin levels (>200 ng/mL) (n=145) as these states can have a range of underlying causes (e.g. thalassemia, chronic disease, hemochromatosis) that per se may negatively affect pregnancy outcomes. Furthermore, women who participated in a randomized controlled trial (RCT) on intravenous and oral iron treatments[15] in their pregnancy were excluded (n=48) since the allocated interventions rather than their Hb and iron status could affect pregnancy outcomes and lead to bias. Subsequently, the cohort was categorized into three groups as follows:

1. Iron deficient anemic cases (ferritin <30 ng/mL and Hb <110 g/L)
2. Iron deficient non-anemic cases (ferritin <30 ng/mL and Hb $\geq$ 110 g/L)
3. Iron replete non-anemic references (ferritin 30–200 ng/mL and Hb $\geq$ 110 g/L)

Finally, hematological changes were assessed in a subgroup of women with first trimester iron deficiency (i.e. group 1 and 2 above) who attended a follow-up within 2–8 weeks after their screening sample.

Obstetric and perinatal outcomes

Detailed definitions of obstetric and perinatal outcomes are included as supplementary information (Online Resource 1). Obstetric outcomes included: hypertensive disorders in pregnancy, gestational diabetes (GDM), induction of labor, oxytocin augmentation of labor, instrumental vaginal birth (vacuum or forceps), emergency cesarean, labor dystocia (emergency cesarean and/or oxytocin augmentation and/or instrumental vaginal birth), postpartum hemorrhage (PPH $\geq$ 500 mL), and severe PPH (>1,000 mL).

Perinatal outcomes included: stillbirth, birth asphyxia (umbilical artery pH <7.05 and/or Apgar Score <7 at 5 minutes), PTB (<37+0 weeks' gestation), post-term birth (>42+0 weeks' gestation), LBW (<2,500 g), small for gestational age (SGA), macrosomia (>4,500 g), and large for gestational age (LGA). SGA and LGA were defined as birthweight <-2 and >+2 standard deviation differences from the expected sex-specific birthweight for the given gestational age, respectively[16]. The Danish national standard for determining the ultrasound based due date is at the combined first trimester screening examination performed at 11-14 weeks' gestation.

Statistical analysis

Descriptive statistics were used to summarize the distribution of first trimester Hb and ferritin in the total cohort and present characteristics of the three iron status groups. Among women with iron deficiency at screening (i.e. group 1 and 2), all attending a follow-up blood sampling 2–8 weeks after screening contributed with paired data in analyses of Hb and ferritin changes. In these comparative analyses, McNemar exact test was used for categorical data and paired t-test and Wilcoxon signed rank test as appropriate for continuous data.

For the primary aim, obstetric and perinatal outcomes were compared between the grouped women using the iron replete non-anemic women as a reference group. All comparisons were made using univariate and multivariable logistic regression, and results were presented as odds ratios (OR) and adjusted OR (aOR) with 95% confidence intervals (CI). The multivariable logistic regression analyses adjusted for the following potential confounding variables selected based on previous research: multiparity (parity $\geq$ 1); maternal age >35; maternal tobacco-smoking during pregnancy; child's sex; maternal body mass index (BMI) >25 kg/m²; Non-White race-ethnicity. Race-ethnicity data were collected and included in the adjusted model because anemia and adverse pregnancy outcomes may be higher among Non-White women. Race-ethnicity had been classified either by a caregiver or self-reporting and was categorized as White, Non-White, or unknown.

Hb concentrations were reported as mmol/L, and we converted them to g/L using conversion factors recommended by The International Council for Standardization in Haematology[17]. The analysis plan was finalized post-hoc. The statistical software program R was used for all statistical analyses and the MASS-package for logistic regression analyses[18, 19]. A p-value <0.05 was considered statistically significant.

Results

In total, 7,391 women were screened with first trimester Hb and ferritin, of which approximately 20% were missing outcome data in the Obstetric Database, were multiple pregnancies, or missing first trimester samples. Accordingly, 5,763 unselected singleton pregnant women were included in the study cohort (Fig. 2). Screened women with and without obstetric and perinatal data seem to have been comparable in measures of average Hb, ferritin, and age (data not shown).

Of the 5,763 women, 284 were excluded from the regression analyses due to non-iron deficient anemia (n=91), high ferritin levels (n=145), and participation in a RCT on intravenous and oral iron treatments (n=48). The remaining 5,479 women were categorized in the following three groups: (1) iron deficient anemia cases (n=64), (2) iron deficient non-anemic cases (n=778), and (3) iron replete non-anemic references (n=4,637) (Fig. 2). Pre-post treatment assessments included women in group 1 and 2 (i.e. iron deficient women) who attended a new Hb and ferritin sampling 2–8 weeks after their primary sample.

First trimester Hb and iron status

First trimester Hb and ferritin levels of the 5,763 included women are presented in Table 1. A total of 890 (15.4%) were iron deficient: 821 (14.2%) were iron deficient non-anemic and 69 (1.2%) women were iron deficient anemic, respectively. Overall, 91 (1.6%) women had anemia due to other causes than iron deficiency (Hb <110 g/L and ferritin ≥30 ng/mL). No cases of severe anemia (Hb <70 g/L) were observed. A total of 2,376 (41.2%) women had ferritin concentrations ≥70 ng/mL.

Table 1
Distribution of first trimester^a Hb and ferritin in singleton pregnancies (n=5,763)

<i>Category</i>	
Hb (g/L)	
mean ±SD	128.2±9.0
median (IQR)	128.8 (109.5-115.9)
range	75.7-162.6
Ferritin (ng/mL)	
mean ± SD	72.5±51.6
median (IQR)	60 (14-22)
range	5-775
Ferritin (n, (%))	
Ferritin <30 ng/mL	890 (15.4)
<15 ng/mL	161 (2.8)
15–29 ng/mL	729 (12.6)
Ferritin >30 ng/mL	4,873 (84.6)
30–69 ng/mL	2,497 (43.4)
70–200 ng/mL	2,223 (38.6)
>200 ng/mL	153 (2.7)
Hb (n, (%))	
<110 g/L	160 (2.8)
110-130 g/L	3,011 (52.2)
>130 g/L	2,592 (45.0)
Anemic (Hb <110 g/L) (n, (%))	
Ferritin ≥30 ng/mL	91 (1.6)
Ferritin <30 ng/mL	69 (1.2)
Ferritin <15 ng/mL	40 (0.7)
Ferritin 15-29 ng/mL	29 (0.5)
Non-anemic (Hb ≥110 g/L) (n, (%))	
Ferritin ≥30 ng/mL	4,782 (83.0)
Ferritin <30 ng/mL	821 (14.2)
Ferritin <15 ng/mL	121 (2.1)
Ferritin 15-29 ng/mL	700 (12.1)
^a Less than 14 weeks of gestation. Hb, hemoglobin.	

Hb and Ferritin changes after intensified iron supplementation

In groups 1 and 2, a total of 842 women had first trimester iron deficiency and were recommended oral iron supplementation in daily doses according to Figure 1. Among these women, 557 (66.2%) had a new blood test taken within 2–8 weeks after their primary sample, and their hemoglobin and ferritin changes are presented in Table 2. In 47 women who initially had iron deficiency anemia, the mean Hb concentration increased in the subsequent blood samples (102.6 vs. 107.9 g/L, $p<0.001$) and anemia (Hb <110 g/L) resolved in 19 (40.4%). Among 510 initially iron deficient non-anemic women, the mean Hb concentration remained within the normal range though it decreased from baseline to follow-up (126.7 vs. 123.6 g/L, $p<0.001$), and 480 (94.1%) remained non-anemic (Hb $\geq$ 110 g/L). Median ferritin concentrations increased both in the initially iron deficient anemic (11.0 vs. 20.0 ng/mL, $p<0.001$) and iron deficient non-anemic women (23.0 vs. 25.0 ng/mL, $p<0.001$). The overall prevalence of iron deficiency decreased in both groups, however, most women remained iron deficient: 78.7% of initially iron deficient anemic and 67.8% of initially iron deficient non-anemic women, respectively. No initially iron deficient anemic women developed high Hb (>130 g/L), and the prevalence of high Hb decreased in the initially iron deficient non-anemic women (34.9% vs. 21.6%, $p<0.001$).

Table 2
Biochemical data in singleton pregnancies with first trimester ferritin <30 ng/mL and follow-up samples within 2-8 weeks

Outcome	Iron deficient anemic (n=47)			Iron deficient non-anemic (n=510)		
	At screening	At follow-up	Screening vs. follow-up	At screening	At follow-up	Screening vs. follow-up
Hb (g/L)	102.6±6.6	107.9±9.4	MD 5.2 g/L (95% CI 3.0 – 7.5, $p<0.001^a$)	126.7±7.8	123.6±8.1	MD -3.1 g/L (95% CI -3.6 – -2.5, $p<0.001^a$)
Ferritin (ng/mL)	11.0 (9.0 – 20.5)	20.0 (13.0 – 26.0)	$p<0.001^b$	23.0 (18.0 – 27.0)	25.0 (20.0 – 32.0)	$p<0.001^b$
Ferritin <30 ng/mL	100%	78.7% (95% CI 64.3 – 89.3)	NA	100%	67.8% (95% CI 63.6 – 71.9)	NA
Ferritin <15 ng/mL	59.6% (95% CI 44.3 – 73.6)	31.9% (95% CI 19.1 – 47.1)	OR 0.3 (95% CI 0.1 – 0.8, $p=0.01^c$)	14.3% (95% CI 11.4 – 17.7)	7.1% (95% CI 5.0 – 9.6)	OR 0.3 (95% CI 0.2 – 0.6, $p<0.001^c$)
Hb <110 g/L	100%	59.6% (95% CI 44.3 – 73.6)	NA	0%	5.9% (95% CI 4.0 – 8.3)	NA
Hb >130 g/L	0%	0%	NA	34.9% (95% CI 30.8 – 39.2)	21.6% (95% CI 18.1 – 25.4)	OR 0.3 (95% CI 0.2 – 0.4, $p<0.001^c$)
Screening and follow-up concentrations expressed as mean $\pm$ SD for normally distributed continuous data (i.e. Hb concentrations), median and (IQR) for non-normally distributed continuous data (i.e. ferritin concentrations), and as percentage for categorical data with 95% CI in parenthesis for proportions <100% and >0%. Bold font indicates statistical significance. NA indicates that comparison was not possible due to proportion at screening and/or follow-up equal to 100% or 0%.						
^a Paired t-test.						
^b Wilcoxon signed rank test.						
^c McNemar exact test.						
Hb, hemoglobin; MD, mean difference; CI, confidence interval; OR, odds ratio; SD, standard deviation; IQR, interquartile range; NA, not applicable.						

Baseline characteristics

Demographics and baseline characteristics of women in group 1–3 are summarized in Table 3. The proportion women with BMI <18.5 kg/m², parity $\geq$ 1, Non-White race-ethnicity, hyperemesis, and previous bariatric surgery appeared to be unevenly distributed between groups with the highest prevalence in iron deficient anemic women and lowest prevalence in iron replete non-anemic women (although mean BMI appeared comparable across groups). In addition, iron deficient anemic women seemed more likely to be non-smokers and deliver a female child than the women in the other two groups. No other obvious differences were observed between the groups.

Table 3
Demographics/characteristics in 5,479 singleton pregnancies divided by first trimester iron status

Category	Iron status		
	Iron deficient, anemic (n = 64)	Iron deficient, non-anemic (n = 778)	Iron replete, non-anemic (n = 4,637)
Maternal age (years), mean $\pm$ SD	31.7 $\pm$ 5.2	31.8 $\pm$ 5.0	31.7 $\pm$ 4.7
≤ 35 years ^a	46 (71.9)	576 (74.0)	3,531 (76.1)
>35 years	18 (28.1)	202 (26.0)	1,106 (23.9)
Maternal BMI (kg/m ²), mean $\pm$ SD	23.8 $\pm$ 4.3	23.8 $\pm$ 4.4	24.0 $\pm$ 4.5
WHO weight classification by BMI (kg/m ²)			
<18.5/underweight, n (%)	5 (7.8)	41 (5.3)	171 (3.7)
18.5-24.9/normal weight, n (%)	41 (64.1)	492 (63.2)	3,040 (65.6)
25.0-29.9/pre-obesity, n (%)	13 (20.3)	161 (20.7)	945 (20.4)
≥ 30 /obesity, n (%)	5 (7.8)	80 (10.3)	459 (9.9)
BMI unknown ^b	0 (0)	4 (0.5)	22 (0.5)
Parity, mean $\pm$ SD	2.1 $\pm$ 1.0	1.9 $\pm$ 0.9	1.6 $\pm$ 0.7
0, n (%)	20 (31.3)	274 (35.2)	2,589 (55.8)
1, n (%)	25 (39.1)	339 (43.6)	1,594 (34.4)
2, n (%)	12 (18.8)	124 (15.9)	376 (8.1)
≥ 3 , n (%)	7 (10.9)	41 (5.3)	78 (1.7)
Race/ethnicity			
White	46 (71.9)	609 (78.3)	4189 (90.3)
Non-white	17 (26.6)	158 (20.3)	405 (8.7)
Unknown	1 (1.6)	11 (1.4)	43 (0.9)
Maternal tobacco smoking during pregnancy ^c , n (%)	<3	90 (11.6)	450 (9.7)
Maternal use of drugs and/or alcohol in pregnancy ^{d,e} , n (%)	<3	8 (1.0)	44 (0.9)
Essential hypertension ^d , n (%)	<3	<3	16 (0.3)
Diabetes mellitus ^d , n (%)	0 (0)	0 (0)	0 (0)
Hyperemesis ^d , n (%)	3 (4.7)	7 (0.9)	70 (1.5)
Inflammatory bowel disease ^d , n (%)	<3	15 (1.9)	62 (1.3)
Previous bariatric surgery ^d , n (%)	4 (6.3)	8 (1.0)	7 (0.2)
Assisted reproductive technology ^d , n (%)	3 (4.7)	34 (4.4)	272 (5.9)
Sex of child (female), n (%)	34 (53.1)	359 (46.1)	2,269 (48.9)
Characteristic and demographic data divided by iron status group expressed as mean $\pm$ SD for continuous data and as number (%) for categorical data.			
^a Among all 5,479 women, three and six women (i.e. 0.01%) were younger than 18 and 19 years, respectively.			
^b Missing values for maternal BMI among iron deficient non-anemic women (four missing) and iron replete non-anemic women (22 missing), respectively.			
^c Assessed by a Danish national result code (RGAB). Coding for non-tobacco-smoker (00) and tobacco use undisclosed (99) defined non-smokers and any known tobacco smoking in pregnancy (all remaining RGAB codes) defined smokers.			
^d Defined by ICD-10 codes as follows: Maternal use of drugs and/or alcohol in pregnancy (Z358M1-17); essential hypertension (O100); diabetes mellitus (O240–241 and/or O243) including type 1, type 2, and non-specified type; hyperemesis (O21); inflammatory bowel disease (all O996 except O996C) including Crohn's disease and ulcerative colitis; previous bariatric surgery (Z980) including gastric bypass and gastric sleeve; assisted reproductive technology (Z358F and/or Z358K) including pregnancy after in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and/or egg donation.			
^e Substances used included alcohol, opioids, cannabis, methadone, and cocaine.			
SD, standard deviation, BMI, body mass index; WHO, World Health Organization.			

Obstetric and perinatal outcomes

The obstetric and perinatal outcomes of women in the three groups and the logistic regression analyses results are shown in Table 4. Women with iron deficiency anemia were more likely to develop GDM (aOR 3.8, 95% CI 1.4–9.0) than the iron replete non-anemic references. Furthermore, the iron deficient non-anemic women had a higher risk of stillbirth (aOR 4.0, 95% CI 1.0–14.3) than the iron replete non-anemic references. The remaining comparisons showed no statistically significant between-group differences.

Table 4

Multivariable logistic regression analyses: obstetric and perinatal outcomes in iron deficient anemic (n=64), iron deficient non-anemic (n=778), and iron replete non-anemic women (n=4,637) with singleton pregnancies

Outcome	Incidence by iron status, n (%)			Iron deficient anemic vs. replete		Iron deficient non-anemic vs. replete	
	Iron deficient, anemic	Iron deficient, non-anemic	Iron replete, non-anemic	Crude OR (95% CI)	Adjusted OR (95% CI) ^a	Crude OR (95% CI)	Adjusted OR (95% CI) ^a
Hypertensive disorders of pregnancy ^b	<3	38 (4.9)	290 (6.3)	0.5 (0.1-1.6)	0.7 (0.1-2.2)	0.8 (0.5-1.1)	1.0 (0.7-1.4)
GDM ^c	6 (9.4)	26 (3.3)	110 (2.4)	4.3 (1.6-9.3)	3.8 (1.4-9.0)	1.4 (0.9-2.2)	1.1 (0.7-1.7)
Induced labor	11 (17.2)	178 (22.9)	1013 (21.8)	1.3 (0.7-2.7)	1.2 (0.6-2.4)	0.9 (0.8-1.1)	0.8 (0.7-1.0)
Oxytocin augmentation of labor	16 (25.0)	174 (22.4)	1241 (26.8)	0.9 (0.5-1.6)	1.3 (0.7-2.4)	0.8 (0.7-0.9)	1.1 (0.9-1.3)
Instrumental vaginal birth ^d	<3	41 (5.3)	410 (8.8)	0.3 (0.1-1.1)	0.4 (0.1-1.5)	0.6 (0.4-0.8)	0.8 (0.5-1.1)
Emergency cesarean	7 (10.9)	66 (8.5)	465 (10.0)	1.1 (0.5-2.3)	1.4 (0.6-3.0)	0.8 (0.6-1.1)	1.0 (0.7-1.3)
Labor dystocia ^e	19 (29.7)	211 (27.1)	1509 (32.5)	0.9 (0.5-1.5)	1.3 (0.7-2.3)	0.8 (0.6-0.9)	1.0 (0.9-1.3)
Postpartum bleeding ^f							
≥500 mL (PPH)	20 (31.7)	213 (27.5)	1265 (27.4)	1.2 (0.7-2.1)	1.2 (0.7-2.1)	1.0 (0.8-1.2)	1.0 (0.9-1.2)
>1,000 mL (severe PPH)	5 (7.9)	52 (6.7)	332 (7.2)	1.1 (0.4-2.5)	1.2 (0.4-2.8)	0.9 (0.7-1.2)	1.0 (0.7-1.3)
Stillbirth	0 (0)	4 (0.5)	8 (0.2)	NA	NA	3.0 (0.8-9.5)	4.0 (1.0-14.3)
Birth asphyxia ^g	<3	11 (1.5)	91 (2.1)	1.7 (0.3-5.5)	2.3 (0.4-7.8)	0.7 (0.4-1.3)	0.8 (0.4-1.5)
Umbilical pH <7.05	<3	10 (1.3)	77 (1.7)				
Apgar <7 at 5 minutes	0 (0)	7 (0.9)	35 (0.8)				
Gestational age at delivery							
Mean ± SD (weeks)	39.5 ± 1.9	39.8 ± 1.7	39.9 ± 1.8				
<37 weeks (PTB)	4 (6.3)	29 (3.7)	201 (4.3)	1.5 (0.4-3.6)	1.7 (0.5-4.2)	0.9 (0.6-1.2)	0.9 (0.6-1.4)
>42 weeks (post-term)	<3	20 (2.6)	131 (2.8)	0.5 (0.0-2.5)	0.6 (0.0-3.0)	0.9 (0.5-1.4)	1.1 (0.7-1.8)
Birthweight ^f							
Mean ± SD (g)	3,425 ± 567	3,508 ± 523	3,497 ± 544				
LBW (<2,500 g)	5 (7.8)	18 (2.3)	163 (3.5)	2.3 (0.8-5.3)	2.4 (0.8-5.6)	0.7 (0.4-1.0)	0.6 (0.4-1.0)
SGA ^h	5 (7.8)	29 (3.7)	195 (4.2)	1.9 (0.7-4.4)	1.8 (0.6-4.2)	0.9 (0.6-1.3)	0.8 (0.5-1.2)
Macrosomia (>4,500 g)	<3	11 (1.4)	108 (2.3)	1.4 (0.2-4.4)	1.3 (0.2-4.3)	0.6 (0.3-1.1)	0.8 (0.5-1.2)
LGA ⁱ	<3	22 (2.8)	151 (3.3)	1.0 (0.2-3.1)	0.9 (0.1-2.8)	0.9 (0.5-1.3)	
Obstetric and perinatal outcomes divided by iron status. Frequencies expressed as number (%). Logistic regressions with the iron replete non-anemic group as reference expressed as odds ratios with 95% CI in parenthesis. Bold font indicates statistical significance. NA indicate that analysis was not possible due to no cases in one or both groups.							
^a Adjusted for nullipara (yes/no) age>35 (yes/no) + smoking (yes/no) + child's sex (female/male) + BMI>25 (yes/no) + Non-White race/ethnicity (yes/no).							
^b Preeclampsia or gestational hypertension. Essential hypertension prior to preeclampsia was present in six women, all in the replete non-anemic group.							
^c Danish diagnostic criteria for GDM follow the Association of Diabetes and Pregnancy Study Groups and the WHO 2013 recommendations.							

Outcome	Incidence by iron status, n (%)			Iron deficient anemic vs. replete		Iron deficient non-anemic vs. replete	
	Iron deficient, anemic	Iron deficient, non-anemic	Iron replete, non-anemic	Crude OR (95% CI)	Adjusted OR (95% CI) ^a	Crude OR (95% CI)	Adjusted OR (95% CI) ^a
^d Vacuum or forceps.							
^e Emergency cesarean and/or oxytocin augmentation and/or instrumental vaginal birth.							
^f Variables with missing values: postpartum bleeding data in the iron deficient anemic group (two missing), iron deficient non-anemic group (five missing), and iron replete non-anemic group (16 missing), respectively; birthweight data in the non-anemic iron deficiency group (one missing).							
^g Umbilical artery pH <7.05 and/or Apgar Score <7 at 5 minutes							
^h Birth weight <-2 SD from the expected sex-specific birth weight for the given gestational age.							
ⁱ Birth weight >+2 SD from the expected sex-specific birth weight for the given gestational age.							
CI, confidence interval, SD, standard deviation, GDM, gestational diabetes; PPH, postpartum hemorrhage; PTB, preterm birth; LBW, low birth weight; SGA, small for gestational age; LGA, large for gestational age; WHO, World Health Organization.							

Discussion

In this cohort of 5,763 women screened in early pregnancy, 14.2% had non-anemic iron deficiency while 1.2% had iron deficiency anemia. GDM were more likely to occur in women with iron deficiency anemia than in iron replete non-anemic women (aOR 3.8, 95% CI 1.4–9.0), whereas stillbirth was more common in iron deficient non-anemic women than in iron replete non-anemic women (aOR 4.0, 95% CI 1.0–14.3). All iron deficient anemic and iron deficient non-anemic women were recommended to intensify oral iron supplementation and new Hb and ferritin measurements after approximately four weeks. Among women with initial iron deficiency anemia, overall Hb concentration increased (102.6 vs. 107.9 g/L), and anemia resolved in 40.4%. In initially iron deficient non-anemic women, the overall Hb concentration remained normal, although it decreased (126.7 vs. 123.6 g/L), and 94.1% remained non-anemic. The prevalence of iron deficiency decreased, yet iron deficiency persisted in most women as ferritin remained below 30 ng/mL in 78.7% of the initially iron deficient anemic women and 67.8% of the initially iron deficient non-anemic women, respectively.

First trimester Hb and ferritin

First trimester iron status has previously been reported, including a Belgian study (Vandevijvere et al.) that found ferritin concentrations <15 ng/mL in 6.1% and the combination of ferritin <15 ng/mL and Hb <110 g/L in 1.9%[20]. In our cohort, the corresponding prevalences were 2.8% and 0.7%, respectively. Among pregnant women in Australia (Khambalia et al.) and the US (Mei et al.), 19.6% and 7.3% have first trimester ferritin <12 ng/mL, respectively[21, 22]. However, the prevalence of ferritin concentrations <30 ng/mL in non-anemic women in our cohort (14.2%) was similar to that in a recent US report including first trimester pregnant women (14% reported by Auerbach et al.)[23]. First trimester ferritin concentrations ≥70 ng/mL, which has been suggested to reflect the amount of body iron required to complete pregnancy without developing iron deficiency[21, 24], were present in 27.5% of the Belgian study population[20] versus 9.7% of women in Australia[21] and 41.2% of our Danish cohort. Risk factors for iron deficiency in pregnancy identified in some of these previous studies included multiparity, Non-White race-ethnicity, younger age and low socioeconomic status[20–22]. The proportion of multiparous women and women of Non-White race-ethnicity were lower in our population compared to the Belgian study and the US study by Mei et al.[20, 22]. In addition, approximately 14% of participants were 12–19 years old in the study by Mei et al.[22] whereas only six (0.01%) women in our study were aged 19 or younger. Furthermore, the first trimester samplings in studies conducted in countries with iron supplemental policies (i.e. Denmark and US)[10, 25] may more frequently have been influenced by iron supplement use than samplings in study settings without such guidelines (i.e. Belgium and Australia). Hence, parity, race-ethnicity, age, and probably also iron intake and socioeconomic status in our cohort was not comparable with that of previous studies[20–23], which may explain why some of our findings differ.

Hb and Ferritin changes after intensified iron supplementation

Guidelines suggest that Hb should increase by at least 10 or 20 g/L two or four weeks after initiation of oral iron supplementation, respectively[26–29]. Similar to our results, a previous comparative Danish trial has reported that concentrations of Hb and ferritin decrease in early pregnancy despite oral supplementation[30], although the average ferritin at baseline in this population was higher than our iron deficient subpopulation.

A Spanish study have previously explored how Hb and ferritin change in first trimester non-anemic women taking iron supplements[31]. All 41 initially iron depleted non-anemic women (defined as Hb ≥110 g/L and ferritin <12 ng/mL determined at 8–12 weeks' gestation) were recommended iron supplements. Despite supplementation (starting on average at 17 weeks' gestation), 81% were still iron depleted and 12% had developed anemia at 24 weeks' gestation[31]. Among women attending two blood samplings in our study, 30 were initially non-anemic with ferritin <12 ng/mL, of whom only 20% still had ferritin below 12 ng/mL and 13% had developed anemia at follow-up (data not shown). Compared to the Spanish study, the 30 women in our cohort were recommended iron supplementation from an earlier mean gestational age (from 11 vs. 17 weeks' gestation) in higher daily dosage (100 vs. 40 mg) and had the follow-up sampling performed at a lower mean gestational age (at 17 vs. 24 weeks' gestation). These differences may explain why we observed ferritin concentrations below 12 ng/mL at follow-up in only 20%.

First trimester Hb and ferritin and obstetric and perinatal outcomes

Our study examined the first trimester iron status and showed significant associations between non-anemic iron deficiency and stillbirth and between iron deficiency anemia and GDM. However, these findings should be interpreted with caution due to few events, wide CIs, and the fact that information on oral iron supplementation was limited to the recommended doses and not what was actually taken.

We are not aware of previous studies investigating the relationship between non-anemic iron deficiency and stillbirth, whereas low Hb in early pregnancy has been associated with an increased risk of perinatal death[32]. In our study, no stillbirths occurred among the iron deficient anemic women, and the overall incidence of stillbirth was low (12 of 5,479 babies). Hence, our stillbirth risk estimate is therefore imprecise.

The higher risk of GDM in women with iron deficiency anemia compared to iron replete non-anemic women was unexpected, as a previous review and meta-analysis conversely concluded that iron deficiency anemia lowers the risk of GDM[33]. In a previous Danish observational study, pregnant women who developed GDM had higher concentrations of first trimester ferritin than healthy pregnant controls, and ferritin was positively associated with GDM risk[34]. In the previously mentioned Australian cohort, first trimester iron deficiency (ferritin <12 ng/mL) was associated with a reduced risk of GDM[21]. Further previous reviews and meta-analyses have shown that elevated ferritin and Hb are associated with GDM, and that the average concentrations of ferritin and Hb are higher in women with GDM[35–37]. However, it should be noted that Hb and iron status were measured at heterogenous times during gestation in the reviewed studies[35–37], and that two of the reviews assessed but did not find an association between prenatal iron supplementation and GDM[35, 36]. The underlying pathophysiological mechanisms of abnormal iron status and GDM is not fully understood, and it remains unknown if the association can be explained by the iron status per se, oral iron exposure, or other factors. Our finding of increased risk of GDM with iron deficiency anemia may be explained by high exposure to iron supplements. However, we did not find a similar increased risk of GDM in the non-anemic iron deficient women (who were also expected to have a high exposure), which does not support this theory. Neither mean BMI nor prevalence of overweight were obviously different between the groups, and furthermore, our model adjusted for several relevant factors including BMI >25 kg/m² and race-ethnicity. The role of socioeconomic status, however, remains unknown.

Strengths and limitations

It is a strength that we assessed baseline Hb and ferritin in the first trimester, and that follow-up samples were measured with a narrow time window. Plasma volume expansion is a normal process in a healthy pregnancy and exceeds the increase in erythrocyte mass [5, 38–41], making differentiation between physiological and pathological anemia difficult as gestation proceeds. This may explain why previous studies have found the association between hematological markers and adverse outcomes more evident in early pregnancy[6], when the impact of the changing plasma dynamics on Hb and ferritin concentrations is minimal. Thus, determining Hb and iron status in early pregnancy is presumably more valid than later in pregnancy. Further strengths include the large sample size and that we were able to adjust for important potential confounders. However, with the observational design, we cannot investigate causality but only generate hypotheses of such. The lack of information about socioeconomic status is a limitation, as this factor may influence both hematological indices and several pregnancy outcomes. Furthermore, women were recommended individualized doses of oral iron supplementation, and no information on compliance was available. However, as compliance to iron policies among pregnant women in Denmark has been reported to be high[42], it is likely that most women in our cohort took iron supplements and that the exposure was higher in the iron deficient than in the iron replete women. Subsequently, associations may potentially be explained by the high exposure to iron supplementation in pregnancy rather than the first trimester iron status per se. On the other hand, the intensified supplementation in iron deficient women might improve their iron status and thereby dilute the true adverse effects of anemic and non-anemic iron deficiency. Lastly, data for the subgroup assessments of Hb and ferritin changes were short-term and affected by missing, as only 557 of the 890 eligible women attended a follow-up sampling.

Conclusions

In conclusion, we have assessed first trimester iron status in an unselected sample of singleton pregnant women. In comparison to iron replete non-anemic women, we found that women with iron deficiency anemia had a higher risk of GDM, and iron deficient non-anemic women had a higher risk of stillbirth, although risk estimates were imprecise due to few events, especially for stillbirth. We found a low prevalence of both anemic and non-anemic iron deficiency, and a high prevalence of ferritin levels estimated as sufficient to complete pregnancy without developing iron deficiency[21, 24]. With this new knowledge of the iron deficiency and repletion prevalence in pregnant women in Denmark we question if routine iron supplementation is the most beneficial approach to maternal and infant health and call for trials that compare short- and long-term effects of routine and individualized iron supplementation

Declarations

Authors' contributions

RH, ALS, VMS, CH, LK and AP were involved in the conception and design of the study. RH and VMS carried out data collection. RH and ALS performed data analyses. RH drafted the manuscript. All authors were involved in the interpretation of the data and reviewed and approved the final manuscript.

Funding

This study has been covered solely by internal funding.

Conflicts of interest / competing interests

When the study was conducted, Rebecka Hansen and Veronika Markova Sommer were investigators and Charlotte Holm primary investigator for a clinical trial sponsored by Pharmacosmos A/S. As investigators, Rebecka Hansen and Veronika Markova Sommer had salary costs funded by Pharmacosmos A/S.

Rebecka Hansen and Charlotte Holm and have served on advisory boards for Pharmacosmos A/S. However, Pharmacosmos A/S did not play any role or had any influence on this cohort study. Only the named authors had influence on the study design, conduct, analysis, interpretation and the manuscript. The authors have no additional conflicts of interest to declare.

Availability of data and material

Requests on data access can be made through contact with the corresponding author. Data may be accessed upon reasonable request and after review and approval by the Danish Data Protection Agency and the Danish Patient Safety Authority.

Code availability

The code (R) will be available from the corresponding author upon reasonable request.

Ethics approval

The study was approved by the Danish Data Protection Agency (J. No.: AHH-2017-031, I-suite number: 05349) and the Danish Patient Safety Authority (J. No.: 3-3013-2410/1). According to Danish legislation, informed written consent from patients is not required for this specific type of study. The reporting of the study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

We acknowledge and are thankful for the data management support from Steen Christian Rasmussen, MSc, MPH.

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Figures

Figure 1
Overview of local standard care screening procedure

Figure 2

Study flow

Supplementary Files

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