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Prevalence of iron deficiency states and risk of haemoconcentration during pregnancy according to initial iron stores and iron supplementation

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Abstract

Objective: To describe the prevalence of iron depletion (ID), iron-deficiency anaemia (IDA) and risk of haemoconcentration during pregnancy and at delivery and to assess the influence of initial Fe stores and Fe supplementation on that prevalence.

Design: Longitudinal study.

Setting: Hospital Universitari Sant Joan de Reus (Catalonia, Spain).

Subjects: Two hundred and eighty-five pregnant women. Serum ferritin and Hb were measured in the first, second and third trimesters and at delivery. Women were classified according to initial Fe stores as ID or no ID (serum ferritin $\geq 12\,\mu g/l$) and according to Fe supplement use as supplemented or non-supplemented.

Results: Initial ID was 16·2 %. At delivery, 45·7 % had ID, 13·5 % IDA and 13·3 % had risk of haemoconcentration. Initial ID and non-supplemented groups had significantly higher prevalences of ID and IDA and lower risk of haemoconcentration at delivery than the other groups. In the multiple logistic models, no initial ID and Fe supplementation exerted a protective effect against ID at delivery (adjusted OR = 0·28; 95 % CI 0·13, 0·58 and adjusted OR = 0·39; 95 % CI 0·22, 0·69, respectively). Moderate Fe supplementation did not seem to clearly prevent IDA (adjusted OR = 0·91; 95 % CI 0·42, 1·96) or to enhance the haemoconcentration (adjusted OR = 1·42; 95 % CI 0·58, 3·50).

Conclusions: The prevalence of ID and IDA was high in late pregnancy in healthy pregnant women, particularly in those with initial ID and/or those not taking supplements. Starting pregnancy with no ID and/or taking moderate Fe supplementation decreased the likelihood of ID at delivery. The risk of haemoconcentration was high at delivery, but did not seem to be promoted by Fe supplementation. Further research is necessary to determine the most appropriate nutritional advice for pregnant women.

Keywords
Pregnancy
Iron-deficiency anaemia
Iron depletion
Iron supplementation

Pregnant women are at special risk of Fe deficiency because Fe requirements increase during pregnancy and are difficult to cover by diet alone[1]. In addition, a significant percentage – between 11 and 36 % – of European women of childbearing age already have depleted Fe stores even before becoming pregnant[2]. As a result of this negative Fe balance, the prevalence of anaemia during pregnancy is about 25 % in Europe[3].

Although the prevalence of anaemia has been extensively evaluated in pregnant women in different countries, the WHO reports that data on the prevalence of Fe deficiency are scarce despite being considered the primary cause of anaemia[3]. Furthermore, there are also few data on the prevalence of risk of haemoconcentration, defined as Hb > 130 g/l by a recent Cochrane meta-analysis[4], which may be produced by excessive Fe supplementation.

However, both Fe deficiency and haemoconcentration during pregnancy are associated with adverse effects on the health of the mother and fetus[4–6].

Recently, some authors have described the importance of Fe stores in early pregnancy, suggesting that although high Fe stores might prevent Fe deficiency at the end of gestation[7,8], the risk of haemoconcentration might increase if pregnant women take Fe supplements[9]. On the other hand, other authors suggest that there is no relationship between Fe supplementation and this risk[6,10] and that women who are supplemented with Fe during pregnancy might present Fe deficiency less often than women who are not.

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Therefore, the present study aimed to assess the prevalence of Fe depletion, Fe-deficiency anaemia and risk of haemoconcentration in pregnant women in an industrialized country during each trimester of pregnancy and at delivery, and to describe the prevalence in relation to initial Fe stores of the mother and Fe supplementation.

Materials and methods

A longitudinal study was carried out with pregnant women through to delivery. Pregnant women were recruited at the Unit of Obstetrics and Gynaecology at the reference clinical centre of Hospital Universitari Sant Joan de Reus (Catalonia, Spain) during their first prenatal care appointment (gestational week 10). The volunteers admitted to the study signed an informed consent, according to the Declaration of Helsinki. The Sant Joan's Hospital ethics committee approved the study.

Inclusion criteria were as follows: Caucasian; over 18 years of age; and recruited between weeks 8 and 12 of gestation. Exclusion criteria were: the presence of any chronic illness or a possible inflammation diagnosed when high serum ferritin (SF) levels (SF $>$ 62 μg/l)[11] and low transferrin saturation (TS $<$ 16 %) occurred simultaneously; taking a higher Fe supplement dose than recommended by the obstetrician (>80 mg/d); and having a multiple pregnancy (twins or triplets).

From a total of 300 pregnant women recruited, twelve were excluded for taking Fe supplement doses over 80 mg/d and three had a possible inflammation that might have altered their biochemical parameters. The final study sample comprised 285 women. Data were collected over four visits and at delivery.

At the first appointment with obstetrics, around the 10th week of gestation, socio-economic status and BMI were recorded, data were collected from the medical history and venous blood was extracted for further laboratory analyses. During the second prenatal appointment, around weeks 13–15 of gestation, the obstetrician recommended Fe supplementation of between 40 and 80 mg/d to all the women and recorded whether they had taken any previously. At subsequent appointments, during the 24th and 34th weeks of gestation and at delivery, further blood samples were taken.

At the same time, using a semi-structured questionnaire designed by the researchers, a trained professional (not a member of the regular health-care staff, in order to not influence the responses of the women) recorded the initial and continuing supplementation month by month, as well as the number of days per week that the supplements were usually taken.

Medical record abstraction

The socio-economic level of the family was assessed using the Hollingshead index[12] and the results were grouped into three categories as 'low', 'medium' or 'high'.

BMI (kg/m^2) was calculated as weight in kilograms divided by the square of height in metres. The amount in milligrams of the total Fe supplementation was calculated as:

$$\text{Total Fe supplementation (mg)} = (\text{supplement Fe content} \times \text{days per week} \times \text{number of weeks}),$$

and the daily Fe supplementation in milligrams was calculated as:

$$\text{Daily Fe supplementation (mg)} = (\text{total Fe supplementation/number of days between the beginning of the supplementation until delivery}).$$

Infant birth weight was measured with a SECA electronic weighbridge (Vogel & Halke GmbH & Co., Hamburg, Germany) within an accuracy of 10 g.

Laboratory analyses

The assessments of SF, TS and Hb were used to estimate the different compartments of body Fe: Fe stores by SF, the circulating level by TS, and the contribution of Fe to the bone marrow by Hb.

From the blood samples, Hb values were immediately measured using a Coulter Gen-S analyser (Coulter, Hialeah, FL, USA). Plasma was processed and stored at −80°C at the Institut d'Investigació Sanitària Pere Virgili (IISPV) Biobanc in Reus (www.iispv.cat) until required for analysis. This allowed all of the samples to be analysed together using the same kit, thereby limiting the potential for bias in the values obtained.

SF was determined by turbidimetric immunoassay as described previously[13]; serum transferrin and serum Fe by spectrophotometry (Biokit SA, Barcelona, Spain and ITC Diagnostics SA, Barcelona, Spain) using standard clinical chemistry techniques.

TS was calculated as[14]:

$$\text{TS (\%)} = [\text{serum Fe (μmol/l)/serum transferrin (g/l)}] \times 3{\cdot}9.$$

Definitions

'Iron depletion' (ID) was defined as SF $<$ 12 μg/l and 'anaemia' as Hb values lower than 110 g/l in the first or third trimester and at delivery and Hb values lower than 105 g/l in the second trimester[15]. 'Iron-deficiency anaemia' (IDA) was defined as anaemia and SF $<$ 12 μg/l simultaneously, and 'risk of haemoconcentration' as Hb values higher than 130 g/l in the second or third trimester of gestation and at delivery[4].

'Inflammation' was defined as high SF levels[11] and low TS levels (defined as TS $<$ 16 %).

'Preterm' was defined as babies born before the 37th week of gestation and 'low birth weight' as babies weighing <2500 g.

Table 1 General, socio-economic and obstetric characteristics of the participants and their newborns; Reus, Catalonia, Spain (*n* 285)

	Mean	SD
Mothers		
Age (years)	31·1	4·4
BMI at the first visit (kg/m^2)	23·3	3·5
Smoker (%)	19·9	
Socio-economic status of the family (%)		
Low	9·4	
Medium	44·8	
High	45·9	
Primipara (%)	52·6	
Gestation length (weeks)	39·1	1·6
Newborns		
Gender (% male)	50·6	
Birth weight (g)	3217·7	439·8
Birth weight adjusted for gender and gestation week (g)	3201·1	250·8
Low birth weight (%)	5·5	
Preterm (%)	4·8	

Values are presented as means and standard deviations, or as percentages.

Women who took Fe supplements on fewer than 2 d/week were included in the non-supplemented group.

Statistical analyses

All statistical analyses were performed with the SPSS statistical software package version 19·0. Categorical data are presented as relative (%) frequencies. Continuous variables were checked for normality of distribution. All continuous variables were normally distributed, except for the SF values, and are presented as means and standard deviations. SF values were log-transformed and are presented as geometric mean and standard deviation. A Student's *t* test was used to compare continuous data and the two-proportion *Z*-test was used to compare categorical data. A two-way ANOVA was applied to detect an interaction between ID and Fe supplementation for continuous variables. When the interaction was significant, *post hoc* pairwise comparisons with *P* values corrected by the Bonferroni method were performed. The magnitude of the association between no initial ID and daily Fe supplementation with ID, IDA or Hb $>$ 130 g/l at delivery was evaluated using multiple logistic regression analyses. First, we ran three models to assess simultaneously the effect of no initial ID and Fe supplementation (independent variables) on each of the dependent variables: ID, IDA and Hb $>$ 130 g/l at delivery. Subsequently the previous models were adjusted for the age of the mother, parity, tobacco use, gestational age and birth weight. In all cases, the level of significance was set at $P < 0{\cdot}05$.

Table 2 Prevalence of iron deficiency states and risk of haemoconcentration in pregnant women; Reus, Catalonia, Spain (*n* 285)

Baseline characteristics	
Hb 10th week (g/l)	
Mean	125·7
SD	8·2
SF 10th week (μg/l)*	
Mean	27·9
SD	2·3
ID (%)	
1st trimester	16·2[a]
2nd trimester	52·3[b]
3rd trimester	66·1[c]
Delivery	45·7[d]
Anaemia (%)	
1st trimester (Hb $<$ 110 g/l)	3·2[a]
2nd trimester (Hb $<$ 105 g/l)	12·9[b]
3rd trimester (Hb $<$ 110 g/l)	27·5[c]
Delivery (Hb $<$ 110 g/l)	18·8[d]
IDA (%)	
1st trimester	1·0[a]
2nd trimester	9·6[b]
3rd trimester	21·5[c]
Delivery	13·5[d]
Hb $>$ 130 g/l (%)	
2nd trimester	1·9[a]
3rd trimester	4·2[b]
Delivery	13·3[c]

SF, serum ferritin; ID, iron depletion; IDA, iron-deficiency anaemia.
ID was defined as SF $<$ 12 μg/l; IDA was defined as anaemia and ID simultaneously; risk of haemoconcentration was defined as Hb $>$ 130 g/l.
Values are presented as means and standard deviations, or as percentages.
[a,b,c,d]Proportions within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
*Geometric mean (antilog SD).

Results

Table 1 describes the general, obstetric and socio-economic characteristics of the group of healthy pregnant women who participated in the study, as well as the characteristics of their newborns. Table 2 shows the prevalence of Fe deficiency states and of risk of haemoconcentration by trimester of gestation in the women. The prevalence of all Fe deficiency states measured in the study increased as pregnancy progressed and decreased at delivery, when haemodilution reduces, except in the case of haemoconcentration, which continued to increase until delivery.

Table 3 Prevalence of iron deficiency states and risk of haemoconcentration as a function of initial iron stores and iron supplementation in pregnant women; Reus, Catalonia, Spain (*n* 285)

	Initial Fe stores					Fe supplementation					
	No ID (*n* 237)		ID (*n* 48)			Yes (*n* 209)		No (*n* 76)			
	Mean	SD	Mean	SD	*P* value	Mean	SD	Mean	SD	*P* value	*P* for interaction
General characteristics											
Hb 10th week (g/l)	126·5	7·7	123·5	9·2	0·016	125·1	8·1	127·6	8·2	0·015	0·224
SF 10th week (μg/l)*	36·3	1·8	7·1	1·6	<0·001	27·3	2·3	28·1	2·3	0·809	0·667
Starting week of supplementation	14·3	9·9	13·5	9·3	0·588	18·3	7·2	–	–	<0·001	0·110
Daily Fe supplementation (mg)	44·2	34·4	36·7	30·3	0·158	57·8	27·7	–	–	<0·001	0·245
Total Fe supplementation (mg)	6321	5088	5543	4710	0·323	8367	4313	–	–	<0·001	0·340
Infant birth weight (g)	3237	441·3	3132	387·3	0·130	3231	431·4	3179	462·5	0·348	0·361
	%		%			%		%			
ID (%)											
1st trimester	0·0[a]		100·0[a]		<0·001	14·9[a]		19·8[a]		0·380	
2nd trimester	46·4[b]		83·3[b]		<0·001	50·0[b]		58·8[b]		0·267	0·326
3rd trimester	63·9[c]		81·3[b,c]		0·019	62·1[c]		77·5[c]		0·026	0·231
Delivery	40·9[b]		70·8[c]			40·2[d]		61·3[b]			0·058
Anaemia (%)											
1st trimester (Hb < 110 g/l)	2·0[a]		6·3[a]		0·123	3·5[a]		2·4[a]		0·910	0·799
2nd trimester (Hb < 105 g/l)	11·7[b]		20·8[b]		0·087	13·3[b]		11·9[b]		0·962	0·977
3rd trimester (Hb < 110 g/l)	25·8[c]		39·6[c]		0·052	27·0[c]		28·9[c]		0·789	0·850
Delivery (Hb < 110 g/l)	16·5[b]		31·3[b,c]		0·017	16·8[b]		24·1[b]		0·176	0·907
IDA (%)											
1st trimester	0·0[a]		6·3[a]		0·004	0·9[a]		1·2[a]		0·697	1
2nd trimester	7·9[b]		20·8[b]		0·015	9·6[b]		9·5[b]		0·839	0·957
3rd trimester	19·5[c]		35·4[c]		0·022	19·3[c]		27·4[c]		0·141	0·708
Delivery	11·9[b]		25·0[b]		0·017	12·7[b]		15·5[c]		0·613	0·326
Hb > 130 g/l (%)											
2nd trimester	1·6[a]		4·2[a]		0·252	1·8[a]		2·4[a,b]		0·931	0·640
3rd trimester	4·8[b]		2·1[a]		0·701	5·3[b]		1·2[a]		0·210	0·997
Delivery	16·1[c]		2·1[a]		0·010	15·0[c]		8·4[b]		0·198	0·990

SF, serum ferritin; ID, iron depletion; IDA, iron-deficiency anaemia.
ID was defined as SF < 12 μg/l; IDA was defined as anaemia and ID simultaneously; risk of haemoconcentration was defined as Hb > 130 g/l.
Values are presented as means and standard deviations, or as percentages.
[a,b,c,d]Proportions within a column with unlike superscript letters were significantly different ($P < 0·05$).
*Geometric mean (antilog SD).

Table 3 shows the baseline characteristics of the participants according to their initial Fe stores and whether or not they took Fe supplements. It also shows the prevalence of ID, IDA and risk of haemoconcentration by trimester of gestation and at delivery.

After checking if there was any interaction between initial ID and Fe supplementation, we observed only a slight interaction regarding the prevalence of ID at delivery ($P = 0·058$). It appeared that women with no initial ID who were taking Fe supplements presented a significantly lower prevalence of ID at delivery than women with initial ID who were taking supplements or women with no initial ID who were not taking supplements (34·0% *v.* 72·7% and 60·9%, respectively). Except in the case previously mentioned, we present the main effects of ID and Fe supplementation.

Regarding initial Fe stores of the pregnant women, it was shown that a greater number of women with no initial ID did not have anaemia or IDA at delivery compared with women initially with ID, although they were more likely to be at risk of haemoconcentration.

From the group with no ID, seventy-six women (30·0%) had initial Hb higher than 130 g/l. Women with Hb > 130 g/l at early pregnancy who took Fe supplements had a significantly higher prevalence of risk of haemoconcentration at delivery than women who were not supplemented with Fe (30·0% *v.* 7·7%; $P = 0·027$).

During pregnancy, 78·6% (*n* 224) took Fe supplements. Among them, 6·7% took the supplements on 1–2 d/week and were therefore classified as non-supplemented (*n* 15); 16·1% took them on 5 d/week and the remaining 77·5% took them on 6–7 d/week.

Women who took Fe supplements had a significantly lower prevalence of ID at delivery than women who did not (40·2% *v.* 61·3%, $P = 0·003$), but had a higher prevalence of risk of haemoconcentration, although not statistically significant (15·6% *v.* 8·4%, $P = 0·198$).

Table 4 describes the risk of ID, IDA and Hb > 130 g/l at delivery as a function of the initial Fe stores and Fe supplementation. We can appreciate that no ID in the first trimester and Fe supplementation protected against ID at delivery even after adjusting for other variables. Regarding the risk of IDA at delivery, no ID in the first trimester exerted a protective effect, although this relationship was not significant when adjusted for other variables. On the other hand, the risk of haemoconcentration at

Table 4 Risk of iron deficiency states and haemoconcentration at delivery as a function of initial iron depletion and iron supplementation in pregnant women; Reus, Catalonia, Spain (*n* 285)

	ID at delivery			IDA at delivery			Hb > 130 g/l at delivery		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Model 1									
Initial ID									
Yes	1·00	Ref.		1·00	Ref.		1·00	Ref.	
No	0·28	0·14, 0·57	<0·001	0·41	0·19, 0·88	0·019	8·85	1·18, 66·1	0·034
Fe supplementation									
No	1·00	Ref.		1·00	Ref.		1·00	Ref.	
Yes	0·41	0·24, 0·70	0·001	0·80	0·39, 1·64	0·536	2·98	1·05, 8·99	0·040
Model 2									
Initial ID									
Yes	1·00	Ref.		1·00	Ref.		1·00	Ref.	
No	0·28	0·13, 0·58	0·001	0·49	0·22, 1·10	0·083	7·34	0·97, 55·6	0·054
Fe supplementation									
No	1·00	Ref.		1·00	Ref.		1·00	Ref.	
Yes	0·39	0·22, 0·69	0·004	0·91	0·42, 1·96	0·810	1·42	0·58, 3·50	0·442
Age of the mother (years)	0·98	0·92, 1·04	0·425	1·02	0·94, 1·11	0·610	0·95	0·87, 1·04	0·271
Parity									
Primipara	1·00	Ref.		1·00	Ref.		1·00	Ref.	
Multipara	1·21	0·71, 2·06	0·484	1·47	0·68, 3·18	0·330	0·60	0·28, 1·30	0·197
Smoker									
No	1·00	Ref.		1·00	Ref.		1·00	Ref.	
Yes	0·56	0·30, 1·05	0·073	0·67	0·26, 1·70	0·401	1·99	0·91, 4·36	0·086
Gestational age (weeks)	0·79	0·66, 0·95	0·010	0·57	0·44, 0·74	<0·001	1·24	0·95, 1·61	0·109

ID, iron depletion; IDA, iron-deficiency anaemia; Ref., reference category; SF, serum ferritin.
ID was defined as SF < 12 μg/l; IDA was defined as anaemia (Hb < 110 g/l) and ID simultaneously; risk of haemoconcentration was defined as Hb > 130 g/l.

delivery increased with no ID in the first trimester and/or Fe supplementation in the non-adjusted model. Once the model was adjusted, this effect remained the same only for no ID at early gestation.

Discussion

The present study described a high prevalence of ID and IDA in late pregnancy, adding to the few data that exist on healthy women from the developed countries in southern Europe. The prevalence was higher when women started pregnancy with their Fe levels already depleted and/or they did not take Fe supplements during gestation. The prevalence of risk of haemoconcentration at delivery was also high but Fe supplementation did not seem to enhance it.

All women in the study were volunteers and therefore are not necessarily representative of our whole population, but all of them were healthy (no obstetric pathology), Caucasian and had similar socio-economic status and smoking habits to the rest of our society and to other industrialized countries[16–19]. The average infant birth weight and the percentage of babies born preterm or with low birth weight were also similar to those found in other industrialized countries[16,17].

SF is considered to be the best biochemical parameter for monitoring a deficient Fe status in pregnancy in the absence of infection or inflammation because it correctly identifies the women without Fe stores[20]. However, a known limitation of SF is that it also increases with acute or chronic inflammation, malignancy or liver disease[21]. As TS does not increase in the presence of inflammation[21], we used both parameters in order to detect inconsistent values (high SF and low TS) that may hide a possible inflammation with Fe deficiency, as suggested by some authors[22,23].

Our results describe the unfavourable evolution of ID, IDA and risk of haemoconcentration during pregnancy.

Frequency of iron depletion (serum ferritin <12 μg/l)

The percentage of pregnant women who began gestation with ID (16·2%) was similar to that found in non-pregnant women in European countries[2,24] and to the great majority of studies conducted in pregnant women of industrialized countries, including at the end of pregnancy when that prevalence is higher[20,25,26], confirming the negative trend in Fe status of pregnant women in developed countries. Regarding ID at delivery, there was a slight decrease in its prevalence, possibly related to the fact that haemodilution almost disappears at this moment in the pregnancy[27]; consequently the real levels of SF in this moment are better shown.

Frequency of iron-deficiency anaemia

The negative evolution in Fe stores during pregnancy also leads to a negative evolution in the levels of Hb, increasing the prevalence of anaemia. Frequently, the term 'anaemia' has been used as an approximation of IDA[28], which would overestimate its prevalence. Indeed, this relationship between anaemia and IDA can be seen in our

study, where there are about 30 % more cases of anaemia than of IDA, in agreement with other studies[7,26].

Our results at the end of pregnancy are similar to those published by the WHO in its global database on anaemia[3] and also to the results from other European studies[9,29–31], confirming that Fe deficiency is elevated even in industrialized countries. However, the WHO itself indicates that the data provided include values measured at different moments of gestation, suggesting that estimates in the report[3] are not very accurate, something that we have improved in our study.

Frequency of risk of haemoconcentration

Haemoconcentration may be caused by an inadequate plasma volume expansion and can be confused with relatively high values of Hb due to a good Fe status. Regardless of its cause, a Cochrane meta-analysis used the cut-off value of Hb > 130 g/l to define the risk of haemoconcentration[4]; in the present study we decided to use the same cut-off value as we were not able to determine the reason for high Hb levels.

As far as we are concerned, even if the previous meta-analysis states that the clinical significance of haemoconcentration remains uncertain, some recent studies have found an association of high Hb levels with poor outcomes such as low birth weight or intra-uterine growth retardation[30,32,33]. Recently, our research team also analysed this association and found that haemoconcentration significantly increases the risk of low birth weight (adjusted OR = 11·48; 95 % CI 1·13, 116·6)[33]. Therefore, it is important to establish the percentage of pregnant women who may be at risk.

In the present study, we observed that risk of haemoconcentration increased during pregnancy, from 1·9 % to 13·3 % at delivery. In the previously mentioned Cochrane meta-analysis, the prevalence of this risk during the second or third trimester of gestation described for developed countries ranges from 8·7 % in Finland to 42 % in Norway[4], but with no data available from southern Europe. Our study can therefore provide some data for this part of Europe. The high prevalence of risk of haemoconcentration found in some of these studies might be due to only selecting women who were not anaemic at the beginning of their pregnancy and who therefore had higher values of Hb than in our study.

Importance of initial iron stores and of iron supplementation

As in other studies, we observed that the prevalence of ID and IDA was more common when women begin pregnancy with ID and when they are non-supplemented, and in this situation the risk of haemoconcentration was lower[4,8,34].

In order to further study the differences observed in the prevalence caused by these two risk factors (initial Fe stores and Fe supplementation), we assessed the probability of having ID, IDA and Hb > 130 g/l at delivery using multiple logistic regression analyses that allowed the separation of the effects of each risk factor, each taking into account the other, and also some other variables that may be associated with the studied relationship[35].

Regarding the prevention of ID and IDA, we observed that initially good Fe stores protected against ID and IDA at delivery, although in the case of IDA, we only observed a protective trend that was not statistically significant. This suggests the importance of starting pregnancy with good Fe stores in order to cover any increased Fe needs and to avoid Fe deficiency, reaffirming the findings of Milman *et al.* in a study conducted on 301 healthy, pregnant Danish women[8].

We also found that Fe supplementation (57·8 mg/d on average) exerted a protective effect against ID at delivery, but did not have a clear, observable effect on IDA. Similar results were obtained in a previous study conducted by Ekstrom *et al.*, who reported that a total dose of 2400 mg Fe consumed (30 mg/d) had no additional benefit in increasing Hb levels when the supplementation started late and lasted around 12 weeks[10]. We observed the same lack of effect even when supplementing with higher total doses of Fe of about 8367 mg (57·8 mg/d) and for a longer period (about 22 weeks). High doses of Fe supplementation protect against Fe deficiency states in a great proportion of women, but it has been associated with oxidative stress and haemoconcentration[4,30,31]. Consequently, recent recommendations suggest using doses of Fe lower than 60 mg/d[4,36]. These low or moderate Fe doses taken during this limited number of weeks might be enough to improve Fe stores, but should be taken for a longer period of time to increase significantly the Hb levels at the end of pregnancy. In this situation, we emphasize the need for individualizing Fe prescriptions, although more randomized clinical trials are required.

Concerning the risk of haemoconcentration, it appeared to be related to no initial ID, but we did not observe any association with the moderate Fe supplementation taken in the present study, once adjusted for no initial ID and for some other variables related with the studied relationship.

Currently, there is no clear relationship between Fe supplementation and the risk of haemoconcentration at the end of pregnancy. While on the one hand, the Cochrane meta-analysis indicates that the risk of haemoconcentration is more common among women who receive daily Fe supplements than among those who receive no treatment or take placebo[4], some studies do not observe this relationship and suggest that, in healthy pregnant women, Fe supplementation cannot increase Hb levels beyond what is optimal for a given person[6,10]. However, none of these studies took into account the initial Fe levels of pregnant women as suggested by Milman *et al.* In their study they assessed the effectiveness of different Fe supplementation doses (20, 40, 60, 80 mg/d) according to the initial Fe stores, suggesting that women

having SF ≤ 30 μg/l at early pregnancy, as in our study, should take 80–100 mg ferrous Fe/d, while women with higher initial Fe stores could take less Fe supplementation and still prevent Fe deficiency states[8]. Therefore, women with high Fe levels at early pregnancy may be at increased risk of haemoconcentration if they receive a higher Fe supplementation dose.

There might be other risk factors that favour haemoconcentration in pregnant women. In our opinion, the genetic alterations in the gene *HFE*, which increases the absorption of dietary Fe[37], could be associated with higher initial Hb levels[38]. Therefore, we believe that daily Fe supplementation, which is routinely recommended in pregnancy, may be unnecessary for women with this genetic alteration.

However, it is not clearly known what implications other factors can have, such as initial Fe stores and the genetic alterations of women, in the assessment of the effect of Fe supplementation in anaemic and non-anaemic pregnant women. Therefore, it is unlikely that the same Fe supplementation pattern is optimal for all pregnant women because each one could have different characteristics regarding their genetic and initial Fe levels.

Conclusion

In the present study we observed that 45·7 % of pregnant women reached delivery with ID, 13·5 % with IDA and a 13·3 % at risk of haemoconcentration. The prevalence of ID and IDA almost doubled and the prevalence of risk of haemoconcentration decreased among women who started pregnancy with ID and among those who did not take Fe supplements.

Therefore, starting pregnancy with no ID and/or taking moderate Fe supplementation decreases the likelihood of ID at the end of pregnancy, although it does not appear to be enough to prevent IDA in a large percentage of women. It is unclear whether the effects of Fe supplementation enhance haemoconcentration.

Further studies are necessary to determine the most appropriate nutritional advice that can be tailored to the needs of pregnant women according to their individual characteristics.

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