Intravenous versus oral iron for iron deficiency anaemia in pregnant Nigerian women (IVON): a randomized controlled trial

Clinical Study Report

Trial Name	Intravenous versus oral iron for iron deficiency anaemia in pregnant Nigerian women (IVON): a randomized controlled trial
EudraCT Number:	XXXXX
ISRCTN Number /Clinical trials.gov Number:	XXXXX
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1 Synopsis

Anaemia in pregnancy is common in low- and middle-income countries (LMICs) including Nigeria, with life-threatening maternal and infant complications. Maternal anaemia is typically treated with oral iron, which has poor tolerance and compliance. Intravenous iron has the potential to be a more efficacious alternative therapy, with fewer adverse events, and minimal patient-facility contact. We therefore conducted an open-label randomized controlled trial to assess the effect of intravenous iron, compared to oral iron, among pregnant women in Nigeria.

The primary endpoints were the prevalence of maternal anaemia (<11 g/dl) at 36 weeks' gestation and the incidence of preterm birth. Important secondary objectives were the prevalence of maternal iron deficiency (serum ferritin <30 µg/dL), iron deficiency anaemia (serum ferritin <30 µg/dl and haemoglobin <11g/dl), and depression using the validated Edinburgh Postnatal Depression Scale (EPDS).

1,056 women were individually randomized in a 1:1 ratio to a single dose of 20mg/kg IV ferric carboxymaltose (not exceeding 1000mg) or daily 200mg (65mg elemental iron) thrice daily oral ferrous sulphate. The study was conducted at five health facilities in each of Kano and Lagos states, the two largest states in Nigeria.

To be eligible for inclusion, participants were pregnant women aged 15 to 49 years with haemoglobin <10g/dl. Participants with medically confirmed significant bleeding or blood transfusion in preceding three months, severe symptomatic anaemia or anaemia of other known causes were excluded.

The main analyses were by intention to treat (ITT). Continuous outcomes were summarized using the n (non-missing sample size), mean and standard deviation (SD) while categorical outcomes were summarized using the n, frequency and percent of observed levels. Linear and log-binomial regression models were used to assess the treatment effect on continuous and categorical outcomes respectively. In subgroup analysis, the extent to which the treatment effect differed in subgroups of women who were iron-deficient (vs. not iron-deficient) at enrolment was evaluated.

Baseline characteristics did not differ by treatment arm. The majority of the participants had previously had 0 – 2 pregnancies born alive or carried beyond the age of viability. The gestational age at enrolment was 25 weeks, on average, and ranged from 16 – 32 weeks. Most (81%) completed at least secondary education and were in the lower socioeconomic status (66%).

The mean haemoglobin at enrolment was 9.2 g/dl (SD: 0.7). Most participants had moderate anaemia (98%). The prevalence of iron deficiency was 39.8% at enrolment.

The treatment effect of IV vs. oral iron on the prevalence of maternal at 36 weeks (*P*-value = 0.36) and the incidence of preterm delivery (*P*-value = 0.66) did not significantly differ. The prevalence of maternal anaemia at 36 weeks was 57.8% in the IV arm and 60.6% in the oral arm. The incidence of preterm delivery was 14.1% in the IV arm and 15.0% in the oral arm.

The treatment effect of IV vs. oral iron on the incidence of preterm delivery varied by state (*P*-value = 0.018). While IV iron reduced the incidence of preterm deliveries in Lagos (RR=0.62, 95% CI: 0.38 – 0.98), it had no significant effect in Kano (RR=1.28; 95% CI: 0.86 – 1.91). The treatment effect on the prevalence of maternal anaemia did not significantly vary. Facility type (primary, secondary or tertiary) did not significantly modify the treatment effect of either primary endpoint.

IV iron reduced the risk of iron deficiency (ID) by 73% (95% CI: 58% – 83%) and iron deficiency anaemia (IDA) by 78% (95% CI: 58% – 88%), compared to the oral arm. The prevalence of ID at 36 weeks was 4.5% in the IV arm and 16.4% in the oral arm. The prevalence of IDA at 36 weeks was 2.1% in the IV arm and 9.6% in the oral arm.

The prevalence of maternal depression was 5.7% in the IV arm and 4.5% in the oral arm. There was no difference in the effect of IV and oral iron on the prevalence of maternal depression (*P*-value = 0.40).

The mean (±SE) maternal haemoglobin concentration four weeks post-enrollment was 10.4 (±0.04) in the IV arm and 10.2 (±0.04) in the oral arm. The increase in maternal haemoglobin concentration was significantly greater in the IV arm than in the oral arm (*P*-value = 0.003), though the magnitude of the difference seems minimal. In the subgroup of women who were iron-deficient at baseline, the increase in haemoglobin in the IV arm was slightly steeper than in the oral arm (*P*-value = 0.008).

The other outcomes evaluated were moderate to severe anaemia, postpartum haemorrhage, need for blood transfusion, low birthweight, small-for-gestational age (SGA), stillbirth, neonatal death, breastfeeding and vaccine up-to-date. The effect of IV and oral iron on the other outcomes did not significantly differ. There were also no significant differences in the subgroup analyses.

In subgroup analyses, IV iron (vs. oral) was significantly more effective to prevent maternal anaemia in the IDA subgroup, when IDA at enrolment was defined using ferritin <30µg/L. IV iron (vs. oral) was also more effective to prevent moderate to severe anaemia and ID in the IDA subgroup when IDA at enrolment was defined using ferritin <15µg/L. These effects were not seen in the NIDA subgroup.

There were 47 adverse event reports in 41 participants. Individuals in the IV arm were 2.14-times (95% CI: 1.22, 4.78) more likely to experience adverse events. The most common adverse event was mild hypotension occurring during the enrolment visit after administering IV iron (n=22). Three serious adverse events were identified: diarrhea (n=1), hypertension (n=1) and postpartum haemorrhage (n=1). The proportion of participants who experienced hypophosphataemia during follow-up (after 4 weeks post-enrollment) was 10.7% in the IV arm and 1.0% in the oral arm. The risk of hypophosphataemia during follow-up was 10.4-times (95% CI: 4.63, 29.6) in the IV arm compared to the oral arm.

In conclusion, the effect of IV iron on the risk of maternal anaemia and preterm birth did not significantly differ. IV iron had a greater effect to reduce the risk of ID and IDA than oral iron in

the overall population, and a greater response to prevent maternal anaemia and ID among those with IDA at baseline. Hypophosphataemia was a safety concern in the IV iron arm.

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List of Tables and Figures

3 List of Abbreviations

Acronym	Definition
AE	Adverse Events

AIP	Anaemia in pregnancy
ANC	Antenatal care
CI	Confidence interval
CTCAE	Common Terminology Criteria for Adverse Events
EGA	Estimated gestational age
EPDS	Edinburgh Postnatal Depression Scale
FCM	ferric carboxymaltose
FS	Ferrous sulphate
GCP	Good Clinical Practice
HIV	human immunodeficiency virus
ID	Iron deficiency
IDA	Iron deficiency anaemia
ITT	intention to treat
IV	Intravenous
LMIC	Low- and middle-income countries
MD	Mean difference
MIC	Minimally important change
mITT	modified intention-to-treat
NIDA	non-iron deficiency anaemia
RR	Risk ratio or relative risk
SAE	Serious adverse events
SAP	Statistical Analysis Plan
SD	standard deviation
SE	Standard error
SES	Socioeconomic status
SGA	small-for-gestational age

4 Ethics

4.1 Institutional Review Board

Ethical approval was obtained from the Health Research and Ethics committees of the Lagos University Teaching Hospital, Aminu Kano Teaching Hospital, Lagos State Health Service Commission and the Kano State Ministry of Health.

4.2 Ethical Conduct of the Study

This trial was registered with the ISRCTN Registry (ID: 63484804) and on ClinicalTrials.gov (ID: NCT04976179). All study investigators and site coordinators were trained and certified in Good Clinical Practice (GCP) and research ethics. All research staff will be adequately trained to monitor, recognize and manage any significant AEs.

4.3 Participant Information and Consent

At the screening visit, pregnant women presenting to antenatal care clinics were approached by research nurses, counselled on anaemia in pregnancy, informed about the IVON trial, and assessed for eligibility.

Participants who met all eligibility criteria were further counselled about the study, including its risks, benefits and what is expected as a participant, as part of the informed consent process. Potential participants then completed the informed consent forms immediately or after discussing with their families.

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6 Introduction

6.1 Background

Anaemia in pregnancy (AIP) is common in many low- and middle- income countries (LMICs) including Nigeria(1,2). It leads to substantial or life-threatening maternal and infant complications which can potentially be prevented if anaemia is promptly and adequately treated(3,4). Iron deficiency is the commonest cause of AIP(1); in LMICs, its treatment is typically by oral iron(5), which is often-poorly tolerated and not fully complied with(6).

Intravenous iron requires minimal patient-facility contact and corrects anaemia much faster than oral preparations(7,8). Recent intravenous iron preparations have been found to be well tolerated and with fewer adverse effects than the previously available high molecular weight iron dextrans(9).

In the Nigerian setting, pregnant women seek antenatal care late, and have poor antenatal care (ANC) clinic attendance(10). Thus, the use of a minimally dosed iron formulation that is safe, rapidly effective, and cost-effective can improve the likelihood of prompt and appropriate IDA treatment and potentially reduce the risk of complications(11).

Prior evidence has not shown oral iron to be impactful for important clinical outcomes such as low birthweight and preterm delivery; thus, intravenous iron may be more effective in this regard. Findings from this study could potentially protect significant proportions of pregnant women and neonates in LMICs from severe morbidity and mortality.

7 Study Objectives and Endpoints

7.1 Primary Objective

To determine the effect of intravenous ferric carboxymaltose on the prevalence of maternal anaemia (<11 g/dl) at 36 weeks' gestation and the incidence of preterm births after administration compared with oral ferrous sulphate in pregnant women with iron deficiency anaemia.

7.2 Important Secondary Objectives

To assess:

1. The prevalence of maternal iron deficiency (ID) and iron deficiency anaemia (IDA) at 36 weeks' gestation
2. Incidence of depression linked to emotional well-being of mothers using the validated Edinburgh Postnatal Depression Scale (EPDS).

7.3 Other Secondary Objectives

To assess:

3. Increase in maternal haemoglobin levels at 4 weeks post-initiation of treatment.
4. prevalence of maternal moderate to severe anaemia (<10 g/dl) at 36 weeks' gestation
5. The safety and tolerability of intravenous ferric carboxymaltose versus oral ferrous sulphate, including the incidence of hypophosphatemia and severity of maternal adverse effects.
6. Severe maternal events, specifically, postpartum haemorrhage, sepsis, shock, and the need for blood transfusion.
7. The incidence of
    a. low infant birthweight (<2.5 kg),
    b. stillbirth and,
    c. neonatal mortality (birth till 28 days of life),
8. Proportion of infants
    a. being breastfed at 2 and 6 weeks of life, and
    b. having received vaccines up-to-date (BCG, oral polio and hepatitis vaccination) in same time period.
9. The incidence of small for gestational age (birthweight less than the 10th percentile for gestational age).

8 Investigational Plan

8.1 General Study Design and Plan

Multicenter, parallel, open label individually randomized controlled trial, with 1,056 women allocated in a 1:1 ratio in conjunction with a cost-effectiveness analysis.

Single dose of 20mg/kg IV ferric carboxymaltose (not exceeding 1000mg). Intravenous route

Daily administration of 200mg (65mg elemental iron) thrice daily oral ferrous sulphate. Oral route.

The study was conducted at health facilities in Kano and Lagos states, the two largest states in Nigeria. Five study sites (one tertiary, two secondary, and two primary health facilities) were selected per state, for a total of 10 sites (**Figure 1**). To be considered for inclusion, health facilities had to be publicly funded, with antenatal clinic attendance of at least 60 pregnant women per month, delivery rate of at least 20 women per month, consistent 24-h routine vaginal delivery services and onsite testing for haemoglobin, human immunodeficiency virus (HIV) and malaria.

Detailed description of the protocol has been published(11).

8.1.1 Screening/Baseline Visit

At the screening visit, pregnant women presenting to antenatal care clinics were approached by research nurses, counselled on anaemia in pregnancy, informed about the IVON trial, and assessed for eligibility based on the selection criteria in section 8.3. Consenting pregnant women were screened for haemoglobin by finger prick using the Hemocue® haemoglobinometer.

Those with haemoglobin < 10g/dl who met other eligibility criteria were further counselled about the study, including its risks, benefits and what is expected as a participant, as part of the informed consent process. Potential participants then completed the informed consent forms innediately or afer discussing with their families.

8.1.2 Treatment Duration

Participants in the oral arm received the oral iron daily from enrolment until six weeks postpartum. Participants in the IV arm received the IV iron at enrolment into the study.

8.1.3 Follow-up

Participants were seen in clinic every 4 weeks till 28 weeks' gestation and every 2 weeks until 36 weeks, then weekly until delivery. The final study visit occurred at 6 weeks' postpartum.

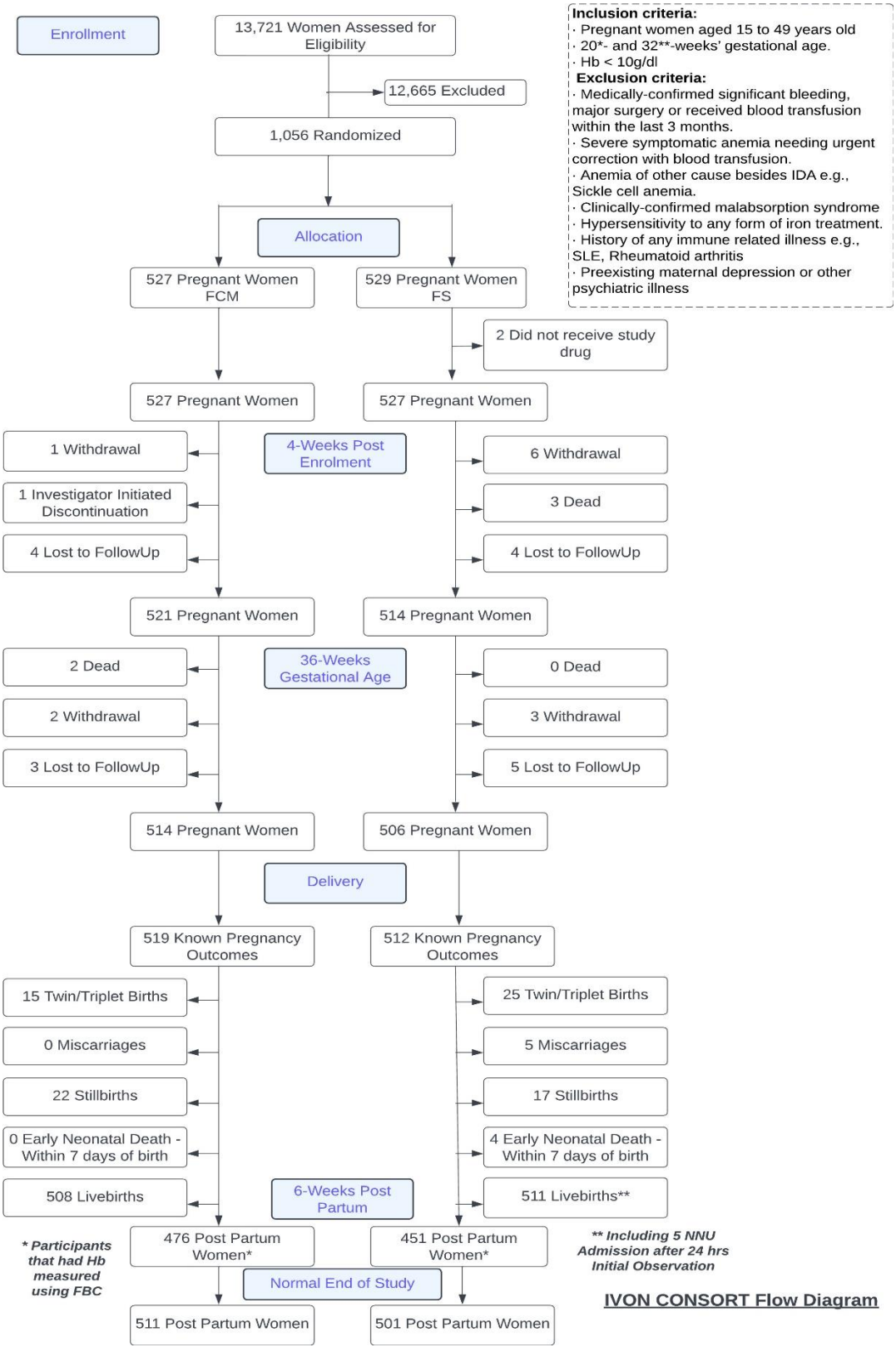

Figure 1. Study design flowchart

8.2 Discussion of Study Design (Including Changes in Study Design and choice of control groups)

There were no changes to the study design or choice of control groups.

8.3 Treatments

8.3.1 Treatments Administered

In addition to study drugs, participants received other routine medication (malaria prophylaxis, tetanus toxoid prophylaxis and folic acid supplementation) per standard of care.

Table 1. Schedule of treatments

Dose Group	No. Participants	Dose	Route	Day 0	Daily
Ferrous carboxymaltose (Ferinject®, FCM)	527	20 mg/kg	IV	Yes	
Ferrous sulphate (Fesulf®, FS)	529	200 mg	Oral	Yes	Yes
Folic acid	1056	5 mg	Oral	Yes	Yes
Vitamin C	1056	100 mg	Oral	Yes	Yes

8.3.2 Investigational Product(s)

The investigational products of interest were IV ferric carboxymaltse (FCM) and oral ferrous sulphate (FS). FCM was administered as a single dose of 20 mg/kg up to a maximum of 1000 mg diluted in 200 ml of 0.9% sodium chloride and infused over 15 – 20 min.

FS was given as one 200-mg tablet containing 65 mg of elemental iron, three times daily, to be taken 1 h before meals or 2 h after meals until 6 weeks postpartum.

8.4 Selection of Study Population

8.4.1 Inclusion criteria

Only participants who met the following inclusion criteria were eligible for enrolment in the study.

- Pregnant women aged 15 to 49 years old between 20- and 32-weeks' gestational age
    - 20 weeks was chosen as lower limit because Nigerian women register for ANC care in the second trimester, typically at 20 weeks or later.
    - 32 weeks as the upper limit to enable assessment of impact of both intervention and standard of care on perinatal events by evaluating their haemoglobin concentration by 36 weeks.
- Baseline (enrolment) laboratory-confirmed moderate or severe anaemia (Hb < 10g/dl).

8.4.2 Exclusion criteria

Any participants who met the following inclusion criteria were could not participate in the study.

- Medically confirmed significant bleeding, major surgery or received blood transfusion within the last 3 months.

- Severe symptomatic anaemia needing urgent correction with blood transfusion.
- Anaemia of other known causes besides IDA e.g., sickle cell anaemia, thalassemia, autoimmune diseases, chronic kidney disease, cancer, HIV.
- Clinically confirmed malabsorption syndrome
- Hypersensitivity to any form of iron treatment.
- History of any immune related illness e.g., SLE, Rheumatoid arthritis
- Preexisting maternal depression or other psychiatric illness
- Severe allergic reactions such as severe asthma
- History of known drug allergy

8.4.3 Removal of participants from therapy or assessment

Participants were withdrawn or withdrew from the study for the following reasons:

Table 2. Removal of participants from study

	Oral (N=529)	IV (N=527)	Overall (N=1056)
Death	3 (0.6%)	2 (0.4%)	5 (0.5%)
Lost to FollowUp (LTFU)	12 (2.3%)	10 (1.9%)	22 (2.1%)
Participant Discontinued Study Drug	1 (0.2%)	0 (0%)	1 (0.1%)
Withdrawal of consent	12 (2.3%)	3 (0.6%)	15 (1.4%)
Investigator Initiated Discontinuation	0 (0%)	1 (0.2%)	1 (0.1%)

More participants in the oral arm (n=12) than in the IV arm (n=3) withdrew consent to participate in the study

The remaining follow-up safety evaluations were conducted if the participant agreed. If a participant was discontinued by the investigator because of an adverse event, the Participant was followed until resolution of the event.

8.4.3 Method of Assigning Participants to Groups

At the enrolment visit, a pregnant woman who is found to have AIP through haemoglobin testing, using the Hemocue® haemoglobinometer, with a haemoglobin concentration of 9.9g/dL or lower, who meets the eligibility criteria and gives informed consent will be enrolled. Eligible participants will be consecutively enrolled. They will be randomized to one of the two treatments groups. Individual randomization and allocation concealment were done by Sealed Envelope, UK, through a web-based randomisation service, in a 1:1 ratio in blocks stratified according to center (www.sealedenvelope.com).

8.4.4 Blinding

The study was open-label given the obvious morphological differences of the intervention (intravenous solution) and control (oral tablet). Both participants and the site teams knew which arm the participants were randomized to.

The Senior Data Manager had access to the fully unblinded study data. All other members of the study team, including the Trial Biostatistician, did not have access to the fully unblinded study data until the database was locked.

8.4.5 Treatment Compliance

Compliance for the oral arm was assessed using pill counts.

8.5 Efficacy and Safety Variables

8.5.1 Schedule of assessments

The following table presents the efficacy and safety variables that were assessed in the study

Table 3. Schedule of assessments

Visit	Treatment (Baseline)	4 weeks' post-enrollment	36 weeks' EGA	Delivery	2 wks pp	6 wks pp
Socio-demographics	X					
Physical exam	X	X	X	X	X	X
Haemoglobin	X	X	X	X	X	X
Malaria	X					
Full blood count	X + 4 weeks after	X	X	X		X
Iron panel	X + 4 weeks after	X	X	X		X
Maternal serum phosphate	X + 4 weeks after	X	X	X		X
EPDS	X	X	X		X	
Adverse events	X	X	X	X	X	X
Child immunization status						X

Analysis time windows below defined which observations were eligible to be included in assessments for each time point.

Table 4. Analysis Time Windows

Visit (target day)	Lower bound (days)	Upper bound (days)
Baseline (0)	N/A	N/A
36 weeks EGA	-6	Any time before delivery
Delivery	0	+2
2 wks pp	10	18
4 wks pp	19	34
6 wks pp	35	49

8.5.2 Efficacy assessments

Efficacy was primarily assessed using haemoglobin at 36 weeks' gestation and assessments conducted at childbirth.

8.5.3 Safety assessments

Blood samples for maternal serum phosphate assessment was the bass for evaluating safety.in addition, in clinical information regarding adverse events were collected.

8.5.4 Appropriateness of Measurements

All efficacy and safety measurements were standard, reliable, and widely recognized as appropriate.

8.5.5 Endpoints

8.5.5.1 Primary endpoints

1. The prevalence of maternal anaemia (haemoglobin <11 g/dl) at 36 weeks' gestation
2. Incidence of preterm delivery
    a. Based on gestational age at birth, which was based on an ultrasound scan done before 22 weeks EGA or the last menstrual period if an early ultrasound scan was unavailable.

8.5.5.2 Important secondary endpoints

1. The prevalence of maternal iron deficiency (ID) and iron deficiency anaemia (IDA) at 36 weeks' gestation
2. Incidence of depression linked to emotional well-being of mothers using the validated Edinburgh Postnatal Depression Scale (EPDS).

8.5.5.3 Other secondary endpoints

1. Increase in maternal haemoglobin levels at 4 weeks post-initiation of treatment.
2. prevalence of maternal moderate to severe anaemia (<10 g/dl) at 36 weeks' gestation
3. The safety and tolerability of intravenous ferric carboxymaltose versus oral ferrous sulphate, including the incidence of hypophosphatemia and severity of maternal adverse effects.
4. Severe maternal events, specifically, postpartum haemorrhage, sepsis, shock, and the need for blood transfusion.
5. The incidence of
    a. low infant birthweight (<2.5 kg),

b. prematurity (<37 weeks' gestation as dated from the last menstrual period or early ultrasound scan done not later than 22 weeks gestational age if unsure of LMP) (25)

c. stillbirth and,

d. neonatal mortality (birth till 28 days of life),

6. Proportion of infants

    a. being breastfed at 2 and 6 weeks of life, and

    b. having received vaccines up-to-date (BCG, oral polio and hepatitis vaccination) in same time period.

7. The incidence of small for gestational age (birthweight less than the 10th percentile for gestational age).

8.6 Data Quality Assurance

Data was entered directly by the clinician (or other appropriate study personnel) into REDcap electronic data capture tool, hosted at the College of Medicine, University of Lagos.

Data were handled in accordance with Good Clinical Practice, federal regulations, and study protocol. All forms were filled out completely by the examining personnel or the study coordinator and an identifier for the study personnel auto-filled in on the form. Any updates to the electronic data required reasons for the edits to be specified. Updates made at the site were audited by the study data team.

8.7 Statistical Methods

This section describes the statistical analyses that were planned as per the protocol and Statistical Analysis Plan (SAP). The final analysis was performed on the final unblinded dataset, after data cleaning is completed and database is locked.

The Trial Statistician conducted the statistical analysis in RStudio1.0.153(12–14). An independent statistician reviewed the codes.

8.7.1 Statistical and Analytical Plans

The final SAP is available at this link.

8.7.2 Sample Size

At the 5% significance and precision level, 1,056 pregnant women (528 in each study arm) were required to detect a difference in improvement in the prevalence of AIP at term by 14%, between the control group (70% corrected) and the intervention group (84% corrected). This

was based on a multi-country international study in Europe, Asia and Australia(15) at 90% power, adjusting for 15% attrition and protocol violations(16).

To assess the outcome of increase in haemoglobin concentration: at the 5% significance level, 990 pregnant women (495 in each study arm) are required to detect a difference in improvement in the haemoglobin level after 4 weeks among anaemic pregnant women at term by 1g/l, between the control group and the intervention group. This was based on 90% power, adjusting for a 15% attrition and protocol violations, giving a superiority and two-tailed tests of hypotheses(16). A systematic review reported a pooled confidence interval of mean difference of haemoglobin between treatment and control arm as 3.9 to +10.9 g/L(17) while Kochhar et al. in India(18) reported a difference in mean haemoglobin of 2g/dl. We therefore assumed a conservative clinically relevant effect size of 1g/dl to achieve the current sample size.

There was no previous study in our environment describing the efficacy of intravenous iron administration on the outcome of preterm deliveries among pregnant women with anaemia. Prevalence of preterm birth in Nigeria is between 16.8% and 32.9%(19–21). According to a systematic review, there was about 1.6-fold risk of preterm delivery among anaemic mothers. (RR: 1.56, 95%CI: 1.25 – 1.95)(22). Thus, the prevalence of preterm delivery among anaemic mothers is assumed to be between 28.9% and 52.6%.

Hence, we utilized the power calculator in Stata version 17 statistical software (StataCorp. 2021. *Stata Statistical Software: Release 17*. College Station, TX: StataCorp LLC) to calculate the minimum sample size to be 892 women (446 per arm) based on the assumption that prevalence of preterm deliveries among anaemic pregnant women was 28.9%, given 90% power, a protective relative risk of intravenous iron against preterm delivery of 0.65 and a 20% loss to follow -up.

A total of 1,056 pregnant women with GA between 20 and 32 weeks were enrolled into the IVON study.

9 Study Participants

9.1 Analysis Populations

9.1.1 Intention to Treat (ITT) population

The intention to treat population referred to all subjects who were randomized. Following the intention-to-treat principle, patients were analyzed according to the treatment they were assigned to at randomization.

There were 1,056 participants in the ITT population. Of these, 529 were in the IV arm and 527 were in the oral arm.

9.1.2 Modified Intention to Treat (mITT) population

All ITT patients that completed 36 weeks were eligible for a secondary assessment of the primary endpoints.

There were 889 participants in the mITT population. Of these, 454 were in the IV arm and 435 were in the oral arm.

9.2 Analysis of Baseline Characteristics

For the ITT populations, baseline covariates were summarized to describe the population. Continuous variables were summarized using the following descriptive statistics, n (non-missing sample size), mean, standard deviation (SD), medians, minimum and maximum. The frequency and percentages (based on the non-missing sample size) of observed levels were reported for all categorical measures. Summary tables were presented for each treatment, and annotated with the total population size relevant to that table/treatment. The number of missing observations will be presented in the footnote of each table.

9.3 Results: Baseline Characteristics

The baseline characteristics were approximately balanced in the two study arms, indicating that randomization was successful.

Participants were predominantly in their twenties, with the youngest being 16 years and the oldest 49 years. The majority had previously had 0 – 2 pregnancies born alive or carried beyond the age of viability. The gestational age at enrollment was 25 weeks, on average, and ranged from 16 – 32 weeks. Only 6.5% had no formal education and most (81%) completed at least secondary education. Most (66%) were in the lower socioeconomic status (SES) per the Ibadin SES classification(23). Participants were predominantly married and resident in urban areas of Kano and Lagos.

The mean haemoglobin at enrollment was 9.2 g/dL (SD: 0.7). Most participants had moderate anaemia (98%). The prevalence of iron deficiency was 39.8% based on ferritin <30µg/L and 18.5% based on ferritin <15µg/L.

Table 5. Participants' baseline characteristics by treatment arm

	Oral (N=529)	IV (N=527)	Overall (N=1056)
Age			
Mean (SD)	28.6 (5.96)	28.0 (6.07)	28.3 (6.02)
Median [Min, Max]	28.2 [16.7, 48.8]	27.6 [16.0, 47.5]	28.0 [16.0, 48.8]
Age_Categories			
<20 y	22 (4.2%)	30 (5.7%)	52 (4.9%)
20 - <30 y	296 (56.0%)	296 (56.2%)	592 (56.1%)
30 - <40 y	191 (36.1%)	188 (35.7%)	379 (35.9%)
40 and above, y	20 (3.8%)	13 (2.5%)	33 (3.1%)
Parity			
0	187 (35.3%)	192 (36.4%)	379 (35.9%)
1 or 2	187 (35.3%)	195 (37.0%)	382 (36.2%)
3 or 4	78 (14.7%)	78 (14.8%)	156 (14.8%)
5 or more	74 (14.0%)	61 (11.6%)	135 (12.8%)
gestation_age_weeks			
Mean (SD)	25.1 (3.44)	25.3 (3.51)	25.2 (3.47)
Median [Min, Max]	25.0 [20.0, 32.0]	25.0 [16.0, 32.0]	25.0 [16.0, 32.0]
Enrollment_GA			
<20 wk	0 (0%)	5 (0.9%)	5 (0.5%)
20 - <28 wk	381 (72.0%)	351 (66.6%)	732 (69.3%)
28 - <42 wk	148 (28.0%)	171 (32.4%)	319 (30.2%)
Educational_attainment			
No formal education	26 (4.9%)	43 (8.2%)	69 (6.5%)
Completed Primary	67 (12.7%)	58 (11.0%)	125 (11.8%)
Completed Secondary	280 (52.9%)	264 (50.1%)	544 (51.5%)
Completed Tertiary	141 (26.7%)	150 (28.5%)	291 (27.6%)
Postgraduate	13 (2.5%)	11 (2.1%)	24 (2.3%)
SES			
Lower	347 (65.6%)	345 (65.5%)	692 (65.5%)
Middle	167 (31.6%)	162 (30.7%)	329 (31.2%)
Upper	13 (2.5%)	19 (3.6%)	32 (3.0%)
Enrollment_Haemoglobin			
Mean (SD)	9.15 (0.689)	9.14 (0.695)	9.15 (0.692)
Median [Min, Max]	9.30 [5.90, 9.90]	9.30 [6.00, 9.90]	9.30 [5.90, 9.90]
Married			
No	19 (3.6%)	27 (5.1%)	46 (4.4%)
Yes	510 (96.4%)	500 (94.9%)	1010 (95.6%)
Place_of_Residence			
Rural	46 (8.7%)	47 (8.9%)	93 (8.8%)
Urban	483 (91.3%)	480 (91.1%)	963 (91.2%)
State			
Kano	270 (51.0%)	267 (50.7%)	537 (50.9%)
Lagos	259 (49.0%)	260 (49.3%)	519 (49.1%)
Ethnicity			
Hausa	257 (48.6%)	259 (49.1%)	516 (48.9%)
Igbo	74 (14.0%)	59 (11.2%)	133 (12.6%)
Yoruba	157 (29.7%)	177 (33.6%)	334 (31.6%)
Others	39 (7.4%)	32 (6.1%)	71 (6.7%)
Anaemia_Grade			
Moderate	521 (98.5%)	516 (97.9%)	1037 (98.2%)
Severe	8 (1.5%)	11 (2.1%)	19 (1.8%)
Iron deficiency (ferritin <30µg/L)			
No	326 (61.6%)	303 (57.5%)	629 (59.6%)

	Oral (N=529)	IV (N=527)	Overall (N=1056)
Yes	200 (37.8%)	220 (41.7%)	420 (39.8%)
Iron deficiency (ferritin <15µg/L)			
No	429 (81.1%)	425 (80.6%)	854 (80.9%)
Yes	97 (18.3%)	98 (18.6%)	195 (18.5%)

There were missing observations for parity (N=4), educational attainment (N=3), SES (N=3), ethnicity (N=2) and iron deficiency (N=7). Note that all participants with iron deficiency at baseline had iron deficiency anaemia since all participants in the study were anaemic at baseline.

9.4 Other contextual variables

A sizeable proportion of participants delivered outside of clinical study sites despite the study team's efforts. The proportion of participants did not meaningfully differ by treatment arm.

	Oral (N=529)	IV (N=527)	Overall (N=1056)
Place of delivery			
On-Site	331 (62.6%)	339 (64.3%)	670 (63.4%)
Other IVON Site	12 (2.3%)	13 (2.5%)	25 (2.4%)
Government Hospital	16 (3.0%)	32 (6.1%)	48 (4.5%)
Home	119 (22.5%)	110 (20.9%)	229 (21.7%)
Private Hospital	19 (3.6%)	13 (2.5%)	32 (3.0%)
TBA	10 (1.9%)	8 (1.5%)	18 (1.7%)

10 Pregnancy and neonatal outcomes

10.1 Definition of variables

The pregnancy and neonatal were defined as below:

Table 6. Pregnancy and neonatal outcomes - definition of variables

Variable	Description
Gestational age at delivery	Calculated based on an ultrasound scan done before 22 weeks EGA or the last menstrual period if an early ultrasound scan was unavailable.
Preterm birth	Measured from gestational age at birth ▪ The first second primary endpoint is preterm birth ▪ The lower limit of acceptable range of gestational age is 20 weeks. The usual upper limit of gestational age is 44 weeks, beyond which baby is unlikely to have survived.
Low birthweight	Calculated from birthweight <2,500g ▪ Birthweight was measured to the nearest 10g
Small-for-gestational age (SGA)	Binary variable (0, 1). Determined from the birthweight for the gestational age at birth benchmarked to the threshold established by Oken and colleagues(24) based on US infants
Stillbirth	Binary variable (0, 1). Gestational age must be ≥28 weeks, the age of viability.
Neonatal death	Binary variable (0, 1). Defined as infant age at death <42 days. Mothers who had stillbirths were excluded.

10.2 Relevant analysis methods

The frequency of occurrence of pregnancy and neonatal outcomes are presented as N and percent of the total study population by treatment group (IV iron vs. oral iron) for categorical variables. Continuous variables were summarized using the following descriptive statistics, n (non-missing sample size), mean, standard deviation (SD), medians, minimum and maximum.

10.3 Results

The mean gestational age at birth was 39.1 weeks, and 14.2% of participants had a preterm birth. Mean birthweight was 3,120 grams. The incidence of low birthweight and SGA were 6.3% and 14.1% respectively. The incidence of stillbirth and neonatal death were 3.0% and 1.3% respectively. None of the pregnancy and neonatal outcomes differed meaningfully by treatment arm.

Table 7. Pregnancy and neonatal outcomes by treatment arm

	Oral (N=529)	IV (N=527)	Overall (N=1056)
Gestational age at delivery, weeks			
Mean (SD)	39.0 (2.82)	39.2 (2.33)	39.1 (2.58)
Median [Min, Max]	39.4 [24.1, 45.6]	39.4 [25.9, 44.3]	39.4 [24.1, 45.6]
Preterm birth			
No	435 (82.2%)	446 (84.6%)	881 (83.4%)
Yes	77 (14.6%)	73 (13.9%)	150 (14.2%)
Birthweight, grams			
Mean (SD)	3120 (542)	3110 (488)	3120 (515)
Median [Min, Max]	3100 [1200, 4800]	3100 [1000, 4700]	3100 [1000, 4800]
Low birthweight			
No	365 (69.0%)	370 (70.2%)	735 (69.6%)
Yes	31 (5.9%)	36 (6.8%)	67 (6.3%)
SGA			
No	329 (62.2%)	323 (61.3%)	652 (61.7%)
Yes	66 (12.5%)	83 (15.7%)	149 (14.1%)
Stillbirth			
No	497 (94.0%)	502 (95.3%)	999 (94.6%)
Yes	15 (2.8%)	17 (3.2%)	32 (3.0%)
Neonatal death			
No	464 (87.7%)	463 (87.9%)	927 (87.8%)
Yes	9 (1.7%)	5 (0.9%)	14 (1.3%)

11 Efficacy Analyses of primary endpoints

11.1 Definition of variables

The primary endpoints were defined as below:

Table 8. Primary endpoints - definition of variables

Variable	Description
Maternal anaemia	Calculated from haemoglobin variable, <11g/dL. ▪ The first primary endpoint is anaemia at 36 weeks' gestation ▪ Usually, the lower limit of the measured range of haemoglobin is 3 g/dL and the upper limit is 20 g/dL. ▪ 880 participants had haemoglobin testing at 36 weeks. For others, post-enrollment haemoglobin results collected between 30 weeks and delivery, whichever was closer, was included in this analysis.
Preterm birth	Measured from gestational age at birth ▪ The first second primary endpoint is preterm birth ▪ The lower limit of acceptable range of gestational age is 20 weeks. The usual upper limit of gestational age is 44 weeks, beyond which baby is unlikely to have survived.

11.2 Relevant analysis methods

The main analysis was conducted in the ITT population. The frequency of occurrence of the two categorical primary endpoints are presented as N and percent of the total study population by treatment group (IV iron vs. oral iron). Log-binomial regression models were used to evaluate the effect of the treatment group on the risk of the primary endpoints, and risk ratios and confidence intervals presented (see section 10.2).

The analyses were repeated in the mITT population (see section 10.3). The prevalence of maternal anaemia was re-analyzed in the ITT population using haemoglobin <11 g/dl.

To obtain the relative risk of anaemia accounting for the state and facility type, logistic generalized linear mixed regression models were used, and the beta coefficients exponentiated(25). Relevant measures of uncertainty (confidence intervals and p-values) are reported (see section 10.4). The p-values were obtained from likelihood ratio tests comparing models with an interaction term for the treatment arm and the modifier (state or facility type) to models without the interaction term.

11.3 Primary Efficacy Analyses

11.3.1 Maternal anaemia at 36 weeks

The first primary endpoint of the trial was the prevalence of maternal anaemia (<11 g/dL) at 36 weeks in the intention-to-treat (ITT) population. The prevalence was 57.8% in the IV arm and 60.6% in the oral arm. There was no difference in the effect of IV and oral iron on the prevalence (*P*-value = 0.36).

```
##                                          Oral                IV
##                                         (N=503)            (N=517)
## ---------------------------------------------------------------------------
## Maternal anaemia at 36 weeks
##   No                                198.00 (39.36%)    218.00 (42.17%)
##   Yes                               305.00 (60.64%)    299.00 (57.83%)
## Unstratified Response Analysis
##   Difference in Response Rates (%)                         -2.80
##   95% CI (Wald, with correction)                         (-8.83, 3.23)
##   p-value (Chi-Squared Test)                               0.3625
##   Risk Ratio (95% CI)                                  0.95 (0.86 - 1.06)
```

There were missing observations in the oral (n=26) and IV arms (n=10).

11.3.2 Preterm delivery

The second primary endpoint of the trial was the incidence of preterm delivery in the ITT population. The incidence of preterm delivery was 14.1% in the IV arm and 15.0% in the oral arm. There was no difference in the effect of IV and oral iron on the incidence of preterm delivery (*P*-value = 0.66).

```
##                                          Oral                IV
##                                         (N=512)            (N=519)
## ---------------------------------------------------------------------------
## Preterm delivery
##   No                                435.00 (84.96%)    446.00 (85.93%)
##   Yes                               77.00 (15.04%)      73.00 (14.07%)
## Unstratified Response Analysis
##   Difference in Response Rates (%)                         -0.97
##   95% CI (Wald, with correction)                         (-5.28, 3.33)
##   p-value (Chi-Squared Test)                               0.6576
##   Risk Ratio (95% CI)                                  0.94 (0.70 - 1.26)
```

There were missing observations in the oral (n=17) and IV arms (n=8).

11.4 Secondary Analysis of Primary Endpoints

11.4.1 Maternal anaemia at 36 weeks

The prevalence of maternal anaemia (<11 g/dl) at 36 weeks in the modified intention-to-treat (mITT) population was secondarily analyzed. The prevalence was 56.6% in the IV arm and 58.8% in the oral arm. There was no difference in the effect of IV and oral iron on the prevalence in the mITT population (*P*-value = 0.52).

```
##                                              Oral                IV
##                                             (N=434)            (N=454)
## ———————————————————————————————————————————————————————————————————————
## Maternal anaemia at 36 weeks
##   No                                    179.00 (41.24%)    197.00 (43.39%)
##   Yes                                   255.00 (58.76%)    257.00 (56.61%)
## Unstratified Response Analysis
##   Difference in Response Rates (%)                              -2.15
##   95% CI (Wald, with correction)                             (-8.65, 4.35)
##   p-value (Chi-Squared Test)                                    0.5173
##   Risk Ratio (95% CI)                                      0.96 (0.86 - 1.08)
```

There was a missing observation in the oral arm (1/435) and no missing observation in the IV arm (0/454).

11.4.2 Preterm delivery

The incidence of preterm delivery in the mITT population was also secondarily analyzed. The incidence of preterm delivery was 5.3% in the IV arm and 4.2% in the oral arm. There was no difference in the effect of IV and oral iron on the incidence of preterm delivery in the mITT population (*P*-value = 0.42).

```
##                                              Oral                IV
##                                             (N=434)            (N=454)
## ———————————————————————————————————————————————————————————————————————
## Preterm delivery
##   No                                    416.00 (95.85%)    430.00 (94.71%)
##   Yes                                    18.00 (4.15%)      24.00 (5.29%)
## Unstratified Response Analysis
##   Difference in Response Rates (%)                              1.14
##   95% CI (Wald, with correction)                             (-1.65, 3.92)
##   p-value (Chi-Squared Test)                                    0.4242
##   Risk Ratio (95% CI)                                      1.27 (0.70 - 2.31)
```

There was a missing observation in the oral arm (1/435) and no missing observation in the IV arm (0/454).

11.5 Primary Endpoints by region and facility type

There were no differences in proportion of participants who were enrolled into either treatment arm from Kano (vs. Lagos) or from either primary, secondary or tertiary facilities.

Table 9. Participants' state and facility type by treatment arm

	Oral (N=529)	IV (N=527)	Overall (N=1056)
State			
Kano	270 (51.0%)	267 (50.7%)	537 (50.9%)
Lagos	259 (49.0%)	260 (49.3%)	519 (49.1%)
Facility_Type			
Primary	131 (24.8%)	126 (23.9%)	257 (24.3%)
Secondary	358 (67.7%)	359 (68.1%)	717 (67.9%)
Tertiary	40 (7.6%)	42 (8.0%)	82 (7.8%)

11.5.1 State differences

Maternal anaemia

The prevalence of maternal anaemia (<11 g/dl) at 36 weeks in Lagos was 57.5% in the IV iron arm and 62.0% in the oral iron arm. In Kano, the prevalence was 58.1% in the IV arm and 59.3% in the oral arm. The treatment effect did not significantly vary by state (*P*-value = 0.40).

Table 10. Regional variation in the effect of intravenous (vs. oral) iron on maternal moderate to severe anaemia

Outcome	State	level	IV	Oral	ES	CI	Pvalue
Maternal anaemia, RR	Lagos	No	107 (42.5)	93 (38.0)	Ref		0.5962
		Yes	145 (57.5)	152 (62.0)	0.93	(0.80, 1.07)	
	Kano	No	111 (41.9)	105 (40.7)	Ref		
		Yes	154 (58.1)	153 (59.3)	0.98	(0.85, 1.13)	

There were missing observations in the oral (n=26) and IV arms (n=10).

Preterm

The incidence of preterm delivery in Lagos was 9.8% in the IV iron arm and 15.9% in the oral iron arm. In Kano, the incidence was 18.1% in the IV arm and 14.2% in the oral arm. The treatment effect on preterm delivery significantly varied by state (*P*-value = 0.018). While IV iron reduced the incidence of preterm deliveries in Lagos (RR=0.62, 95% CI: 0.38 – 0.98), it had no significant effect in Kano (RR=1.28; 95% CI: 0.86 – 1.91).

Table 11. Regional variation in the effect of intravenous (vs. oral) iron on preterm delivery

Outcome	State	level	IV	Oral	RR	CI	Pvalue
Preterm birth	Lagos	No	229 (90.2)	211 (84.1)	Ref		0.01838
		Yes	25 (9.8)	40 (15.9)	0.62	(0.38, 0.98)	
	Kano	No	217 (81.9)	224 (85.8)	Ref		
		Yes	48 (18.1)	37 (14.2)	1.28	(0.86, 1.91)	

There were missing observations in the oral (n=17) and IV arms (n=8).

11.5.1 Facility type differences

Maternal moderate to severe anaemia

The prevalence of maternal moderate to severe anaemia at 36 weeks in primary facilities was 65.6% in the IV iron arm and 64.0% in the oral iron arm. In secondary facilities, the prevalence was 55.8% in the IV arm and 60.9% in the oral arm. In tertiary facilities, the prevalence was 52.4% in the IV arm and 47.4% in the oral arm. The treatment effect did not significantly vary by facility type (*P*-value = 0.50).

Table 12. Facility type variation in the effect of intravenous (vs. oral) iron on maternal moderate to severe anaemia

Outcome	Facility_type	level	IV	Oral	ES	CI	Pvalue
Maternal anaemia, RR	Primary	No	42 (34.4)	45 (36.0)	Ref		0.4984
		Yes	80 (65.6)	80 (64.0)	1.02	(0.85, 1.23)	
	Secondary	No	156 (44.2)	133 (39.1)	Ref		
		Yes	197 (55.8)	207 (60.9)	0.92	(0.81, 1.04)	
	Tertiary	No	20 (47.6)	20 (52.6)	Ref		
		Yes	22 (52.4)	18 (47.4)	1.11	(0.71, 1.76)	

There were missing observations in the oral (n=26) and IV arms (n=10).

Preterm

The incidence of preterm deliveries in primary facilities was 17.1% in the IV iron arm and 12.7% in the oral iron arm. In secondary facilities, the incidence was 13.8% in the IV arm and 16.7% in the oral arm. In tertiary facilities, the incidence was 7.1% in the IV arm and 7.9% in the oral arm. The treatment effect on the incidence of preterm deliveries did not vary by facility type (*P*-value = 0.39).

Table 13. Facility type variation in the effect of intravenous (vs. oral) iron on preterm delivery

Outcome	Facility_type	level	IV	Oral	RR	CI	Pvalue
Preterm birth	Primary	No	102 (82.9)	110 (87.3)	Ref		0.3936
		Yes	21 (17.1)	16 (12.7)	1.34	(0.74, 2.50)	
	Secondary	No	305 (86.2)	290 (83.3)	Ref		
		Yes	49 (13.8)	58 (16.7)	0.83	(0.58, 1.18)	
	Tertiary	No	39 (92.9)	35 (92.1)	Ref		
		Yes	3 (7.1)	3 (7.9)	0.90	(0.18, 4.64)	

There were missing observations in the oral (n=17) and IV arms (n=8).

12 Important secondary endpoints – Efficacy Analyses

12.1 Definition of variables

The important secondary endpoints were defined as below:

Table 14. Important secondary endpoints - definition of variables

Variable	Description
Iron deficiency (ID)	Calculated in two alternative ways – using ferritin <15 µg/L(26) and ferritin <30 ug/L ▪ ID at baseline will be the basis for subgroup analyses ▪ ID was also an important secondary endpoint ▪ 862 participants had ferritin testing at 36 weeks. For others, post-enrollment ferritin results collected between 30 weeks and delivery, whichever was closer, was included in this analysis.
Iron deficiency anaemia (IDA)	Calculated in two alternative ways – from haemoglobin <11g/dL and ferritin <15 µg/L, and from haemoglobin <11g/dL and ferritin <30 µg/L ▪ IDA was an important secondary endpoint.
Depression	Binary variable (0,1). Calculated from the EPDS score(27). Depression will be defined as EPDS score ≥10 any time after birth. Given the EPDS is assessed multiple times, the highest score will be used. Any woman who commits or attempts suicide will be regarded as depressed, regardless of her score. Any woman who self-reports depressive illness or whose family member or physician reports a depressive illness will also be regarded as depressed.
EPDS score	Continuous variable. Individuals identified as depressed despite EPDS being <10 will have their EPDS corrected to 10/median EPDS for the depressed subgroup

12.2 Relevant analysis methods

This analysis was conducted in the ITT population. The frequency of occurrence of the ID, IDA, and maternal depression are presented as N and percent of the total study population by treatment group (IV iron vs. oral iron). The proportion of participants that attained the minimally important change (MIC) of four points was estimated, compared by treatment group, and presented as N and percent of the total study population by treatment group. Log-binomial regression models were used to evaluate the effect of the treatment group on the risk of these endpoints, and risk ratios and confidence intervals presented (see section 11.2, 11.3, 11.4.1 and 11.4.3).

The EPDS score was summarized as a continuous outcome using the mean, standard deviation (SD), median, minimum and maximum, overall and by treatment group, at baseline and 36 weeks (see section 11.4.2).

12.3 Iron deficiency

12.3.1 Using ferritin <30 µg/L

Iron deficiency (ID) and iron deficiency anaemia (IDA) at 36 weeks in the ITT population were important secondary endpoints.

The prevalence of ID at 36 weeks was 4.5% in the IV arm and 16.4% in the oral arm. IV iron significantly reduced the risk of ID, compared to oral iron (*P*-value < 0.0001). The risk of ID at 36 weeks was 73% lower (95% CI: 58% – 83%) in the IV arm compared to the oral arm.

Note that the prevalence of ID was 39.8% at baseline.

```
##                                          Oral                IV
##                                         (N=500)            (N=516)
## ──────────────────────────────────────────────────────────────────────
## ID at 36 weeks
##   No                                418.00 (83.60%)    493.00 (95.54%)
##   Yes                               82.00 (16.40%)      23.00 (4.46%)
## Unstratified response
##   Difference in Response Rates (%)                        -11.94
##   95% CI (Wald, with correction)                       (-15.64, -8.24)
##   p-value (Chi-Squared Test)                              <0.0001
##   Risk Ratio (95% CI)                                0.27 (0.17 - 0.42)
```

There were missing observations in the oral (n=29) and IV arms (n=11).

12.3.2 Using ferritin <15 µg/L

We also reanalysed based on ferritin <15 µg/L. The prevalence was 1.2% in the IV arm and 5.0% in the oral arm. IV iron significantly reduced the risk of ID, compared to oral iron (*P*-value = 0.0004). The risk of ID at 36 weeks was 77% lower (95% CI: 44% – 90%) in the IV arm compared to the oral arm.

Note that the prevalence was 18.5% at baseline.

```
##                                          Oral                IV
##                                         (N=500)            (N=516)
## ──────────────────────────────────────────────────────────────────────
## ID at 36 weeks
##   No                                475.00 (95.00%)    510.00 (98.84%)
##   Yes                                25.00 (5.00%)       6.00 (1.16%)
## Unstratified response
##   Difference in Response Rates (%)                         -3.84
##   95% CI (Wald, with correction)                        (-5.96, -1.71)
##   p-value (Chi-Squared Test)                               0.0004
##   Risk Ratio (95% CI)                                0.23 (0.10 - 0.56)
```

There were missing observations in the oral (n=29) and IV arms (n=11).

12.4 Iron deficiency anaemia

12.4.1 Using ferritin <30 µg/L and haemoglobin <11g/dl

The prevalence of IDA at 36 weeks was 2.1% in the IV arm and 9.6% in the oral arm. IV iron significantly reduced the risk of IDA, compared to oral iron (*P*-value < 0.0001). The risk of IDA at 36 weeks was 78% lower (95% CI: 58% – 88%) in the IV arm compared to the oral arm.

Note that the prevalence was 39.8% at baseline.

```
##                                          Oral                IV
##                                         (N=498)            (N=516)
## ---------------------------------------------------------------------
## IDA at 36 weeks
##   No                                450.00 (90.36%)    505.00 (97.87%)
##   Yes                                48.00 (9.64%)      11.00 (2.13%)
## Unstratified response
##   Difference in Response Rates (%)                        -7.51
##   95% CI (Wald, with correction)                       (-10.38, -4.63)
##   p-value (Chi-Squared Test)                              <0.0001
##   Risk Ratio (95% CI)                                0.22 (0.12 - 0.42)
```

There were missing observations in the oral (n=31) and IV arms (n=11).

12.4.2 Using ferritin <15 µg/L and haemoglobin <11g/dl

We also reanalysed based on ferritin <15 µg/L. The prevalence was 0.4% in the IV arm and 4.0% in the oral arm. IV iron significantly reduced the risk of IDA, compared to oral iron (*P*-value < 0.0001). The risk of IDA at 36 weeks was 90% lower (95% CI: 59% – 98%) in the IV arm compared to the oral arm.

Note that the prevalence was 18.5% at baseline.

```
##                                          Oral                IV
##                                         (N=498)            (N=516)
## ---------------------------------------------------------------------
## IDA at 36 weeks
##   No                                478.00 (95.98%)    514.00 (99.61%)
##   Yes                                20.00 (4.02%)       2.00 (0.39%)
## Unstratified response
##   Difference in Response Rates (%)                        -3.63
##   95% CI (Wald, with correction)                       (-5.43, -1.82)
##   p-value (Chi-Squared Test)                              <0.0001
##   Risk Ratio (95% CI)                                0.10 (0.02 - 0.41)
```

There were missing observations in the oral (n=31) and IV arms (n=11).

12.5 Maternal depression

12.5.1 Prevalence of depression

The prevalence of maternal depression was 5.7% in the IV arm and 4.5% in the oral arm. There was no difference in the effect of IV and oral iron on the prevalence of maternal depression (*P*-value = 0.40).

```
##                                          Oral                IV
##                                        (N=426)           (N=438)
## ──────────────────────────────────────────────────────────────────
## Maternal Depression at 36 weeks
##   No                                407.00 (95.54%)    413.00 (94.29%)
##   Yes                                19.00 (4.46%)      25.00 (5.71%)
## Unstratified response
##   Difference in Response Rates (%)                         1.25
##   95% CI (Wald, with correction)                        (-1.68, 4.17)
##   p-value (Chi-Squared Test)                              0.4043
##   Risk Ratio (95% CI)                                  1.28 (0.72 - 2.29)
```

There were missing observations in the oral (n=103) and IV arms (n=89). Participants who had a preterm delivery did not have a suitable depression measure at 36 weeks gestation.

12.5.2 EPDS Treatment effect

The mean EPDS at 36 weeks GA was also similar in the IV and oral arms: 4.3 (3.2) vs. 4.4 (3.3). There was no difference in the mean EPDS at 36 weeks EGA in the IV and oral iron arms (*P*-value = 0.75).

	Oral (N=426)	IV (N=438)	P-value
epds_sum_enroll			
Mean (SD)	4.70 (3.24)	4.69 (3.38)	0.972
Median [Min, Max]	4.50 [0, 16.0]	4.00 [0, 17.0]	
epds_sum_36Wks_GA			
Mean (SD)	4.36 (3.25)	4.29 (3.24)	0.746
Median [Min, Max]	4.00 [0, 19.0]	4.00 [0, 20.0]	

12.5.1 Minimally important change (MIC) in EPDS

The proportion achieving the MIC for EPDS between enrollment and 36 weeks GA was also similar in the IV and oral arms: 13.7% vs. 14.1%. There was no difference in the effect of IV and oral iron on whether the MIC was achieved or not (*P*-value = 0.87).

```
##                                          Oral                IV
##                                        (N=426)           (N=438)
## ──────────────────────────────────────────────────────────────────
## Minimally important change in EPDS
##   Achieved                          60.00 (14.08%)      60.00 (13.70%)
##   Not achieved                      366.00 (85.92%)    378.00 (86.30%)
## Difference in % attaining the MIC
##   Difference in Response Rates (%)                          -0.39
##   95% CI (Wald, with correction)                         (-5.00, 4.23)
##   p-value (Chi-Squared Test)                                0.8698
##   Risk Ratio (95% CI)                                  0.97 (0.70 - 1.36)
```

There were missing observations in the oral (n=103) and IV arms (n=89). Participants who had a preterm delivery did not have a suitable depression measure at 36 weeks gestation.

13 Other secondary endpoints – Efficacy Analyses

13.1 Definition of variables

The other secondary endpoints were defined as below:

Table 15. Other secondary endpoints - definition of variables

Variable	Description
Moderate to severe anaemia	Calculated from haemoglobin variable, <10g/dL
Low birthweight	Measured from birthweight
Stillbirth	Binary variable (0, 1). Gestational age must be ≥28 weeks, the age of viability.
Postpartum haemorrhage	Binary variable (0, 1). Blood loss postpartum > 1,000 ml based on visual or weight method, whichever is greater(28).
Neonatal mortality	Binary variable (0, 1). Defined as infant age at death <42 days. Mothers who had stillbirths were excluded.
Vaccination up-to-date	Binary (0,1) at 1, 2 and 4 weeks. Calculated from BCG, oral polio and hepatitis vaccination.
SGA	Binary variable (0,1). Calculated from the birthweight and gestational age, with thresholds based on the Oken cutoffs for all births(24).

13.2 Relevant analysis methods

This analysis was conducted in the ITT population. The frequency of occurrence of the categorical endpoints are presented as N and percent of the total study population by treatment group (IV iron vs. oral iron). Continuous variables are presented as mean, standard deviation, median, minimum and maximum.

Log-binomial regression models were used to evaluate the effect of the treatment group on the risk of categorical endpoints, and risk ratios and confidence intervals presented (see section 12.4, 12.5, 12.7, 12.8, 12.9, 12.10, 12.11 and 12.12).

Linear regression models were used to evaluate the effect of the treatment group on the risk of continuous endpoints, and mean difference and confidence intervals presented (see section 12.6). The four-week increase in maternal haemoglobin from treatment initiation was presented in figures (see section 12.3) and p-values obtained from a model with an interaction term for the timepoint (0 vs. 4 week) and the treatment arm (IV vs. oral iron).

13.3 Increase in maternal haemoglobin levels at 4 weeks post-initiation of treatment

The mean (±SE) maternal haemoglobin concentration at enrollment was 9.14 (±0.04) in the IV arm and 9.15 (±0.04) in the oral arm. Four weeks later, the mean (±SE) haemoglobin concentration was 10.4 (±0.04) in the IV arm and 10.2 (±0.04) in the oral arm.

The increase in maternal haemoglobin concentration was significantly greater in the IV arm than in the oral arm (*P*-value = 0.003), though the magnitude of the difference seems minimal.

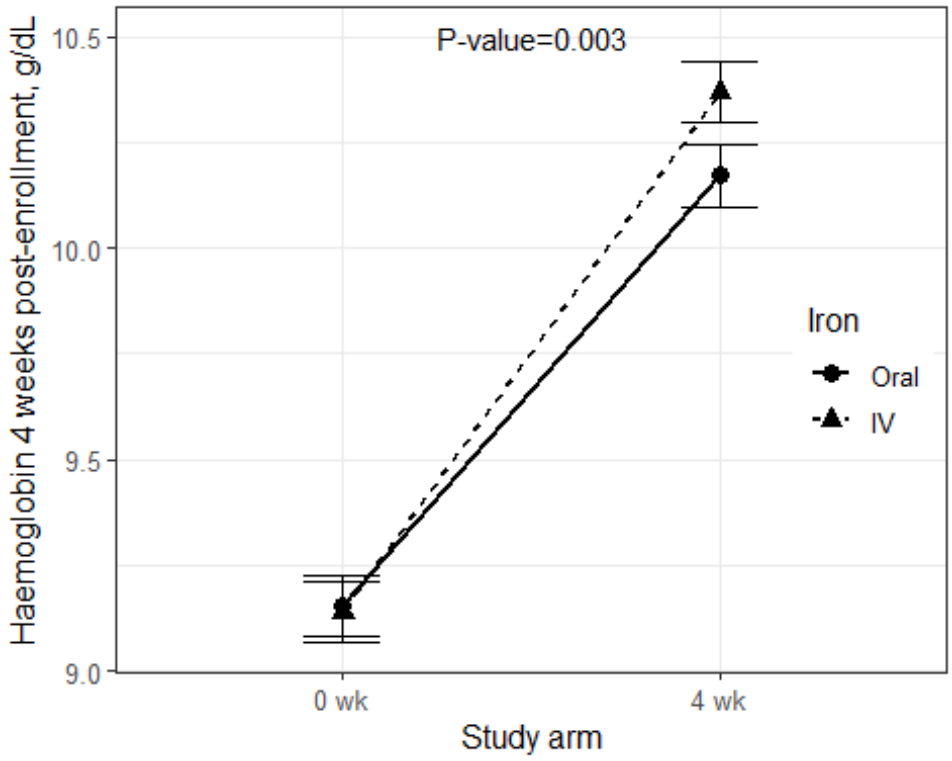

Figure 2. Treatment effect on the increase in maternal haemoglobin concentration

13.4 Other secondary outcomes

Table 16. Treatment effect on secondary efficacy outcomes

Outcome	level	IV	Oral	RR	CI	P-value
Mod. to sev anaemia	No	413 (79.9)	388 (77.1)	Ref		
	Yes	115 (22.9)	104 (20.1)	0.88	(0.70, 1.11)	0.29
Postpartum haemorrhage	Abnormal	7 (1.3)	5 (0.9)	1.41	(0.45, 4.72)	0.56
	Normal	520 (98.7)	524 (99.1)	Ref		
Blood transfusion	No	524 (99.4)	525 (99.2)	Ref		
	Yes	3 (0.6)	4 (0.8)	0.75	(0.15, 3.42)	0.71
Low birthweight	No	370 (91.1)	365 (92.2)	Ref		
	Yes	36 (8.9)	31 (7.8)	1.13	(0.72, 1.80)	0.60
SGA	No	323 (79.6)	329 (83.3)	Ref		
	Yes	83 (20.4)	66 (16.7)	1.22	(0.91, 1.64)	0.18
Stillbirth	No	502 (96.7)	497 (97.1)	Ref		
	Yes	17 (3.3)	15 (2.9)	1.12	(0.56, 2.25)	0.75
Neonatal death	No	463 (98.9)	464 (98.1)	Ref		
	Yes	5 (1.1)	9 (1.9)	1.00	(0.88, 1.14)	>0.99
Breastfeeding at 2 wks postpartum	No	32 (7.0)	55 (12.0)	Ref		
	Yes	423 (93.0)	403 (88.0)	1.06	(0.92, 1.21)	0.43
Breastfeeding at 6 wks postpartum	No	49 (10.7)	60 (13.1)	Ref		
	Yes	407 (89.3)	397 (86.9)	1.03	(0.89, 1.18)	0.70
Vaccines up-to-date	No	44 (10.1)	33 (7.8)	Ref		
	Yes	390 (89.9)	388 (92.2)	0.98	(0.85, 1.12)	0.72

Table 17. Treatment effect on related continuous outcomes

Outcome	IV	Oral	MD	CI	P-value
Birthweight, grams	3108 (488)	3125 (542)	-16.5	(-87.8, 54.9)	0.65
GA at delivery, days	274 (16)	273 (20)	0.8	(-1.4, 3.0)	0.46

13.4.1 Moderate to severe anaemia

The incidence of moderate to severe anaemia (haemoglobin <10 g/dl) was 20.1% in the IV arm and 22.9% in the oral arm (Table 10), and did not significantly differ by treatment arm (*P*-value = 0.29).

Note that there were missing observations in the IV (n=10) and oral arms (n=26).

13.4.2 Postpartum haemorrhage

The incidence of postpartum haemorrhage was 1.3% in the IV arm and 0.9% in the oral arm (Table 10), and did not significantly differ by treatment arm (*P*-value = 0.56).

Note that there were missing observations in the IV (n=48) and oral arms (n=66).

13.4.3 Need for blood transfusion

Only three individuals in the IV arm (0.6%) and four individuals in the oral arm (0.8%) received blood transfusion (Table 10). The need for blood transfusion did not differ by treatment arm (*P*-value = 0.71).

13.4.4 Low birthweight

The mean (SD) birthweight was 3,108 (488) in the IV arm and 3,125 (542) in the oral arm.

The incidence of low birthweight was 8.9% in the IV arm and 7.8% in the oral arm, and did not significantly differ by treatment arm (*P*-value = 0.60).

Note that there were missing observations in the IV (n=123) and oral arms (n=131).

13.4.5 Small-for-gestational age (SGA)

The incidence of SGA was 20.4% in the IV arm and 16.7% in the oral arm, and did not significantly differ by treatment arm (*P*-value = 0.18).

Note that there were missing observations in the IV (n=121) and oral arms (n=134).

13.4.6 Gestational age at delivery

The mean (SD) gestational age was 274 (16) days in the IV arm and 273 (20) days in the oral arm, and did not significantly differ by treatment arm (*P*-value = 0.46).

Note that there were missing observations in the IV (n=8) and oral arms (n=17).

13.4.7 Stillbirth

There were 32 stillbirths. There were 17 (3.3%) in the IV arm and 15 (2.9%) in the oral arm (Table 10). The incidence of stillbirths did not significantly differ by treatment arm (*P*-value = 0.75).

Note that we had delivery information for 1,031 participants. Data was missing for 25 participants – 8 in the IV arm and 17 in the oral arm.

13.4.8 Neonatal death

There were 14 neonatal deaths – five (1.1%) in the IV arm and nine (1.9%) in the oral arm (Table 10). The incidence of neonatal deaths did not significantly differ by treatment arm (*P*-value = >0.99).

Of the 999 participants with livebirths, vital status information at six weeks postpartum was missing in 58 participants – 34 in IV arm and 24 in oral arm.

13.4.9 Breastfeeding at 2 weeks postpartum

The prevalence of breastfeeding at 2 weeks postpartum was 93.0% in the IV arm and 88.0% in the oral arm (Table 10), and did not significantly differ by treatment arm (*P*-value = 0.43).

Of the 999 participants with livebirths, information about breastfeeding at 2 weeks postpartum was missing in 86 participants – 45 in IV arm and 41 in oral arm.

13.4.10 Breastfeeding at 6 weeks postpartum

The prevalence of breastfeeding at 6 weeks postpartum was 89.3% in the IV arm and 86.9% in the oral arm (Table 10), and did not significantly differ by treatment arm (*P*-value = 0.70).

Of the 999 participants with livebirths, information about breastfeeding at 6 weeks postpartum was missing in 86 participants – 45 in IV arm and 41 in oral arm.

13.4.11 Vaccine uptake

While vaccines were up-to-date in 89.9% infants in the IV arm, they were only up-to-date in 92.2% in the oral arm (Table 10). Vaccine uptake did not significantly differ by treatment arm (*P*-value = 0.86).

Of the 999 participants with livebirths, information about vaccine uptake was missing in 119 participants – 58 in IV arm and 61 in oral arm.

14 IDA vs. NIDA: Subgroup analyses

14.1 Relevant analysis methods

The ITT population was used for analysis. The outcomes of interest were the primary and secondary endpoints. The analyses were conducted among those with IDA at enrolment compared to those with NIDA. IDA was defined using haemoglobin <11g/dl and ferritin <30µg/L.

Continuous endpoints were summarized using N, mean, and standard deviation (SD), in the overall IDA (vs. NIDA population) and by treatment group, at 36 weeks. Linear regression models were used to obtain mean difference and 95% CI in each subgroup.

The frequency of occurrence of the dichotomous endpoints were presented as N and percent of the IDA (vs. NIDA) population, and by treatment group (IV iron vs. oral iron).

To obtain the relative risk and 95% CI of each dichotomous endpoint, log-binomial regression models were used. In some cases, the log-binomial models may not converge and log-Poisson models, which provide consistent but not fully efficient estimates of the relative risk, and its confidence intervals will be used(29). Likelihood ratio tests were used to compare models with an interaction term for IDA status and treatment to those without.

Subgroup analysis based on haemoglobin <11g/dl and ferritin <15µg/L was repeated and respective figures and tables are in the appendix.

14.2 Increase in maternal haemoglobin levels at 4 weeks post-initiation of treatment

In the subgroup of women who were iron-deficient at enrollment (Figure 2A), the mean (95% CI) maternal haemoglobin concentration at enrollment was 9.3 (9.1, 9.4) in the IV arm and 9.2 (9.1, 9.3) in the oral arm. Four weeks later, the mean (95% CI) was 10.6 (10.4, 10.7) in the IV arm and 10.3 (10.1, 10.4) in the oral arm. The slope of the increase in maternal haemoglobin in the IV (vs. oral) arm differed slightly (*P*-value = 0.049).

In the subgroup of women who were not iron-deficient at enrollment (Figure 2B), the mean (95% CI) maternal haemoglobin concentration at enrollment was 9.1 (9.0, 9.2) in the IV arm and 9.1 (9.1, 9.2) in the oral arm. Four weeks later, the mean (95% CI) was 10.2 (10.1, 10.3) in the IV arm and 10.1 (10.0, 10.2) in the oral arm. The slope of the increase in the maternal haemoglobin in the IV arm was steeper than in the oral arm (*P*-value = 0.038).

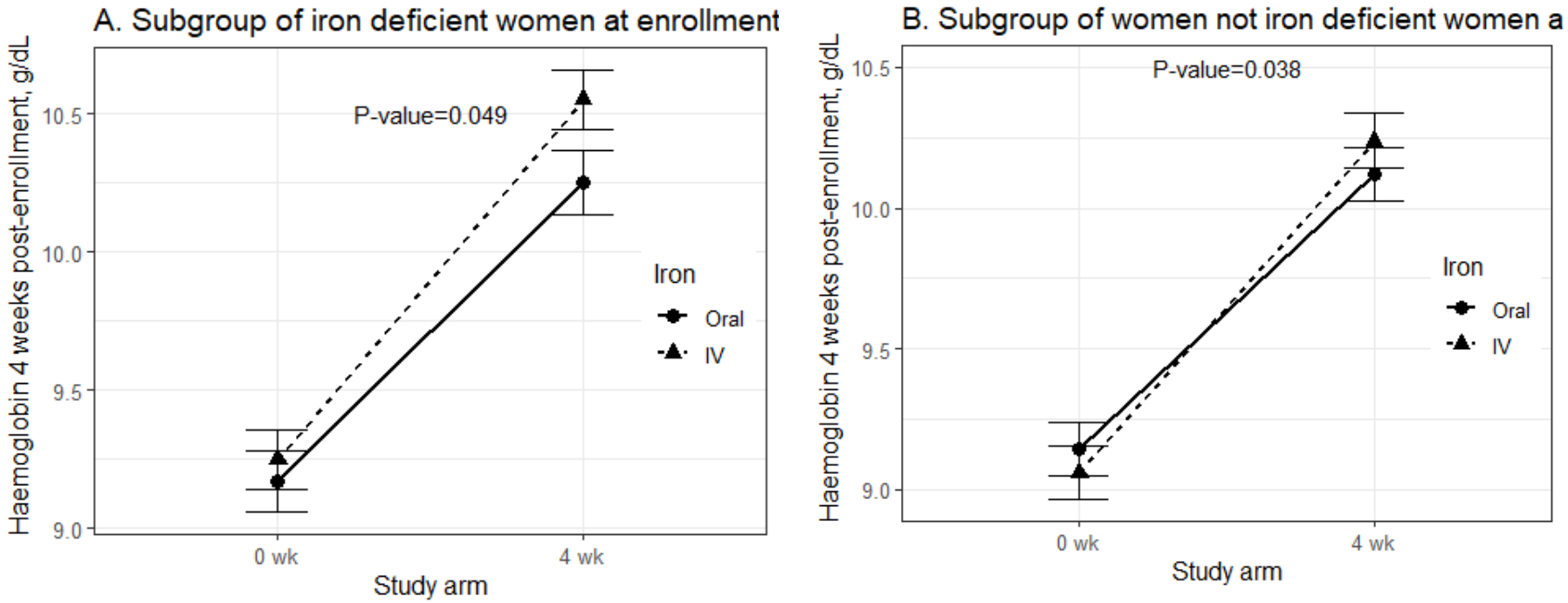

Figure 3. Change in hemoglobin from enrolment to 4 weeks post-enrolment

14.3 Subgroup analyses of binary outcomes

In subgroup analysis based on ferritin <30µg/L at enrollment (Table 18), IV iron was significantly more effective to prevent maternal anaemia (haemoglobin <11g/dl) at 36 weeks in the IDA subgroup than in the NIDA subgroup compared to oral iron (*P*-value = 0.039). While IV iron reduced the risk of maternal anaemia compared to oral iron in the IDA subgroup (RR=0.83; 95% CI: 0.71, 0.98), it had no effect in the NIDA subgroup (RR=1.04; 95% CI: 0.91, 1.18).

The effect of IV vs. oral iron on the other binary outcomes considered did not vary by the presence of iron deficiency at enrolment.

Our findings in subgroup analysis based on ferritin <15µg/L at enrollment (Appendix 1) were somewhat different from the findings from the subgroup analysis based on 30µg/L. IV iron was significantly more effective to prevent moderate to severe anaemia (haemoglobin <10g/dl) at 36 weeks in the IDA subgroup than in the NIDA subgroup compared to oral iron (P-value = 0.045). While IV iron reduced the risk of moderate to severe anaemia compared to oral iron in the IDA subgroup (RR=0.50; 95% CI: 0.25, 0.92), it had no effect in the NIDA subgroup (RR=0.98; 95% CI: 0.76, 1.27). IV iron was significantly more effective to prevent ID at 36 weeks (serum ferritin <15µg/L) in the IDA subgroup than in the NIDA subgroup compared to oral iron (*P*-value = 0.042). While IV iron reduced the risk of ID compared to oral iron in the IDA subgroup (RR=0.06; 95% CI: <0.01, 0.30), it had no significant effect in the NIDA subgroup (RR=0.49; 95% CI: 0.15, 1.36).

Table 18. Subgroup analyses of iron deficient vs. non-iron deficient

		IDA subgroup				NIDA subgroup				
Outcome[a]	level	IV	Oral	RR	95% CI	IV	Oral	RR	95% CI	Pvalue
Maternal anaemia	No	101 (45.9)	67 (35.1)	Ref		117 (39.7)	130 (41.8)	Ref		
	Yes	119 (54.1)	124 (64.9)	0.83	(0.71, 0.98)	178 (60.3)	181 (58.2)	1.04	(0.91, 1.18)	0.039
Moderate to severe anaemia	No	185 (84.1)	147 (77.0)	Ref		226 (76.6)	240 (77.2)	Ref		
	Yes	35 (15.9)	44 (23.0)	0.69	(0.46, 1.03)	69 (23.4)	71 (22.8)	1.02	(0.77, 1.37)	0.116
ID at 36 weeks	No	216 (98.2)	173 (90.6)	Ref		292 (99.3)	301 (97.7)	Ref		
	Yes	4 (1.8)	18 (9.4)	0.19	(0.06, 0.51)	2 (0.7)	7 (2.3)	0.30	(0.04, 1.23)	0.655
IDA at 36 weeks	No	219 (99.5)	175 (92.1)	Ref		293 (99.7)	302 (98.4)	Ref		
	Yes	1 (0.5)	15 (7.9)	0.06	(0.00, 0.28)	1 (0.3)	5 (1.6)	0.21	(0.01, 1.28)	0.401
Maternal Depression	No	167 (91.3)	148 (94.3)	Ref		244 (96.4)	258 (96.3)	Ref		
	Yes	16 (8.7)	9 (5.7)	1.53	(0.71, 3.51)	9 (3.6)	10 (3.7)	0.95	(0.38, 2.33)	0.435
Hypophosphataemia	<0.8075	3 (1.5)	1 (0.6)	Ref		1 (0.4)	6 (2.1)	Ref		
	>=0.8075	200 (98.5)	167 (99.4)			269 (99.6)	278 (97.9)	0.18	(0.01, 1.02)	0.061
Postpartum haemorrhage	Abnormal	5 (2.3)	1 (0.5)	4.55	(0.74, 86.69)	2 (0.7)	4 (1.2)	0.54	(0.07, 2.74)	0.094
	Normal	215 (97.7)	199 (99.5)	Ref		301 (99.3)	322 (98.8)	Ref		
Preterm birth	No	187 (85.0)	162 (83.1)	Ref		257 (86.5)	272 (86.1)	Ref		
	Yes	33 (15.0)	33 (16.9)	0.89	(0.57, 1.38)	40 (13.5)	44 (13.9)	0.97	(0.65, 1.44)	0.774
Low birthweight	No	161 (92.5)	144 (94.1)	Ref		208 (90.0)	221 (90.9)	Ref		
	Yes	13 (7.5)	9 (5.9)	1.27	(0.56, 3.00)	23 (10.0)	22 (9.1)	1.10	(0.63, 1.93)	0.776
SGA	No	142 (81.6)	138 (90.8)	Ref		180 (77.9)	191 (78.6)	Ref		
	Yes	32 (18.4)	14 (9.2)	2.00	(1.13, 3.73)	51 (22.1)	52 (21.4)	1.03	(0.73, 1.45)	0.051
Stillbirth	No	212 (96.4)	192 (98.5)	Ref		288 (97.0)	304 (96.2)	Ref		
	Yes	8 (3.6)	3 (1.5)	2.36	(0.69, 10.68)	9 (3.0)	12 (3.8)	0.80	(0.33, 1.86)	0.158
Neonatal death	No	196 (98.0)	179 (97.8)	Ref		264 (99.6)	283 (98.3)	Ref		
	Yes	4 (2.0)	4 (2.2)			1 (0.4)	5 (1.7)	1.00	(0.85, 1.18)	>0.99
Breastfeeding at 2 weeks postpartum	No	10 (5.3)	19 (10.7)	Ref		20 (7.6)	34 (12.2)	Ref		
	Yes	180 (94.7)	159 (89.3)	1.06	(0.86, 1.31)	242 (92.4)	244 (87.8)	1.05	(0.88, 1.26)	0.956
Breastfeeding at 6 weeks postpartum	No	19 (10.0)	24 (13.6)	Ref		28 (10.6)	34 (12.2)	Ref		
	Yes	171 (90.0)	153 (86.4)	1.04	(0.84, 1.30)	235 (89.4)	244 (87.8)	1.02	(0.85, 1.22)	0.876
Vaccines up-to-date, RR	No	14 (7.4)	12 (7.1)	Ref		30 (11.8)	36 (13.5)	Ref		
	Yes	174 (92.6)	157 (92.9)	1.00	(0.80, 1.24)	225 (88.2)	231 (86.5)	1.02	(0.85, 1.23)	0.871

[a]Maternal outcomes, except postpartum haemorrhage, assessed at 36 weeks EGA

15 Safety

15.1 Definition of variables

Adverse events (AEs) were any unfavourable and unintended clinical sign, symptom or disease newly identified in the course of the trial, whether or not they were causally related to the study treatments(30).

The Common Terminology Criteria for Adverse Events (CTCAE) were used to classify the severity of AEs. Serious adverse events (SAE) were adverse events that were classified as grades 3, 4, or 5. These were medically significant, fatal or life-threatening, resulted in significant disability, constituted a congenital anomaly, required inpatient hospitalization or prolonged existing hospitalization.

15.2 Relevant analysis methods

The number and proportion of participants who experience adverse events were analysed and presented. Log-Poisson regression models were used to compare the risk of experiencing an adverse event by treatment arm, and risk ratios and confidence intervals presented (see section 14.3).

The count of adverse events were also analysed and present. Poisson regression models were used to compare the mean number of adverse events by treatment arm.

A listing of the serious adverse events is also presented, including the timing, relation to treatment and the action taken.

15.3 Adverse Events

There were 47 adverse event reports in the trial – 32 in the IV arm and 15 in the oral arm. Most adverse events occurred during the enrolment visit, in the IV arm, and were cases of mild hypotension.

Table 19. Occurrence of Adverse Events by Treatment Arm

Timepoint[a]	AE	IV	Oral
Enrollment	No	501 (95.1)	528 (99.8)
	Yes	26 (4.9)	1 (0.2)
Follow-up	No	446 (98.9)	434 (97.7)
	Yes	5 (1.1)	10 (2.3)
Delivery	No	452 (99.8)	442 (99.8)
	Yes	1 (0.2)	1 (0.2)
Total	No	498 (94.0)	517 (97.2)
	Yes	32 (6.0)	15 (2.8)

[a]These refer to the number of adverse event reports.

The mean number of adverse events was 1.03-times (95% CI: 1.01, 1.06) in the IV arm compared to the oral arm. The risk of experiencing an adverse event was 2.14-times greater (95% CI: 1.18, 4.07) in the IV arm compared to the oral arm.

Table 20. Treatment effect on the occurrence of adverse event

Outcome[a]	level	IV	Oral	ES	CI	Pvalue
Adverse events, counts	No	498 (94.0)	517 (97.2)	1.03	(1.01, 1.06)	0.01
	Yes	32 (6.0)	15 (2.8)	Ref		
Adverse events, people	No	498 (94.5)	517 (97.7)	2.14	(1.18, 4.07)	0.01
	Yes	29 (5.5)	12 (2.3)	Ref		

[a]Maternal outcomes, except postpartum haemorrhage, assessed at 36 weeks EGA

Most of the adverse events that occurred were in ≤3 participants, and did not meaningfully differ by study arm. However, 23 participants in the IV arm experienced mild hypotension and six participants in the oral arm had diarrhoea.

Table 21. Nature of adverse events experienced by treatment arm

Timepoint	Adverse Event	IV	Oral
Enrollment	Congenital familial and genetic disorders	1	0
	Hypertension	1	0
	Hypotension	23	0
	Nausea	1	1
Follow-up	Chills	1	0
	Cough	0	1
	Diarrhea	0	6
	Fatigue	2	1
	Headache	2	1
	Nausea	0	1
	Vomiting	0	3
Delivery	Hypertension	0	1
	Postpartum haemorrhage	1	0

15.4 Serious Adverse Events

There were three SAEs in the study. These were diarrhea, hypertension and postpartum haemorrhage. All SAEs resolved following treatment and care.

Table 22. List of Serious Adverse Events

study_id	Timepoint[a]	SAE	Adverse_event	Relationship	Action
02-0318	Follow-up	Yes	Diarrhea	Possibly Related - AE has a reasonable temporal relationship to study drug	Patient hospitalized/patient's hospitalization prolonged
06-0118	Delivery	Yes	Hypertension	Unrelated - AE has no reasonable temporal relation to study drug	No action taken (i.e. further observation only)
06-0070	Delivery	Yes	Postpartum haemorrhage	Unrelated - AE has no reasonable temporal relation to study drug	Patient hospitalized/patient's hospitalization prolonged

[a]Serious adverse events include adverse events that are medical significant, fatal or life-threatening, resulting in significant disability, constitute a congenital anomaly, requiring inpatient hospitalization or prolongation of existing hospitalization

16 Hypophosphataemia – Safety analysis

16.1 Definition of variables

Hypophosphataemia was the laboratory safety event assessed during the study. It was defined as serum phosphate <0.8075 mmol/L (equivalent to 2.5 mg/dL)(31).

16.2 Relevant analysis methods

The number and proportion of participants who experience adverse events were analysed and presented. Log-binomial regression models were used to evaluate the effect of the treatment group on the occurrence of hypophosphataemia, and risk ratios and confidence intervals presented (see section 14.2).

16.3 Analysis findings

The proportion of participants who experienced hypophosphataemia during follow-up (after 4 weeks post-enrolment) was 10.7% in the IV arm and 1.0% in the oral arm. The risk of hypophosphataemia during follow-up was 10.4-times (95% CI: 4.63, 29.6) in the IV arm compared to the oral arm.

The occurrence of hypophosphataemia did not significantly differ by treatment arm at the other timepoints.

Table 23. Treatment effect on hypophosphatemia risk

Timepoint	level	IV	Oral	ES	CI	Pvalue
Enrollment	<0.8075	7 (1.3)	8 (1.5)	Ref		
	>=0.8075	516 (98.7)	518 (98.5)	0.88	(0.31, 2.43)	0.80
Follow-up 4 weeks post enrollment	<0.8075	54 (10.7)	5 (1.0)	Ref		
	>=0.8075	451 (89.3)	480 (99.0)	10.37	(4.63, 29.59)	<0.01
Delivery	<0.8075	4 (0.8)	7 (1.5)	Ref		
	>=0.8075	471 (99.2)	446 (98.5)	0.54	(0.14, 1.79)	0.33
6 weeks postpartum	<0.8075	5 (1.0)	1 (0.2)	Ref		
	>=0.8075	475 (99.0)	448 (99.8)	4.68	(0.76, 89.42)	0.16

17 Discussion

We conducted an open-label randomized controlled trial of IV versus oral iron supplementation among pregnant women in Nigeria. We found that there were no significant differences in the effect of IV and oral iron to prevent maternal anaemia at 36 weeks' gestation and preterm birth, the primary efficacy endpoints. IV iron was more effective than oral iron to prevent ID and IDA at 36 weeks' gestation. There were no significant differences in the effect of IV and oral iron to prevent postpartum depression. In subgroup analysis, individuals with IDA at baseline were more likely to benefit from IV iron to prevent moderate to severe anaemia and ID at 36 weeks' gestation

Anaemia is very common among pregnant women in LMICs, including Nigeria(1,2). Though oral iron supplementation is the main stay of prevention and treatment, there are numerous concerns about its effectiveness related to poor adherence, inadequate gastrointestinal absorption and bioavailability of orally ingested iron. and the role of infections as a cause of a substantial proportion of anaemia(32–34). We hypothesized that IV iron supplementation would more effectively treat anaemia than oral iron, while also preventing preterm births and other adverse outcomes of pregnancy. Our findings suggest that any superiority of IV iron supplementation may be limited to preventing ID and IDA, and, in addition, to preventing moderate to severe anaemia among individuals with ID at enrolment. Clinical consequences of anaemia and iron depletion are more likely with increasing severity(35). This suggests a role for IV supplementation as an important alternative to oral supplementation when targeted based on hematologic indices.

Our negative findings with respect to the other outcomes are potentially related to limited sample size. For instance, we estimated the sample size of the study expecting preterm births to occur among 16.8% and 32.9%(19–21). The incidence of preterm births in the current trial was however 14.5%. It is also possible that there is no true difference in the biologic effect of IV and oral iron on these outcomes, especially given that most participants were enrolled into the trial after 20 weeks' gestation. Randomized trials of oral supplementation vs. placebo have not found consistent effects(36–38).

There were no important remarkable differences in the safety of IV and oral iron, especially with respect to the occurrence of SAEs. Hypophosphatemia and mild hypotension were more likely in the IV arm and adverse gastrointestinal events were more likely in the oral arm. Clinicians planning to administer these therapies should bear these in mind.

18 Conclusion

We conclude that there were no differences in the efficacy of IV vs. oral iron with respect to the primary endpoints of maternal anaemia at 36 weeks' gestation and preterm birth. IV iron was however more effective than oral iron to prevent ID and IDA at 36 weeks' gestation. Individuals who were iron-deficient at enrolment were also more likely to benefit from the effect of IV iron to prevent anaemia and ID, compared to oral iron.

References

1. Balarajan Y, Ramakrishnan U, Özaltin E, Shankar AH, Subramanian S. Anaemia in low-income and middle-income countries. The lancet. 2011;378(9809):2123–35.

2. Weze K, Abioye AI, Obiajunwa C, Omotayo M. Spatio-temporal trends in anaemia among pregnant women, adolescents and preschool children in sub-Saharan Africa. Public Health Nutr. 2021 Aug;24(12):3648–61.

3. Parks S, Hoffman M, Goudar S, Patel A, Saleem S, Ali SA, et al. Maternal anaemia and maternal, fetal, and neonatal outcomes in a prospective cohort study in India and Pakistan. BJOG Int J Obstet Gynaecol. 2019;126(6):737–43.

4. Daru J, Zamora J, Fernández-Félix BM, Vogel J, Oladapo OT, Morisaki N, et al. Risk of maternal mortality in women with severe anaemia during pregnancy and post partum: a multilevel analysis. Lancet Glob Health. 2018;6(5):e548–54.

5. Tunçalp Özge, Pena-Rosas JP, Lawrie T, Bucagu M, Oladapo OT, Portela A, et al. WHO recommendations on antenatal care for a positive pregnancy experience-going beyond survival. Bjog. 2017;124(6):860–2.

6. Auerbach M, Gafter-Gvili A, Macdougall IC. Intravenous iron: a framework for changing the management of iron deficiency. Lancet Haematol. 2020;7(4):e342–50.

7. Jimenez K, Kulnigg-Dabsch S, Gasche C. Management of iron deficiency anemia. Gastroenterol Hepatol. 2015;11(4):241.

8. Bonovas S, Fiorino G, Allocca M, Lytras T, Tsantes A, Peyrin-Biroulet L, et al. Intravenous versus oral iron for the treatment of anemia in inflammatory bowel disease: a systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore). 2016;95(2).

9. Auerbach M. Commentary: Iron deficiency of pregnancy-a new approach involving intravenous iron. Reprod Health. 2018;15(1):109–15.

10. Nigeria Population Commission. Nigeria demographic and health survey 2018. NPC, ICF; 2019.

11. Afolabi BB, Babah OA, Akinajo OR, Adaramoye VO, Adeyemo TA, Balogun M, et al. Intravenous versus oral iron for iron deficiency anaemia in pregnant Nigerian women (IVON): study protocol for a randomised hybrid effectiveness-implementation trial. Trials. 2022;23(1):1–15.

12. Yoshida K, Bohn J, Yoshida MK. Package 'tableone.' R Found Stat Comput Vienna Austria 30 Novemb 2016. 2020;

13. RStudio Team. RStudio: integrated development for R. RStudio Inc Boston MA URL Httpwww Rstudio Com. 2015;42:14.

14. Fox J, Weisberg S, Friendly M, Hong J, Andersen R, Firth D, et al. Package 'effects.' Url Httpwww R-Proj Org Httpsocserv Socsci Mcmaster Cajfox. 2016;

15. Breymann C, Milman N, Mezzacasa A, Bernard R, Dudenhausen J, investigators FA. Ferric carboxymaltose vs. oral iron in the treatment of pregnant women with iron deficiency anemia: an international, open-label, randomized controlled trial (FER-ASAP). J Perinat Med. 2017;45(4):443–53.

16. Bolarinwa OA. Sample size estimation for health and social science researchers: the principles and considerations for different study designs. Niger Postgrad Med J. 2020;27(2):67.

17. Qassim A, Grivell RM, Henry A, Kidson-Gerber G, Shand A, Grzeskowiak LE. Intravenous or oral iron for treating iron deficiency anaemia during pregnancy: systematic review and meta-analysis. Med J Aust. 2019;211(8):367–73.

18. Kochhar PK, Kaundal A, Ghosh P. Intravenous iron sucrose versus oral iron in treatment of iron deficiency anemia in pregnancy: a randomized clinical trial. J Obstet Gynaecol Res. 2013;39(2):504–10.

19. Bello M, Pius S, Ibrahim BA. Characteristics and predictors of outcome of care of preterm newborns in resource constraints setting, Maiduguri, Northeastern Nigeria. J Clin Neonatol. 2019;8(1):39.

20. Azeez B, Chinyere E, Osayame E, Weathers N, Ladd J, Iretiola F, et al. Characteristics and risk factors of preterm births in a tertiary center in Lagos, Nigeria. Pan Afr Med J. 2016;24.

21. Olusanya BO, Ofovwe GE. Predictors of preterm births and low birthweight in an inner-city hospital in sub-Saharan Africa. Matern Child Health J. 2010;14:978–86.

22. Rahmati S, Azami M, Badfar G, Parizad N, Sayehmiri K. The relationship between maternal anemia during pregnancy with preterm birth: a systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2020;33(15):2679–89.

23. Ibadin MO, Akpede GO. A revised scoring scheme for the classification of socio-economic status in Nigeria. Niger J Paediatr. 2021;48(1):26–33.

24. Oken E, Kleinman KP, Rich-Edwards J, Gillman MW. A nearly continuous measure of birth weight for gestational age using a United States national reference. BMC Pediatr. 2003;3:1–10.

25. Pedroza C, Truong VTT. Estimating relative risks in multicenter studies with a small number of centers—which methods to use? A simulation study. Trials. 2017;18(1):1–10.

26. World Health Organization. WHO guideline on use of ferritin concentrations to assess iron status in populations. World Health Organization; 2020.

27. Chandrasekaran N, De Souza LR, Urquia ML, Young B, Mcleod A, Windrim R, et al. Is anemia an independent risk factor for postpartum depression in women who have a cesarean section?-A prospective observational study. BMC Pregnancy Childbirth. 2018;18:1–7.

28. Wormer K, Jamil R, Bryant S. StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. Acute Postpartum Hemorrhage. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499988/

29. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004;159(7):702–6.

30. US Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 [Internet]. 2017. Available from: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf

31. Sharma S, Hashmi M, D. C. StatPearls. Treasure Island (FL): StatPearls Publishing; 2022. Hypophosphatemia. Available from: https://www.ncbi.nlm.nih.gov/books/NBK493172/

32. Abioye AI, Hughes MD, Sudfeld CR, Premji Z, Aboud S, Hamer DH, et al. The effect of iron supplementation on maternal iron deficiency anemia does not differ by baseline anemia type among Tanzanian pregnant women without severe iron deficiency anemia. Eur J Nutr. 2022 Nov 8;

33. Abioye AI, Fawzi W. Nutritional Anemias. In: Stallings V, editor. Present Knowledge in Nutrition. Elsevier; 2020.

34. Lynch S, Pfeiffer CM, Georgieff MK, Brittenham G, Fairweather-Tait S, Hurrell RF, et al. Biomarkers of Nutrition for Development (BOND)-Iron Review. J Nutr. 2018 Jun 1;148(suppl_1):1001s–67s.

35. Brabin BJ, Hakimi M, Pelletier D. An analysis of anemia and pregnancy-related maternal mortality. J Nutr. 2001;131(2):604S-615S.

36. Etheredge AJ, Premji Z, Gunaratna NS, Abioye AI, Aboud S, Duggan C, et al. Iron Supplementation among Iron-Replete and Non-Anemic Pregnant Women:A Randomized Placebo-Controlled Trial in Tanzania. JAMA Pediatr. 2015;169(10):947–55.

37. Mwangi MN, Roth JM, Smit MR, Trijsburg L, Mwangi AM, Demir AY, et al. Effect of daily antenatal iron supplementation on Plasmodium infection in Kenyan women: a randomized clinical trial. Jama. 2015;314(10):1009–20.

38. Mwangi MN, Prentice AM, Verhoef H. Safety and benefits of antenatal oral iron supplementation in low-income countries: a review. Br J Haematol. 2017 Jun;177(6):884–95.

Appendix

Appendix 1. IDA Subgroup analysis based on haemoglobin <11g/dl and ferritin <15µg/L

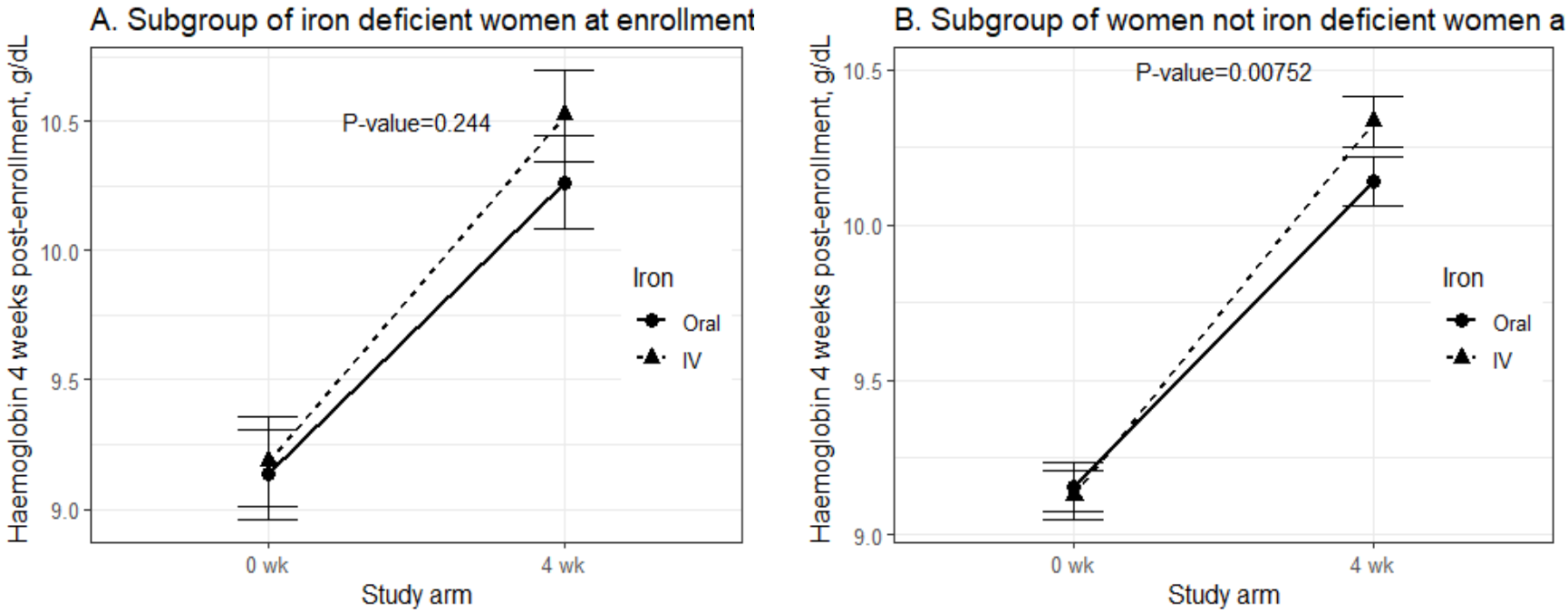

Figure 4. 4-week increase in maternal haemoglobin in subgroups of iron-deficient and non-deficient women

Table 24. Subgroup analyses based on ferritin <15ug/L

		IDA subgroup				NIDA subgroup				
Outcome[a]	level	IV	Oral	RR	CI	IV	Oral	RR	CI	Pvalue
Maternal anaemia	No	51 (52.0)	37 (39.8)	Ref		167 (40.0)	160 (39.1)	Ref		
	Yes	47 (48.0)	56 (60.2)	0.80	(0.61, 1.04)	250 (60.0)	249 (60.9)	0.98	(0.88, 1.10)	0.144
Mod to sev anaemia	No	86 (87.8)	70 (75.3)	Ref		325 (77.9)	317 (77.5)	Ref		
	Yes	12 (12.2)	23 (24.7)	0.50	(0.25, 0.92)	92 (22.1)	92 (22.5)	0.98	(0.76, 1.27)	0.045
ID at 36 weeks	No	97 (99.0)	78 (83.9)	Ref		411 (98.8)	396 (97.5)	Ref		
	Yes	1 (1.0)	15 (16.1)	0.06	(<0.01, 0.30)	5 (1.2)	10 (2.5)	0.49	(0.15, 1.36)	0.042
IDA at 36 weeks	No	97 (99.0)	80 (86.0)	Ref		415 (99.8)	397 (98.3)	Ref		
	Yes	1 (1.0)	13 (14.0)	0.07	(<0.01, 0.36)	1 (0.2)	7 (1.7)	0.14	(0.01, 0.77)	0.667
Maternal Depression	No	73 (96.1)	71 (94.7)	Ref		346 (96.1)	342 (97.7)	Ref		
	Yes	3 (3.9)	4 (5.3)	0.74	(0.15, 3.25)	14 (3.9)	8 (2.3)	1.70	(0.74, 4.21)	0.332
Postpartum haemorrhage	Abnormal	3 (3.1)	1 (1.0)	2.97	(0.39, 59.46)	4 (0.9)	4 (0.9)	1.01	(0.24, 4.24)	0.404
	Normal	95 (96.9)	96 (99.0)	Ref		421 (99.1)	425 (99.1)	Ref		
Preterm birth	No	78 (79.6)	81 (84.4)	Ref		366 (87.4)	353 (85.1)	Ref		
	Yes	20 (20.4)	15 (15.6)	1.31	(0.71, 2.45)	53 (12.6)	62 (14.9)	0.85	(0.60, 1.19)	0.220
Low birthweight	No	63 (86.3)	68 (94.4)	Ref		306 (92.2)	297 (91.7)	Ref		
	Yes	10 (13.7)	4 (5.6)	2.47	(0.87, 8.67)	26 (7.8)	27 (8.3)	0.94	(0.56, 1.58)	0.108
SGA	No	59 (80.8)	62 (87.3)	Ref		263 (79.2)	267 (82.4)	Ref		
	Yes	14 (19.2)	9 (12.7)	1.51	(0.71, 3.42)	69 (20.8)	57 (17.6)	1.18	(0.86, 1.63)	0.558
Stillbirth	No	93 (94.9)	95 (99.0)	Ref		407 (97.1)	401 (96.6)	Ref		
	Yes	5 (5.1)	1 (1.0)	4.90	(0.81, 92.92)	12 (2.9)	14 (3.4)	0.85	(0.39, 1.82)	0.084
Neonatal death	No	84 (96.6)	89 (97.8)	Ref		376 (99.5)	373 (98.2)	Ref		
	Yes	3 (3.4)	2 (2.2)			2 (0.5)	7 (1.8)	1.00	(0.87, 1.15)	>0.99
Breastfeeding										
At 2 weeks postpartum	No	3 (3.7)	7 (7.9)	Ref		27 (7.3)	46 (12.5)	Ref		
	Yes	78 (96.3)	82 (92.1)	1.05	(0.77, 1.43)	344 (92.7)	321 (87.5)	1.06	(0.91, 1.23)	0.936
At 6 weeks postpartum	No	8 (9.9)	10 (11.4)	Ref		39 (10.5)	48 (13.1)	Ref		
	Yes	73 (90.1)	78 (88.6)	1.02	(0.74, 1.40)	333 (89.5)	319 (86.9)	1.03	(0.88, 1.20)	0.944
Vaccines up-to-date	No	3 (3.8)	2 (2.4)	Ref		41 (11.3)	46 (13.1)	Ref		
	Yes	77 (96.2)	83 (97.6)	0.99	(0.72, 1.34)	322 (88.7)	305 (86.9)	1.02	(0.87, 1.19)	0.843

[a]Maternal outcomes, except postpartum haemorrhage, assessed at 36 weeks EGA