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Comparison of routine prenatal iron prophylaxis and screening and treatment for anemia: pregnancy results and preliminary birth from a pragmatic randomized controlled trial (PROFEG) in Maputo, Mozambique

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ABSTRACT

Objective: To present the pregnancy results and interim birth results of a pragmatic randomized controlled trial comparing routine iron prophylaxis with screening and treatment for anemia during pregnancy in a setting of endemic malaria and HIV.

Design: A pragmatic randomized controlled trial

Setting: Two health centers (1º de Maio and Machava) in Maputo, Mozambique, a setting of endemic malaria and high prevalence of HIV.

Participants: Pregnant women (≥12 wk gestation; ≥18 years old; non-high-risk pregnancy, *N*=4326) attending prenatal care consultation at the two health centers were recruited to the trial

Interventions: The women were randomly allocated to either Routine iron (n=2184; 60 mg ferrous sulphate plus 400 µg of folic acid daily throughout pregnancy) or Selective iron (n=2142; screening and treatment for anemia and daily intake of 1 mg of folic acid).

Outcome measures: The primary outcomes were preterm delivery (delivery <37 weeks of gestation) and low birth weight (<2500 grams). The secondary outcomes were symptoms suggestive of malaria and self-reported malaria during pregnancy; birth length; cesarean section; maternal and child health status after delivery.

Results: The number of follow-up visits was similar in the two groups. Between the first and fifth visits, the two groups were similar regarding the occurrence of fever, headache, cold/chills, nausea/vomiting, and body aches. There was a suggestion of increased incidence of self-reported malaria during pregnancy (odds ratio 1.37, 95% confidence interval 0.98-1.92) in the Routine iron group. Birth data were available for 1109 (51%) in the Routine iron and for 1149 (54%) in the Selective iron groups. The birth outcomes were relatively similar in the two groups. However, there was a suggestion (statistically non-significant) of poorer outcomes in the Routine iron group with regard to long hospital stay after birth (relative risk [RR] 1.43, 95% CI 0.97-1.26; risk difference

[RD] 0.02, 95% CI -0.00-0.03) and unavailability of delivery data (RR 1.06, 95% CI 1.00-1.13; RD 0.03, 95% CI -0.01-0.07).

Conclusions: These interim results suggest that routine iron prophylaxis during pregnancy did not confer advantage over screening and treatment for anemia regarding maternal and child health. Complete data on birth outcomes are being collected for firmer conclusions.

Trial registration: The trial is registered at ClinicalTrials.gov, number NCT00488579 (June 2007). The first women were randomized to the trial proper April 2007- March 2008. The pilot was November 2006-March 2008. The 3-month lag was due to technical difficulties in completing trial registration.

Funding: The study was funded by two grants from the Academy of Finland (2004: 210631; 2010: 139191).

Keywords: iron, pregnancy, birth, malaria, HIV, pragmatic trial, Mozambique

ARTICLE SUMMARY:

Article focus:

- The benefits of iron prophylaxis during pregnancy on maternal and child health in developing country settings with endemic malaria and high prevalence of HIV is unclear.
- Iron has been linked to increased risk of infections.
- Among children less than three years, there are indications of harm of universal iron prophylaxis.

Key messages:

- Routine iron prophylaxis during pregnancy did not suggest better maternal and child health outcomes than screening and treatment for anemia in a setting of endemic malaria and HIV.

Strengths and limitations of this study

- So far, this represents the largest trial investigating the benefits of prophylactic iron during pregnancy on maternal and child health in malaria-endemic settings.
- The compliance of the study nurses with the trial protocol and that of the women with regards to uptake of the iron and folic acid tablets was good, as was the follow-up during pregnancy.
- The collection of delivery data was challenging, resulting in up to an estimated 40% of missing birth data, which are now been traced with using various methods.

INTRODUCTION

Despite widespread recommendation of routine iron prophylaxis during pregnancy, its benefits and risks for the mother and child, beyond the reduction of the risk of anemia, remain unclear, particularly in low-income settings. Reviews of randomized controlled trials (RTCs) done for the Cochrane Collaboration and the World Health Organization (WHO) have failed to conclude whether routine iron prophylaxis during pregnancy is beneficial or harmful to pregnancy outcomes.[1,2] There is some evidence that high hemoglobin concentration in late pregnancy may be associated with adverse effects on pregnancy.[3,4] It has also been suggested that iron increases the risk of infections.[5-7] The host requires iron for biochemical functioning, but iron may as well promote the replication of infectious agents.[6] For developing country settings which are still plagued by infectious diseases, such as malaria and HIV, the potential of iron to increase the risk of infections raises serious public health concerns.[8,9]

Previous trials conducted in malarial developing country settings that have evaluated the effects of iron supplementation during pregnancy on maternal and child outcomes have been hampered by small samples, large dropouts, and several outcome-related exclusions.[10-14] The findings from the trials were conflicting on the role of prophylactic iron supplementation on birth weight, prematurity, perinatal mortality, incidence of malaria, and other pregnancy and birth outcomes. Consequently, the evidence they provide is insufficient in addressing the question of the advantages and disadvantages of prenatal prophylactic iron. The results of studies from non-malarial areas[15-21], although of better quality, may not be relevant due to different settings.[15-21] Although, results were also conflicting in a number of outcomes, the main findings included slightly longer birth length, longer gestational age, and reduced risk of preterm delivery, intrapartum hemorrhage, low birth weight, and infant and child mortality in the iron-folic acid group (Nwaru et al *Submitted*).

This limited evidence and the importance of iron prophylaxis in prenatal programs call for further investigation on the benefits of prenatal iron supplementation in areas of endemic malaria and high prevalence of HIV. Using a pragmatic randomized controlled trial, we investigated the effects of routine iron prophylaxis throughout pregnancy compared to screening and treatment for anemia on maternal and child health in Maputo, Mozambique. The present paper presents the pregnancy results and interim birth results. About 40% of births were missed by the original data collection method (Nwaru et al *Submitted)*, and missing birth data are currently being retrieved with various complementary methods. The completed birth results will be presented later.

MATERIALS AND METHODS

Study design and population

The details of the PROFEG trial have been described elsewhere (Nwaru et al *Submitted*) and only the main features are given here. The trial was a pragmatic randomized controlled trial to compare two iron administration policies (routine iron prophylaxis versus screening and treatment for anemia during pregnancy) on maternal and child health in Maputo, Mozambique. The trial was carried out in two health centers, 1º de Maio in Maputo City (the capital) Machava 2, in Maputo Province, in 2007-2008; the completion of collection of birth data continued until 2012.

Recruitment

During the routine early morning health education sessions, all women who came for their first prenatal visit were given general information about the study. Recruitment into the study occurred during individual consultations and was carried out by study nurses who were employed and trained by the project. They carried a recruitment book in which they entered the information of the recruited women. In 1º de Maio health center, the women visited the study nurses after their routine

prenatal care consultations with the maternal and child health (MCH) nurses. In Machava, the study nurse and the routine MCH nurse saw the women in the same room. The study nurses checked for women's eligibility to participate in the study. All pregnant women attending their first prenatal visit were the target group. The exclusion criteria were: woman missed attending a visit with the study nurses; too early in pregnancy (< 12 weeks); with high obstetric risk; and aged less than 18 years. The nurses asked the women to join the study if they did not meet any of the exclusion criteria. Oral and written informed consent was obtained.

Randomization

The participating women were randomized into either the Routine iron group (i.e., routine iron prophylaxis from the first to the last prenatal visit) or the Selective iron group group (i.e. regular screening for hemoglobin level and treatment for anemia). Researchers (OA) used the STATA statistical software (StataCorp LP, Texas, USA) was used to generate sequential random numbers separately for the two centers and the women were assigned to either of the groups with a probability of 50%. The codes for the groups were put into sealed and numbered opaque envelopes; the number was the woman's study number and was repeated in the documents in the envelope. The envelope contained a study identification card (yellow for the Routine iron group and pink for the Selective, 10 x 20cm) and the informed consent form.

Sample size

Because of lack of prior reliable data on the baseline rates of the impact of iron, the sample size was calculated with various assumptions of the base-line rates, power (85% and 90%), significance level of 5%, and the size of the difference to be detected (20% and 30%) for pre-term delivery, low birth weight, clinical malaria, and perinatal mortality. Based on these calculations and the expected

feasibility, we decided a target sample size of 2000 women in each of the two groups. The STATA statistical software was used to estimate the sample size.

Interventions

Women in the Routine iron group received 30 tablets (supply of one month) of 60 mg ferrous sulphate plus 400 µg of folic acid per day. Women in the Selective iron were given 30 tablets of 1 mg of folic acid per day. In the Selective group, at each visit, the nurses measured the hemoglobin using a rapid hemoglobin measure, HemoCue® Hb 201+, (Hemocue AB, Ängelholm, Sweden). If their hemoglobin was below the cut-off of <9g/l Hb, they received a monthly double dose of iron (60 mg + 60 mg) for the treatment of anemia. The tablets were given in a plastic bag having the drug's name and dose on it.

Data collection and follow-up

Data were collected on standard study data forms by three methods: 1) study nurses abstracted data from mothers' maternity cards, 2) study nurses asked women additional questions at the time of the prenatal visits, and 3) researchers afterwards collected birth data from hospital birth records. Delivery nurses were informed of the study and asked to put the delivery cards into a separate study box. The study women were to be identified by the color of the identification card stapled to their maternity card. However, this did not succeed very well. By excluding estimated late miscarriages (5%), early stillbirths (3 %) and home births (10%), we should have received delivery data for 3547 women (82%) of the 4326 women who participated in the trial. We received birth data for only 2258 (64% of the estimated 3547) women.

Outcome measures

The primary outcomes were preterm delivery (delivery <37 weeks of gestation and low birth weight (<2500 grams). Originally, we had malaria activation as a primary outcome, but the pilot showed that it was not feasible. Secondary outcomes were perinatal mortality (as available from our data collection forms; unlikely to cover early stillbirths or neonatal births occurring at home); complications during pregnancy and labor; symptoms suggestive of malaria (fever, headache, cold/chills, nausea/vomiting, and body aches); and self-reported malaria during pregnancy (the woman was asked for diagnosed malaria since her last visit).

Calculation of gestational age at birth

Only 681 (30%) of the women with delivery data had their gestational age recorded at birth. The gestational age for women without that information was estimated from dates using the following algorithm: gestational age at first visit in days + days between the first visit and delivery; the days were then transformed into weeks. For some women (n = 196), the date of delivery was not available. In these cases, date of discharge from the hospital after delivery (minus the length of stay at the hospital) (n = 22) or the date of admission to the hospital (n = 60 women who did not have the date of discharge) was used.

Adherence

The women were instructed and encouraged at each visit to take the tablets they were given. Women allocated to the Routine iron group could refuse to take the iron tablets and they were classified as non-compliant with the intervention. Women who belonged in the Selective iron group and who wanted iron (even if their hemoglobin level was not below the cut-off level) were given iron; they were classified as non-compliant with the intervention. The following questions were asked on each visit: “Was hemoglobin measured?”; “Was iron/folic acid given to the woman?”; “Number of iron/folic acid tablets given?”; “Did the woman take the tablets during the past week?”

At each subsequent visit, almost all of the Selective iron women (98%) were measured for hemoglobin using the recommended HemoCue® method and the same proportion of women in the Routine iron group were given iron tablets at each subsequent visit.

Ethical approval

Ethical approval for the study was obtained from the Mozambique Ministry of Health Ethics Committee (CNBS [Ref. 84/CNBS/06]). A positive statement was obtained from the National Research and Development Centre for Welfare and Health (STAKES) (now the National Institute for Health and Welfare), Helsinki, Finland (Dno 2571/501/2007). The trial is registered at ClinicalTrials.gov, number NCT00488579.

Statistical analysis

All analyses were done on an intention-to-treat basis. Twin pregnancies (n = 48 pairs) were included in the analysis because their numbers were similar in the two groups and their exclusion did not alter the results. For pregnancy outcomes, all women (n = 4326), and for birth outcomes, women with birth data (n = 2258) were included. Differences in health indicators (fever, headache, cold/chills, nausea/vomiting, body aches, malaria) between the two iron groups at each subsequent visit (up to the 5th visit) during pregnancy were analyzed by using binomial generalized estimating equations (GEE) with an exchangeable correlation structure. GEE takes into account the within person correlation in the setting of repeated measures.

Differences in continuously distributed birth outcomes (birth weight, duration of gestation, length of hospital stay) were analyzed by using the two sample Student's t-test. Categorical outcomes were analyzed by using Pearson Chi-square test or Fisher's exact test (in the case of cells with less than 5 cases). To estimate the risk ratios of the effect of iron, the binary birth outcomes (low birth weight

[< 2500 g], preterm birth [< 37 weeks], cesarean section delivery, child and maternal ill-health or death at birth, negative fetal heart beat, delivery in a reference health center, long hospital stay after birth [≥ 2 days], and unavailability of delivery data) were analyzed by generalized linear models. The result estimates are presented with 95% confidence intervals (95% CI). Statistical significance was set at $P < 0.05$. STATA 11 statistical software was used for the analyses.

RESULTS

Of the 4326 women recruited to the trial, 2184 were randomly allocated to the Routine iron group and 2142 to the Selective iron group (Figure 1). The total number of prenatal visits varied but the maximum number of visits was seven. The number of follow-up visits was similar in the two groups (Figure 1). About 40% of delivery data were missed when using the original data collection method and the interim birth data were available for 1109 (51%) in the Routine iron group and for 1149 (54% of women) in the Selective.

Table 1 compares maternal background characteristics between the groups by the availability of birth data. The occurrence of symptoms suggestive of malaria (fever, headache, cold/chills, nausea/vomiting, and body aches) and self-reported malaria during the current pregnancy prior to the first prenatal visit were similar between the Routine and Selective iron groups. The women in the two groups with and without birth data were comparable.

Between the first and fifth visits, the two groups were similar regarding the occurrence of fever, headache, cold/chills, nausea/vomiting, and body aches (Table 2). There was a suggestion of increased incidence of self-reported malaria during pregnancy (odds ratio 1.37, 95% confidence interval 0.98-1.92) in the Routine iron group (Table 2). Table 2 presents the data for the second and third follow-up visits, but this was the case also in subsequent visits (data not shown).

Tables 3 presents the distribution of birth data by intervention group and Table 4 gives the estimates of the effect sizes on the birth outcomes. The birth outcomes were similar in the two groups. However, there was a suggestion (statistically non-significant) that the Routine iron group had worse outcomes in regard to babies with negative heartbeat at admission, and longer mother's hospital stay after birth (Table 3). The effect of iron on the primary outcomes was similar in the two groups. The groups were also relatively similar concerning most other outcomes. However, there was a suggestion of more babies with negative fetal heartbeat at admission, longer mother's hospital stay after birth and unavailability of delivery data in the Routine iron group (Table 4). By excluding births by cesarean section, the estimates for longer mother's hospital stay remained the same (data not shown).

DISCUSSION

The results from this trial indicate that routine iron prophylaxis during pregnancy was not advantageous over the policy of screening and treatment for anemia with regard to pregnancy and birth outcomes. If anything, screening and treatment for anemia appeared to be better. Among all the trial women there was a suggestion of an increased risk of self-reported malaria during pregnancy seen in the Routine iron group. The interim birth data suggested longer hospital stay after birth and higher risk of negative fetal heart beat in the Routine iron group. However, all these differences were statistically non-significant and the complete birth data are needed to conclude any putative effects of iron on birth outcomes.

One of the strengths of our trial is its large sample. So far, this represents the largest trial investigating the benefits of prophylactic iron during pregnancy on maternal and child health in

malaria-endemic settings. The compliance of the study nurses with the trial protocol and that of the women with regards to uptake of the iron and folic acid tablets was good, as was the follow-up during pregnancy (Nwaru et al *Submitted*). However, during pregnancy, we lacked objective measures of malaria; hence, our results may not reflect the putative effect of iron on clinical malaria. The collection of delivery data was challenging, resulting in up to an estimated 40% of missing birth data. We did not realize the extent of the problem until most deliveries had occurred. We are currently tracing the birth data using various methods (abstracting hospital records and death register data and calling women), with results to be reported separately after finalization.

A comparison of our findings with previous studies conducted in malaria endemic areas is problematic because of key differences: the previous studies have compared iron versus no iron and our study compares two policies of iron administration: routine prophylaxis versus screening and treatment. Nevertheless, the studies from Nigeria[11] and The Gambia[12] found no significant effect of iron prophylaxis on malaria; they had used a more reliable measure of malaria (clinical and parasitological analysis). A Ugandan study[14] did not observe any effect of iron supplementation on the incidence of congenital malaria in the offspring. A Bangladeshi study[10] found a difference in preterm delivery (less in the non-iron group), but no association was seen with other outcomes examined, similar to the Nigerian study[11], including abortion, hypertension, eclampsia, postnatal complications, birth weight, Apgar scores, prematurity, development of diarrhea at 6 weeks, and perinatal mortality. Other benefits reported with iron prophylaxis include increased mean birth weight[12,14], reduced incidence of prematurity[12], and increased birth length and Apgar score.[13]

Although more complete birth data are needed to reach firm conclusions, we can speculate that the potential for higher incidence of unavailable delivery data in the Routine iron group may indicate that these women had more adverse outcomes, such as miscarriage and stillbirths, and consequently did not deliver in the expected health centers. Similarly, the higher likelihood of longer mother's hospital stay after birth in the Routine iron group may also be indicative of more problems at birth. Delivery by cesarean section did not explain the longer hospital stay as the estimate remained the same after excluding the births that occurred by cesarean section.

Anemia has been associated with maternal and child health risks[22-24], and the association between iron and increased risk of infections[5-7] calls for more definitive evidence on the benefits of iron prophylaxis during pregnancy in settings with increased infectious diseases where infections remain a major cause of maternal and child mortality.[8,9] Our trial in Maputo, Mozambique, is an attempt to investigate whether routine iron prophylaxis during pregnancy is more effective than screening and treatment for anemia in improving maternal and child health in an area of endemic malaria and HIV.

CONCLUSIONS

These interim results from this pragmatic randomized controlled trial indicate that routine iron prophylaxis during pregnancy did not suggest better maternal and child health outcomes than the policy of screening and treatment for anemia. If anything, screening and treatment for anemia appeared to be better. The complete birth data are needed for a firm conclusion.

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AUTHORS' CONTRIBUTION

Designed, analyzed, and wrote the paper (BIN, EH, ER, and SP). Designed and responsible for the conception of the PROFEG Trial (EH). Participated in the planning of the PROFEG Trial and made substantial contribution in its execution and participated in interpretation of results and critically reviewing the manuscript (BC, CS, EH, FA, GS, JC, MD, MN, OA, SP). Responsible for data preparation and cleaning (ER, OA).

COMPETING INTEREST

None declared.

DATA SHARING

Statistical codes and dataset are available from the corresponding author bright.nwaru@uta.fi. Informed consent was not obtained for data sharing but the presented data are anonymised and risk of identification is low.

REFERENCES

1. Peña-Rosas JP, Viteri FE. Effects and safety of preventive oral iron or iron+folic acid supplementation for women during pregnancy. Cochrane Database of Systematic Reviews 2009, Issue 4, art. No.: CD004736.
2. Villar J. Merialdi M, Gulmezoglu AM, Abalos E, Carroli G, Kulier R, et al. Nutritional interventions during pregnancy for the prevention or treatment of maternal mortality and preterm delivery: an overview of randomized controlled trials. *J Nutr* 2003; 133:1606-25.
3. Lao TT, Tam KF, Chan LY. Third trimester iron status and pregnancy outcome in non-anaemic women: pregnancy unfavourably affected by maternal iron excess. *Hum Reprod* 2000; 15: 1843-48.
4. Yip R. Significance of an abnormally low or high hemoglobin concentration during pregnancy: special consideration of iron nutrition. *Am J Clin Nutr* 2000 72: 272S-9S.
5. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. *J Nutr* 2001; 131: 616S-35S.
6. Prentice AM. Iron metabolism, malaria, and other infections: what is all the fuss about? *J Nutr* 2008; 138: 2537-41.
7. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. *BMJ* 2002; 325: 1142.
8. Lawn JE, Cousens S, Zupan J, Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? *Lancet* 2005; 365: 891-900.
9. Idemyor V. Human Immunodeficiency Virus (HIV) and Malaria Interaction in Sub-Saharan Africa: The Collision of Two Titans. *HIV Clin Trials* 2007; 8: 246-53.

10. Juncker T, Ameer S, Mmaum A, et al. A trial of iron and folate supplementation during pregnancy in Bangladesh. Abstract presented at the American Public Health Association, 2001, Atlanta, USA.

11. Fleming AF, Ghatoura GB, Harrison KA, Briggs ND, Dunn DT. The prevention of anaemia in pregnancy in primigravidae in the guinea savanna of Nigeria. *Ann Trop Med Parasitol* 1986; 80: 211-33.

12. Menendez C, Todd J, Alonso PL, Francis N, Lulat S, Ceesay S, et al The effects of iron supplementation during pregnancy, given by traditional birth attendants, on the prevalence of anaemia and malaria. *Trans R Soc Trop Med Hyg* 1994; 88: 590-3.

13. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. *Am J Clin Nutr* 1997; 66: 1178-82.

14. Ndyomugyenyi R, Magnussen P. Chloroquine prophylaxis, iron/folic-acid supplementation or case management of malaria attacks in primigravidae in western Uganda: effects on congenital malaria and infant haemoglobin concentrations. *Ann Trop Med Parasitol* 2000; 94: 759-70.

15. Christian P, Khatry SK, Katz J, Pradhan EK, LeClerg SC, Shrestha SR, et al. Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal: a double blind randomized community trial. *BMJ* 2003; 326: 571.

16. Christian P, West KP, Khatry SK, LeClerg SC, Pradhan EK, Katz J, et al. Effects of maternal micronutrient supplementation on fetal loss and infant mortality: a cluster-randomized trial in Nepal. *Am J Clin Nutr* 2003; 78: 1194-1202.

17. Christian P, Darmstadt GL, Wu L, Khatry SK, LeClerg SC, Katz J, et al The effect of maternal micronutrient supplementation on early neonatal morbidity in rural Nepal: a randomized, control, community trial. *Arch Dis Child* 2008; 93: 660-4.

18. Christian P, Khatry SK, LeClerq SC, Dali SM. Effects of prenatal micronutrient supplementation on complications of labor and delivery and puerperal morbidity in rural Nepal. *Int J Gynaecol Obstet* 2009; 106: 3-7.

19. Christian P, Stewart CP, LeClerq SC, Wu L, Katz J, West KP Jr, et al. Antenatal and postnatal iron supplementation and childhood mortality in rural Neap: a prospective follow-up in a randomized, controlled community trial. *Am J Epidemiol* 2009; 170: 1127-36.

20. Christian P, Murray-Kolb LE, Khatry SK, Katz J, Schaefer BA, Cole PM, et al. Prenatal micronutrient supplementation and intellectual and motor function in early school-aged children in Nepal. *JAMA* 2010; 304: 2716-23.

21. Zeng L, Dibley MJ, Cheng Y, Dang S, Chang S, Kong L, et al. Impact of micronutrient supplementation during pregnancy on birth weight, duration of gestation, and perinatal mortality in rural western China: double blind cluster randomized controlled trial. *BMJ* 2008; 337: a2001.

22. Müngen E. Iron supplementation in pregnancy. *J Perinat. Med* 2003; 31: 420-6.

23. Brabin BJ, Hakimi M, Pelletier D. An analysis of anemia and pregnancy-related maternal mortality. *J Nutr*. 2001; 131(suppl): 604S-15S.

24. Stoltzfus RJ, Mullanny L, Black RE. Iron deficiency anaemia. In: Ezzati M, Lopez AD, Rodgers A. et al, eds. Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributable to Selected Major Risk Factors. Vol 1. Geneva, Switzerland: World Health Organization; 2004: 163-209.

Table 1. Characteristics of women at recruitment by availability of delivery data and group allocation, proportions % (numbers)

Characteristics	Delivery data, *N* = 2258		No delivery data, *N* = 2068	
	Routine iron (1109)	Selective iron (1149)	Routine iron (1075)	Selective iron (993)
Maternal age, mean (SD) years	24.7 (5.3)	24.6 (5.4)	24.6 (5.6)	25.0 (5.6)
Maternal age (categorized)				
< 20 years	16.5 (183)	17.5 (201)	19.3 (207)	15.7 (156)
20-24 years	39.9 (443)	41.1 (472)	37.1 (399)	39.0 (387)
25-29 years	23.3 (258)	23.1 (265)	23.4 (252)	23.9 (237)
30-34 years	13.8 (153)	11.3 (130)	13.3 (143)	12.9 (128)
≥ 35 years	5.6 (62)	6.3 (72)	6.5 (70)	7.4 (74)
Missing	0.9 (10)	0.8 (9)	0.4 (4)	1.1 (11)
Previous abortions				
No	86.8 (963)	87.6 (1007)	85.4 (918)	86.0 (854)
Yes	12.8 (142)	12.1 (139)	14.5 (156)	13.6 (135)
Missing	0.4 (4)	0.3 (3)	0.1 (1)	0.4 (4)
Gestational age, mean (SD) weeks	10.2 (5.8)	10.3 (6.0)	10.1 (6.1)	10.5 (6.0)
Gestational age (categorized)				
< 10	26.5 (294)	25.9 (298)	28.8 (310)	25.3 (251)
10-14	31.0 (344)	32.2 (370)	30.2 (325)	30.5 (303)
15-28	31.9 (354)	30.4 (349)	28.5 (306)	31.2 (310)
> 28	10.1 (112)	11.3 (130)	12.4 (133)	12.6 (125)
No information	0.5 (5)	0.2 (2)	0.1 (1)	0.4 (4)
Previous stillbirths				
	92.3 (1024)	91.5 (1052)	91.0 (978)	91.6 (910)

No	7.2 (80)	8.2 (94)	8.9 (96)	7.9 (78)
Yes	0.5 (5)	0.3 (3)	0.1 (1)	0.5 (5)
Missing				
Previous deliveries				
None	30.3 (336)	29.7 (341)	33.8 (363)	29.2 (290)
One	31.7 (352)	31.9 (367)	28.5 (306)	30.6 (304)
Two	17.8 (197)	19.2 (221)	18.6 (200)	17.9 (178)
Three or more	19.8 (220)	18.8(216)	19.0 (205)	22.0 (218)
Missing	0.4 (4)	0.4 (4)	0.1 (1)	0.3 (3)
HIV status				
Negative	81.8 (907)	81.2 (934)	79.0 (849)	76.7 (762)
Positive	18.2 (202)	18.8 (215)	21.0 (226)	23.3 (231)
Twin pregnancy				
No	98.6 (1093)	98.7 (1134)	99.2 (1066)	99.2 (985)
Yes	1.4 (16)	1.3 (15)	0.8 (9)	0.8 (8)
Symptoms during current pregnancy before first prenatal visit				
Fever				
Yes	24.4 (271)	22.9 (264)	23.8 (256)	28.8 (286)
Headache				
Yes	43.5 (482)	41.5 (477)	43.0 (462)	44.3 (440)
Cold/chills				
Yes	18.4 (204)	18.0 (207)	18.8 (202)	20.6 (205)
Nausea/vomiting				
Yes	26.9 (298)	27.5 (316)	28.6 (307)	29.8 (296)

Body aches				
Yes	21.3 (237)	21.8 (251)	23.3 (251)	23.8 (236)
Self-reported malaria				
Yes	6.0 (67)	5.7 (66)	6.3 (68)	5.9 (59)
Had malaria test				
Yes	7.1 (79)	7.0 (80)	7.9 (85)	8.0 (79)

Table 2. Proportions (%) of women (numbers) with outcomes suggesting malaria during pregnancy, and odds ratios (OR) and 95% confidence intervals (95% CI) for group effect, $n = 4326$

Outcomes	Second visit[1] % (n)		Third visit[2] % (n)		Between the first and fifth visit[3] OR (95% CI)		
	Routine iron $n = 1494$	Selective iron $n = 1455$	Routine iron $n = 1106$	Selective iron $n = 1040$	Selective iron	Routine iron	P-value
Fever	10.0 (150)	11.5 (168)	12.1 (134)	11.3 (117)	1.00	0.95 (0.81-1.11)	0.523
Headache	24.3 (363)	24.9 (363)	25.1 (278)	24.9 (259)	1.00	0.98 (0.87-1.10)	0.738
Cold/chills	7.0 (104)	8.2 (120)	7.8 (86)	6.7 (70)	1.00	0.92 (0.76-1.11)	0.361
Nausea/vomiting	10.2 (153)	9.1 (133)	9.6 (109)	8.5 (88)	1.00	1.09 (0.92-1.31)	0.323
Body aches	9.2 (138)	10.1 (147)	9.8 (108)	10.9 (113)	1.00	0.89 (0.75-1.06)	0.180
Self-reported malaria	3.0 (45)	2.4 (35)	2.2 (24)	1.5 (16)	1.00	1.37 (0.98-1.92)	0.068

[1]Betetween first and second visit

[2]Between second and third visit

[3]The effect estimates calculated by binomial generalized estimating equations (with exchangeable correlation structure) to account for the repeated measures of the outcomes.

Table 3. Birth outcomes by group allocation, percentages, % (numbers) of women or babies or means (SD).

Outcomes	Routine iron *n* =1109	Selective iron *n* = 1149	*P*-value[1]
Birth weight, mean (SD) grams	2989.4 (514.9)	2996.3 (508.4)	0.752
Birth weight, % (*n*)			0.443
< 2500 g	12.8 (142)	11.8 (136)	
2500-2999 g	31.1 (345)	30.6 (351)	
3000-3499 g	37.8 (419)	40.5 (465)	
3500-3999 g	13.8 (153)	12.7 (146)	
≥ 4000 g	2.1 (23)	3.0 (34)	
No information	2.4 (27)	1.5 (17)	
Duration of gestation, mean (SD) weeks	38.4 (4.0)	38.3 (4.2)	0.689
Duration of gestation, % (*n*)			0.056
< 37 weeks	27.0 (299)	28.8 (331)	
≥ 37 weeks	66.9 (742)	67.2 (772)	
No information	6.1 (68)	4.0 (46)	
Mode of delivery, % (*n*)			0.235
Normal	89.4 (991)	87.6 (1007)	
Cesarean section	2.0 (22)	1.3 (15)	
No information	8.7 (96)	11.1 (127)	
Child health status at birth, % (*n*)			0.685
Well	92.1 (1022)	94.0 (1080)	
Ill	1.0 (11)	0.7 (8)	
Dead	2.0 (22)	1.8 (21)	
No information	5.0 (55)	3.5 (40)	

Still birth, % (*n*)			0.558
No	79.7 (884)	81.2 (933)	
Yes	2.9 (32)	2.5 (29)	
No information	17.4 (193)	16.3 (187)	
Fetal heart beat at admission, % (*n*)			0.085
Negative	2.6 (29)	1.6 (18)	
Positive	85.2 (945)	85.6 (984)	
No information	12.2 (135)	12.8 (147)	
Mother's health status at birth, % (*n*)			0.895
Well	94.9 (1052)	95.6 (1098)	
Ill	0.4 (4)	0.4 (4)	
Dead	0.2 (2)	0.1 (1)	
No information	4.6 (51)	4.0 (46)	
Length of hospital stay, mean (SD) days	1.63 (1.30)	1.33 (1.21)	0.075
Length of hospital stay after birth, % (*n*)			0.103
≤ 1 day	60.7 (673)	65.1 (748)	
2 days	24.2 (268)	23.5 (270)	
≥ 3 days	5.6 (62)	4.0 (46)	
No information	9.6 (106)	7.4 (85)	
Place of delivery, % (*n*)			0.652
1o de Maio (health center)	42.6 (472)	44.4 (510)	
Machava (health center)	38.2 (424)	35.1 (403)	
Jose Macamo (hospital)	3.6 (40)	3.7 (43)	
Mavalane (hospital)	12.8 (142)	14.3 (164)	

Central Hospital	0.3 (3)	0.3 (3)
At home	1.3 (14)	1.1 (13)
On the way to hospital	0.2 (2)	0.0 (0)
No information	1.1 (12)	1.1 (13)

[1]Based on T-test for continuous outcomes, Pearson Chi-square test or Fisher's exact test for categorical outcomes. Subjects with no information were not included in the tests.

Table 4. Numbers and proportions (%) by iron groups, and relative ratio (RR) and risk difference (RD) and their 95% confidence intervals (95% CI) of the effect of routine iron on birth outcomes

Outcomes	Routine iron *n*	Selective iron *n*	Routine iron %	Selective iron %	Effects of routine iron	
					RR (95% CI)	RD (95% CI)
Primary health outcomes						
Low birth weight (<2500 grams)	142	136	12.8	11.8	1.09 (0.88-1.36)	0.01 (-0.02-0.04)
P-value					0.431	0.469
Preterm delivery (<37 weeks)	299	331	27.0	28.8	0.96 (0.84-1.09)	-0.02 (-0.05-0.02)
P-value					0.185	0.340
Secondary health outcomes						
Cesarean section delivery	22	15	2.0	1.3	1.48 (0.77-2.84)	0.01 (-0.00-0.02)
P-value					0.238	0.191
Negative fetal heart beat at admission	29	18	2.6	1.6	1.66 (0.93-2.96)	0.01 (-0.00-0.02)
P-value					0.089	0.097
Child ill or dead at birth	33	29	3.0	2.5	1.20 (0.73-1.96)	0.01 (-0.01-0.02)
P-value					0.473	0.467
Mother ill or dead at birth	6	5	0.5	0.4	1.25 (0.38-4.09)	0.00 (-0.00-0.01)
P-value					0.711	0.722
Other outcomes						
Delivery in reference center[1]	185	210	16.7	18.3	0.91 (0.76-1.09)	-0.02 (-0.05-0.02)
P-value					0.316	0.317
Long hospital stay after delivery (≥ 3 days)	62	46	5.6	4.0	1.43 (0.97-1.26)	0.02 (-0.00-0.03)
P-value					0.059	0.075
No delivery data	1075	993	49.2	46.4	1.06 (1.00-1.13)	0.03 (-0.01.0.07)
P-value					0.060	0.183

[1]Jose Macamo or Mavalane or Central Hospital

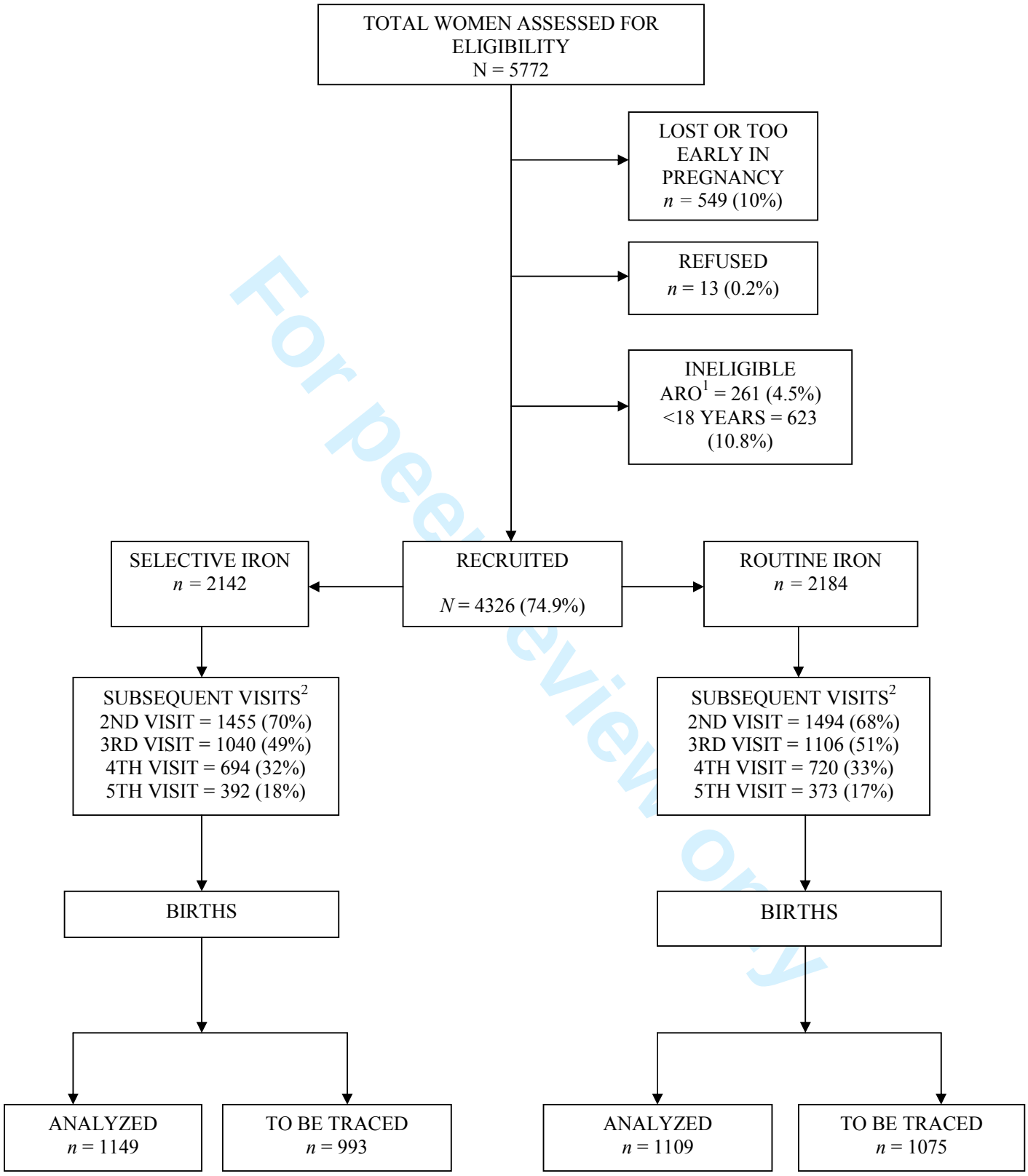

Figure 1. PROFEG Trial Flow Diagram

[1]ARO = high risk pregnancy; [2]After recruitment, % were calculated from recruited, *n* = 4326

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2, 3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5,6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	None
Participants	4a	Eligibility criteria for participants	7
	4b	Settings and locations where the data were collected	6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	None
Sample size	7a	How sample size was determined	7,8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6,7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	Not done.

		assessing outcomes) and how	Pragmatic trial design
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	10,11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Not done
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	11, Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	11, Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6
	14b	Why the trial ended or was stopped	Ended as planned
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	11, Tables 2-4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	11,12, Tables 2-4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Table 4
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	None
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA (benefits and harms not distinguished)
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13, 14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13, 14
Other information			
Registration	23	Registration number and name of trial registry	3
Protocol	24	Where the full trial protocol can be accessed, if available	From Authors
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	3, 15.

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Original Article

A pragmatic randomised controlled trial on routine iron prophylaxis during pregnancy in Maputo, Mozambique (PROFEG): rationale, design, and success

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Abstract

The effects of prophylactic iron during pregnancy on maternal and child health in developing settings with endemic malaria and high prevalence of HIV remain unclear. This paper describes the rationale, implementation and success of a pragmatic randomised controlled trial comparing routine iron supplementation vs. screening and treatment for anaemia during pregnancy. The setting was two health centres in Maputo, Mozambique. Pregnant women (≥12-week gestation; ≥18 years old; and not with a high-risk pregnancy, $n = 4326$) were recruited. The main outcomes are preterm delivery and low birthweight. The women were randomly assigned to one of two iron administration policies: a routine iron group ($n = 2184$) received 60 mg of ferrous sulphate plus 400 μg of folic acid daily while a selective iron group ($n = 2142$) had screening and treatment for anaemia and a daily intake of 1 mg of folic acid. The recruitment, follow-up, and collection of follow-up data were successful; both groups were similar to each other in all the trial stages. Collection of delivery data was challenging and data on about 40% of births is missing. These are currently being traced through different hospitals and health centres. The compliance of the study personnel and the women with regard to regular measurement of haemoglobin and intake of the iron and folic acid tablets was high and similar in both trial arms. Taking into account the various constraints encountered, the stages of the present trial prior to delivery were carried out well.

Keywords: iron, clinical trials, micronutrients, pregnancy, pregnancy outcomes, birth outcomes, developing countries, malaria, infections, HIV.

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Trial Registration: The trial is registered at ClinicalTrials.gov, number NCT00488579 (June 19, 2007). The first women were randomised to the trial in November 2006.

Introduction

Iron deficiency remains a major public health concern in most developing countries, particularly among pregnant women and children (Stoltzfus 2001; McLean *et al*. 2009). Iron-deficiency anaemia in pregnancy is associated with maternal and child health risks (Brabin *et al*. 2001; Stoltzfus *et al*. 2004). On the other hand, high haemoglobin concentrations in late pregnancy also correlate with adverse effects on pregnancy (Lao *et al*. 2000; Yip 2000). Because of poor intake and low bioavailability of iron from many foods, iron supplementation has been widely recommended during pregnancy (Müngen 2003; Peña-Rosas & Viteri 2009). Studies show that prophylactic iron supplementation reduces the risk of anaemia (Yip 2000). But beyond the reduction of anaemia risk, the effects of routine iron prophylaxis on maternal and child health in developing settings remain unclear. Cochrane reviews and the World Health Organization (WHO) overviews of randomised controlled trials (RTCs) and a study in Finland compar-

ing routine and selective iron prophylaxis (based on haemoglobin level) have failed to conclude whether routine iron prophylaxis during pregnancy is beneficial or harmful (Hemminki & Rimpelä 1991; Yip 2000; Villar *et al*. 2003).

The potential of iron to advance infections raises serious concerns for developing settings (Oppenheimer 2001; Gera & Sachdev 2002; Prentice 2008). Infectious agents need iron for replication (Prentice 2008). In sub-Saharan African countries, a large proportion of maternal and child mortality is attributed to infections during pregnancy (Lawn *et al*. 2005; Idemyor 2007), malaria and HIV being the main health risks (Idemyor 2007). Malaria may modify the effects of iron therapy, as some evidence suggests differences in the metabolism of iron between malarial and non-malarial subjects (Gera & Sachdev 2002; Prentice 2008). Some studies show that malaria may be less prevalent and milder among pregnant iron-deficient women (Kabyemela *et al*. 2008). A review by Oppenheimer (2001) showed that five out of nine controlled trials among non-pregnant subjects in malarial areas indicated that iron supplementation increased the rates of clinical episodes of malaria and increased morbidity from other infections. A Tanzanian trial (Sazawal *et al*. 2006) among young children in an endemic malaria area found that those who received iron plus folic acid were more likely to die or be treated in a hospital than those who received placebo. That study recommended treatment of children after screening rather than routine supplementation. On the contrary, a Cochrane review on iron supplementation for preventing or treating anaemia among children in malaria-endemic areas concluded that iron does not increase the risk of clinical malaria when regular screening and treatment of malaria are provided (Ojukwu *et al*. 2009). We found no trial on the effect of iron on HIV infection, but some studies suggest a harmful effect of iron on the progression of HIV infections (Boelaert *et al*. 1996; Gordeuk *et al*. 2001).

The effects of iron on health outcomes in populations with concomitant endemic malaria, iron-deficiency anaemia and on the prevalence of HIV are unexplored (Adetifa & Okomo 2009). There is an urgent need for trials to assess the effects on maternal and child health of prophylactic iron supplementation during pregnancy in populations where iron-deficiency anaemia, endemic malaria and HIV are prevalent (Adetifa & Okomo 2009).

The aim of this paper was threefold: (1) to give the rationale for a pragmatic randomised trial (PROFEG) comparing the effects of two policies on iron administration during pregnancy (routine prophylactic iron supplementation vs. screening and treatment for anaemia) on maternal and newborn health in Maputo, Mozambique, a setting of endemic malaria and high prevalence of HIV; (2) to describe the study design; and (3) to describe the implementation and compliance.

Previous trials on iron supplementation during pregnancy

A PubMed search of trials on the effects of iron supplementation during pregnancy and on birth outcomes in developing countries was carried out up to March 2011. Using a combination of key search terms (iron, iron + folic acid, micronutrients, pregnancy,

Key messages

- Beyond the reduction of the risk of anaemia, the effects of routine iron prophylaxis on maternal and child health in developing settings remain unclear.
- The effects of iron on health outcomes in populations with concomitant endemic malaria, iron-deficiency anaemia and high prevalence of HIV are unexplored.
- We used a pragmatic randomised trial to compare the effects of two policies of iron administration during pregnancy (routine prophylactic iron supplementation vs. screening and treatment for anaemia) on maternal and newborn health in Maputo, Mozambique, a setting of endemic malaria and high prevalence of HIV.
- Taking into account the various constraints encountered, the planned trial proved feasible in an ordinary health care setting in Maputo.

pregnancy outcomes, birth outcomes, developing countries, malaria, infections, HIV), we extracted all relevant RCTs, quasi-RCTs and other controlled clinical trials. The bibliographies of eligible papers were scrutinised to identify additional potential studies. The appendix shows the flow of the literature search process.

We only included studies that had iron or iron plus folic acid only or in combination with other micronutrients as the intervention and a placebo or alternative that has no iron in it as the control. Some studies examined the effects of micronutrient supplementation during pregnancy on pregnancy and birth outcomes in low-income countries (Fall *et al*. 2009). We excluded these studies if iron was both in the intervention and the control groups.

The titles and abstracts of identified studies were checked, and the full text of all potentially eligible studies {except for one unpublished study that had only an extended conference abstract [Juncker *et al*. American Public Health Association (APHA), Atlanta, 2001] } was assessed. Table 1 presents the features and results from the trials included in this summary. The eligible studies were categorised according to the WHO classification (http://www.malaria.org/ABOUT%20MALARIA/Vaccination%20requirement%20and%20malaria%20situation%20WHO.pdf) into malarial and non-malarial areas. The Bangladeshi trial [APHA, Atlanta, 2001 (T. Juncker *et al*., unpublished observations)] was carried out in rural Dhaka, and we classified it into the malarial areas, considering that most parts of the country have malaria, although the city of Dhaka is classified as a non-malarial area (WHO International Travel and Health 2011). We contacted (E-mail correspondence) the authors of that study, but they were unsure of the malaria situation of the trial setting at the time of the study.

Five trials were carried out in malaria-endemic areas. A small trial from Nigeria by Fleming *et al*. (1986) investigated the effects of anti-malarial, iron and folic acid prophylaxis on maternal and child health, including malaria, among primigravid women in comparison with a control group. No significant effects of iron were seen on any of the outcomes investigated. However, because of poor compliance, large number of dropouts, several exclusions by outcomes, particularly severe anaemia, no viable conclusions can be made from that study. The study by Menendez *et al*. (1994) among multigravid poor pregnant women from rural Gambia found a beneficial effect of iron on birthweight {56-g [95% confidence interval (CI) 12–128] increase} and prematurity. However, outcome-related exclusions (27%), such as preterm delivery and anaemia, were problematic. In a rather small trial from Niger (after recruitment at a mean pregnancy of 28 weeks), iron had a beneficial effect on birth length [0.7-cm (95% CI 0.05–1.35) increase] and Apgar score [0.4 (95% CI 0.16–0.96) increase], but not on birthweight (Preziosi *et al*. 1997). However, the small number of women undermines the reliability of the results.

A trial from Uganda found a beneficial effect of iron on birthweight with a 82-g (95% CI 81–83) increase (2999 vs. 2917 g for the iron and placebo arms of the trial, respectively) Ndyomugyenyi & Magnussen 2000). Because of a large number of dropouts and many outcome-related exclusions – inclusion of women who developed anaemia or caught malaria, and late weighing of the baby – the study falls short in addressing our research question. The trial from rural Dhaka, Bangladesh enrolled pregnant women at 24 weeks of pregnancy [APHA, Atlanta, 2001 (T. Juncker *et al*., unpublished observations)]. The study showed no effect of iron-folate on low birthweight, maternal hypertension, antepartum haemorrhage, maternal infection, stillbirth or neonatal death. However, in the iron-folate group, preterm birth [odds ratio (OR), 1.43, 95% CI 1.08–1.89] was more common and birthweight was reduced by 57 g. The results of that study were only available as an extended abstract, so it is difficult to judge the reliability of its findings.

In non-malarial areas, we found two trials that investigated the effect of prenatal iron prophylaxis on maternal and child health (Christian *et al*. 2003a,b, 2008, 2009a,b, 2010; Zeng *et al*. 2008). A trial from rural western China (Zeng *et al*. 2008) showed a 2-day longer gestational age, reduced risk of early preterm delivery (OR 0.50, 95% CI 0.27–0.94), and slightly longer birth length [0.3 cm (95% CI 0.09–0.51)] in the iron-folate group compared with the folate-only group. No difference was seen in the mean

Table I. Previous trials investigating health outcomes of prophylactic iron supplementation during pregnancy, endemic malaria areas and non-malarial areas, developing countries

Author, year of publication (Reference number)	Country	Allocation	Intervention* (n)‡‡‡	Control† (n)‡‡‡	N‡	Start	Population	Results§¶	Potential problems
Malarial area									
Fleming *et al.* 1986**	Nigeria	RCT	60 mg iron; 60 mg + 1 mg folate (40)	Placebo; 600 mg chloroquine + 100 mg proguanil (42)	200	<24 weeks	1st pregnancy	No significant effect of iron found.	Compliance was poor; high dropout rates, and exclusions by outcomes
Menendez *et al.* 1994††	The Gambia	RCT	60 mg iron + 1 mg folate (273)	Placebo + 1 mg folate (277)	757	<34th week	More than 1 pregnancy	▲BW	Outcome-related exclusions 27% (e.g. preterm births, women developing anaemia)
Preziosi *et al.* 1997‡‡	Niger	RCT	100 mg iron (99)	Placebo (98)	197	Mean 28 week (SD 3 weeks)	Healthy women, 66% anemic	▲BL; ▲AS	Small trial
Ndyomugyenyi & Magnussen 2000§§	Uganda	RCT	120 mg iron + 5 mg folate (174)	Placebo (168)	576	<28 week	1st pregnancy, Hb ≥80 g L^{-1}	▲BW	Outcome-related exclusions (women developing anaemia, late baby weighing); many lost to follow-up
APHA, Atlanta, 2001 (T. Juncker *et al.*, unpublished observations)¶¶	Bangladesh	Alternative	66 mg Iron + 250 µg folate (772)	Vitamin B (812)	2007	≥24 week	≥9.0 g/dL Hb at recruitment	▲PD; ▼BW	Only extended abstract available
Non-malarial area									
Zeng *et al.* 2008***	China	Cluster RCT	60 mg iron + 400 µg folate (1565)	400 µg folate (1705)	3929	<28 week	Early pregnancy <28 weeks	▲GA; ▼PD; ▼MA; ▲BL	Outcome-related exclusions 20–26% (withdrawal due to nausea and vomiting, foetal loss, and other medical conditions)
Christian *et al.* 2003a,b, 2008, 2009a,b, 2010†††	Nepal	Cluster RCT	60 mg iron + 400 µg folate (635)	Vitamin A, 1000 µg (628)	2008	Mean 11 week (SD 5.1 weeks)	Recent pregnancy	▼IH; ▼PS; ▼LBW; ▼IM; ▼CM; ▲BW; ▲ IF; ▲MF	>90% of births happened at home; large numbers of dropouts for most outcomes and reduced number at follow-ups

A, abortion; AH, antepartum haemorrhage; ALRI, acute lower respiratory infections; AS, Apgar score; B, bacteriuria; BA, birth asphyxia; BW, birthweight; BL, birth length; CC, chest circumference; CM, child mortality; D, diarrhoea; DL, dysfunctional labour; E, pre-/eclampsia; FL, foetal loss; GA, gestational age; HC, head circumference; IF, intellectual functioning; IH, intrapartum haemorrhage; IM, 3-month infant mortality; LBW, low birthweight; M, malaria; MD, mode of delivery; MF, motor functioning; MH, maternal hypertension; MI, maternal infection; ND, neonatal death; O, ophathalmia; P, pyrexia; PD, preterm delivery; PH, post-partum haemorrhage; PS, puerperal sepsis; PVT, positive venereal disease research laboratory test; PW, placenta weight; RM, preterm premature rupture of membrane; SA, skin abscess; SB, stillbirth; SGA, small-for-gestational age; SS, skin sepsis; UCN, umbilical cord around the neck. *If several intervention groups, only the iron or iron-folate group is presented. †The control group is a placebo or control alternative that excludes iron. ‡Total number of subjects randomised in both iron and control groups. §Comparing the intervention group with the control group. Only results that achieved statistical significance (as reported by the authors) are presented. ¶Symbols: (▲) increase; (▼) decrease. **Outcome assessed: A, ALRI, AS, B, BA, BW, CM, D, E, FL, GA, IH, MD, MH, ND, P, PH, PVT, SA and UCN. ††Outcome assessed: BW, LBW and M. ‡‡Outcome assessed: AS, BW, BL and PW. §§Outcome assessed: BW. ¶¶Outcome assessed: AH, BW, LBW, MH, MI, ND PD and SB. ***Outcome assessed: BW, BL, GA, HC, LBW, PD and SGA. †††Outcome assessed: ALRI, BA, BL, BW, CC, CM, DL, FL, H, HC, IF, IH, IM, LBW, MF, PD, PS, RM and SGA. ‡‡‡Number analysed.

birthweight and head circumference or proportion of low birthweight and small-for-gestational age children. A trial from a rural Nepalese district (Christian *et al*. 2003a,b, 2008, 2009a,b, 2010) followed women from a mean of 11 gestational weeks up to 7 years of follow-up and found among the iron-supplemented group a reduced risk for: intrapartum haemorrhage [risk ratio (RR) 0.59, 95% CI 0.35–0.98]; puerperal sepsis (sepsis 1, measured as ≥100.4°F on ≥2 of the first 10 days, with an RR of 0.72, 95% CI 0.54–0.95; or sepsis 2 measured as ≥100.4°F on 2 or more of the first 10 days plus foul-smelling vaginal discharge for ≥2 days, with an RR of 0.57, 95% CI 0.35–0.91); low birthweight (RR 0.84, 95% CI 72–0.99); 3-month infant mortality (RR 0.53, 95% CI 0.30–0.92); child mortality from birth up to the age of 7 years (RR 0.69, 95% CI 0.49–0.99). Among children whose mothers were supplemented with iron during pregnancy, the trial found an increase in birthweight (37 g, 95% CI –16–90) and better intellectual and motor functioning. Iron supplementation did not influence a number of other outcomes (dysfunctional labour, eclampsia, preterm premature rupture of membrane, birth length, small-for-gestational age, head and chest circumferences, foetal loss, preterm birth, birth asphyxia, acute lower respiratory infections, and hypertension).

In sum, the review of the findings of studies from malarial settings does not allow for a definite conclusion of the benefits of prophylactic iron supplementation on the health of the mother and child. While all of these trials compared iron with either a placebo or a viable control group, no data were available comparing the effects of routine iron supplementation vs. screening and treatment for anaemia. For malaria-prone settings, the suggestion that iron may invigorate the occurrence of infections brings into question the universally routine use of iron prophylaxis (Sazawal *et al*. 2006; Ojukwu *et al*. 2009). None of the previous trials took into account the malaria or HIV status of its participants. Consequently, a clear window of opportunity still exists to further investigate the advantages and disadvantages of routine iron supplementation during pregnancy in settings with concomitant prevalence of malaria and HIV by comparing routine administration of iron to all women vs. administration of iron to only those found to be anaemic.

Methods

Objectives and hypotheses of the PROFEG Trial

The specific objectives of the trial were: (1) to evaluate whether routine iron prophylaxis from the first prenatal visit until delivery (called 'routine iron' subsequently) is better than screening and treatment for anaemia (called 'selective iron') in regard to maternal and child health, such as preterm delivery, low birthweight and perinatal mortality; (2) to assess whether there is a difference in the effects of iron between high and low seasons of malaria; and (3) to assess whether screening and iron treatment for anaemia is more feasible than routine iron prophylaxis in terms of the use of health care providers and overall compliance in Maputo, Mozambique. Originally, we had had malaria activation as one of the primary outcomes, but the pilot showed it to be unfeasible because the needed equipments were not available in the nurses' office; thus, we collected information on symptoms suggestive of malaria (fever, headache, cold, vomiting/nausea, and body aches during pregnancy).

In line with these objectives, we formulated four working hypotheses: (1) preterm delivery, low birthweight and perinatal mortality are more common among women who receive routine iron prophylaxis; (2) (modified after the pilot) routine iron prophylaxis during pregnancy increases symptoms suggestive of malaria; (3) the health problems in hypotheses 1 and 2 are more prominent in the season of high malaria; and (4) screening and treatment for anaemia is equally feasible than routine iron prophylaxis in terms of use of health care providers and overall compliance.

Study context

The study was carried out in the health centre (clinic settings) of 1° de Maio in Maputo City (the capital) (March 2007–December 2008) and in the health centre of Machava 2 in Maputo Province (June 2007–March 2008), Mozambique; Machava is adjacent to Maputo City. The study centres are urban health centres and were chosen on the basis of the following criteria: they did prenatal care and had maternity

ward for child delivery; the two main general hospitals were referral hospitals; the number of births was sufficient to complete the study in the planned time; they had a good accessibility to facilitate the supervision of the study; the centres had an ongoing prevention of vertical transmission of the HIV programme; care providers were interested in the programme; and data collection was feasible. The health profile of Mozambique is typical of sub-Saharan African countries, with nearly 55% of its 23 million people living below the poverty line (The World Bank 2011). The main causes of morbidity and mortality are infectious and parasitic diseases, with malaria accounting for 30–40% mortality. At the time of the trial, the prevalence of HIV/AIDS was estimated to be around 16% nationally, and around 20% in Maputo City (Measure Demographic and Health Surveys 2009). Health care is administered by the state through district, provincial and national systems (Lindlöw *et al*. 2004).

Prenatal consultations are recommended from the third month of pregnancy and are usually carried out, along with delivery, by mother-and-child health (MCH) nurses. Women who come for their first prenatal consultation with a pregnancy of less than 3 months are not seen and are asked to return when the pregnancy becomes visible. Women with problems prior to the third month are referred to a hospital. Seventy-five per cent of women in Maputo City have four or more prenatal consultations, with 50% starting their first prenatal visits by the fourth or fifth month (Lindlöw *et al*. 2004). Like in all public health centres in Mozambique, prenatal care and delivery were free of charge.

Care recommendations at the time of the study included: daily prophylactic iron-folate supplementation (60 mg + 400 μg) throughout pregnancy, one dose of mebendazol 500 mg (for intestinal parasite), malaria prophylaxis with sulfadoxine pyrimethamine, as well as haemoglobin measurement and syphilis screening at the first prenatal visit. Three doses of tetanus vaccine were recommended: at the fifth and seventh months and at delivery. Malaria was diagnosed during prenatal consultations through a laboratory test and by clinical signs. Voluntary HIV testing was offered in many health centres, including our study centres (Mozambiqan Ministry of Health 2002).

Usually, women arrived at the health centre around 6 am–7 am, with the prenatal consultation ending around 1 pm. At the prenatal sessions, women collectively received information and counselling regarding HIV, vaccinations, and advice on diet. After the collective information session, women were individually attended to by MCH nurses, during which time they had a (voluntary) HIV test and tetanus vaccination. After the individual consultation, the women were sent to the heath centre laboratory to have blood tests for syphilis, haemoglobin, and (primiparous women) blood group determination. Haemoglobin was routinely measured only during the first prenatal consultation, but if a woman presented clinical signs of anaemia, she could have further laboratory tests.

Mothers were given a prenatal card on their first visit and were requested to bring it on subsequent visits. The card was to be completed at each prenatal visit and to be given to the birthplace (called hospitals subsequently). After delivery, in some health centres/hospitals the prenatal card was given back to the woman, while for some it was retained in the hospital archives. The prenatal card also had a section covering births, but not all hospitals completed it. Health centres had no individual records for pregnant women; they had a book of first visits including woman's names, age and date of visit. In addition, only the numbers of subsequent visits were recorded, and these were not linked to individual women.

Data on births were collected using separate forms, which were kept by the hospitals. Furthermore, hospitals had other records (admission books, birth books, books for complications, etc) that varied from one hospital to another. Hospital archiving was variable and unreliable. Often, documents were put into a box and retained in a room containing other things too. Post-natal visits were not customary, and no form was used in those visits. The main reason for attending the health centre after delivery was for contraception.

Recruitment

In the two study health centres, general information about the study was given to all women attending their first prenatal visit during the routine early morning health-education sessions. Recruitment

occurred during the individual consultations. The physical locations of the two study centres were slightly different: in the 1° de Maio health centre, a room separate from the prenatal visit room was used, while in the Machava centre, it was the same room. In both centres, the women first went for the voluntary HIV testing; the nurses estimated that about 99% of the women had the HIV test.

In the 1° de Maio health centre, the women first had their routine prenatal care consultation with the MCH nurse, followed by the visit to the study nurse. The study nurse checked for eligibility, and those who met the inclusion criteria were asked if they wanted to join the study. In Machava, it was the MCH nurse who asked if the woman wanted to join the trial. If she agreed, the study nurse sat jointly with the MCH nurse when the information on the woman's history was collected and completed the data collection form simultaneously while the routine nurse completed the routine prenatal form. After the consultation, women in the selective iron group had their haemoglobin measured using HemoCue® (Hemocue AB, Ängelholm, Sweden). Women were then supposed to be guided to the laboratory to have the routine tests.

Study nurses were given a study recruitment book into which they entered the following information on the recruited women: name, age, and number of previous pregnancies and births. The study nurses were retired nurses employed by the project. They were given training and a study manual, which they used to carry out the different steps of the study. In the Machava health centre, the MCH nurses collected the data on subsequent visits. The MCH nurses were paid a little incentive ($10.00 to $25.00 per month, depending on the number of women present at each visit) by the project for accommodating the study and for guiding the study nurses. Recruitment and randomisation into the study took place from November 21, 2006 to March 31, 2008.

Exclusion criteria

All pregnant women having their first prenatal visit were the target group. Women excluded from the study were those who missed attending to the study nurses; those too early in pregnancy (<12 weeks), women with high obstetric risk and those less than 18 years of age.

Interventions

Women in the Routine iron group (i.e. routine iron prophylaxis from the first to the last prenatal visit) received 30 tablets (supply of one month) of 60 mg of ferrous sulphate plus 400 µg of folic acid per day. Women in the Selective iron group (i.e. regular screening for haemoglobin level and treatment for anaemia) were given 30 tablets of 1 mg of folic acid per day. At each visit the nurses measured the haemoglobin using a rapid haemoglobin measure (HemoCue Hb 201+). If their haemoglobin was below the cut-off of <9 g/dL Hb, they received a double dose of iron (60 + 60 mg for treatment of anaemia). The iron plus folic acid tablets were round and red in colour, while the folic acid only tablets were round and yellow in colour. The tablets were given in a plastic bag that had the drug's name and dose on it.

Outcome measures

The main outcomes were preterm delivery (delivery <37 weeks of gestation, estimated from last menstrual period) and low birthweight (<2500 g). Originally, malaria activation was one of the primary outcomes, but as the pilot showed it to be unfeasible, we dropped it. Instead, we collected information on symptoms suggestive of malaria (fever, headache, cold, vomiting/nausea and body aches during pregnancy as secondary outcomes) and self-reported malaria during pregnancy (the woman was asked by the study nurse whether she has had diagnosed malaria since her last visit). Secondary outcomes were perinatal mortality (as available from the local registers; unlikely to cover early stillbirths or neonatal births occurring at home), complications during pregnancy and labour, and symptoms suggestive of malaria.

Sample size

As there was no prior reliable information on baseline rates or what impact iron might have, we calcu-

lated the sample size with various assumptions of the baseline rates, power (85 and 90%), significance level of 5%, and the size of the difference to be detected (20 and 30%) for preterm delivery, low birth rate, (clinical malaria) and perinatal mortality. Based on these calculations and the expected feasibility, the target size chosen was 2000 women for each group. The STATA 7 (StataCorp LP, College Station, TX, USA) was used to estimate the sample size.

Randomisation

Women who agreed to participate and met the inclusion criteria were randomised into either Routine iron group or Selective iron group. The STATA statistical software was used to generate sequential random numbers separately for the two centres, and the women were assigned to either of the groups with a probability of 50%.

The codes for the groups were put into sealed and sequentially numbered opaque envelopes; the woman's study number was repeated on all the documents in the envelope. The envelope contained a study identification card (pink for the Selective group and yellow for the Routine iron group, 10×20 cm) and an informed consent form. The envelopes were put into a box and the study nurses were advised to pick them in order. Before the nurse opened the envelope, she wrote the woman's name on it. After opening, the coloured study identification card was stapled to the maternity card.

Informed consent was requested in two stages: first orally, and again after opening the envelope, this time with written confirmation. An envelope was opened for each woman who had orally agreed to join the trial. Women were asked to sign or thumb-print the informed consent form. Nurses read and explained the text of the form to those who could not read Portuguese. Women were informed about the study on an individual basis. Detailed information was given about the group the woman was assigned to, while it was also explained that the woman had the right not to follow the recommendations. The information included data collection procedures, such as longer first visit and meeting the study nurse at each visit. Those who refused to participate were assured that their decision would not influence their routine care.

Data collection and follow-up

Data were collected through three methods: (1) abstracting data from mothers' maternity cards and birth records around the time of the visit/hospital stay; (2) asking women questions at prenatal visits; and (3) for birth data only, collecting data from hospital records, death registers, as well as calling women to complete missing data. The first two methods were used mainly for data collection during pregnancy, while the last method (involving mixed methods) was used for collection of delivery data. The first two mentioned methods are described here.

In the 1° de Maio health centre and at the first visit at the Machava health centre, data from prenatal visits were collected by the trained study nurses using data collection forms. In subsequent visits to the Machava health centre, data were collected by the MCH nurses who were giving routine care. Study women were identified by the colour study identification card stapled to the maternity card. Clinical data were abstracted from the maternity cards. Additional questions were asked, for example, on whether the woman had had malaria since the previous visit, whether any malaria prophylaxis was taken, and whether the iron and folic acid tablets were taken by the women. Researchers regularly collected these forms from the health centres; coding and data entry were done by research assistants at the Eduardo Mondlane University using Microsoft Access. The data were later transferred to STATA for data analysis.

The study nurses were given diaries to record any incidents at the health centres, any lack of iron tablets, lack of HIV tests (reagents) or any information they felt was valuable. The information from these diaries was regularly checked by the study coordinators.

At delivery, the study women were identified by the colour identification card stapled to their maternity card. Nurses taking care of deliveries at the study health centres were informed of the study and were requested to tear the study identification card from the maternity card, staple it to the (routine) delivery

card, and put the delivery card into a separate study box. The study nurses abstracted data from the delivery cards onto the data collection forms daily. At the two second-level referral hospitals (Mavalane and Jose Macamo), the MCH nurses were informed of the study and asked likewise to put the delivery cards aside. The study coordinators collected the data from these referral hospitals every 1–2 weeks. We could not organise the birth data collection at the central hospital (third-level hospital) or other potential birthplaces.

Compliance

The women were instructed and encouraged at each visit to take the tablets they were given. Women allocated to the Routine iron group could refuse to take the iron tables, in which case they were classified as non-compliant with the intervention. Women who belonged to the Selective iron group and wanted iron (even if their haemoglobin level was not below the cut-off level) were given iron and were classified as non-compliant with the intervention. To assess whether nurses had given the tablets and that women had taken the tablets, a few questions were asked on each subsequent visit, including 'Was haemoglobin measured?'; 'Was iron/folic acid given to the woman?'; 'Number of iron/folic acids given?'; and 'Did the woman take the tablets during the past week?'

Ethical approval

Ethical approval for the study was obtained from the Mozambique Ministry of Health Ethics Committee [CNBS (Ref. 84/CNBS/06)]. A positive statement was obtained from the National Institute for Health and Welfare, Helsinki, Finland (Dno 2571/501/2007).

Monitoring

The study was monitored for reliable data collection and the safety of the intervention. Decreased haemoglobin levels in the screening group were reported to the local ethics committee. The study nurses kept diaries on 'any events', the stock of iron tablets in the health centre, any lack of HIV tests and reagents. They kept a register on the women's attendance to subsequent visits and kept a separate stock of iron tablets purchased for the study; the stock was to be used in the event that the health centre ran out of iron tablets; they reported to the local coordinators. Local coordinators and international coordinators visited the study sites regularly and verified that the study procedures were followed in regard to informing the women, randomisation, recruitment, the technique for measuring haemoglobin using HemoCue, handing out of iron/folic acid tablets and the data collection. Study nurses reported to the local coordinators and local coordinators reported to the international coordinators.

Pilot

A pilot study to study the feasibility of recruitment and follow-up during pregnancy was carried out between November 2006 and March 2007 in the 1° de Maio health centre to test the feasibility of recruitment (Parkkali *et al*. 2008). A total of 781 women were enrolled into the pilot study, 134 of whom were followed until delivery; the pilot did not test the completeness of birth data collection.

The setting up of the pilot study was time consuming and administrative issues and authorisations took longer than expected. However, after practical obstacles had been solved, the study design turned out to be feasible. The mean number of women recruited per week was 43. The women came from various nearby areas. Anaemia prevalence (Hb < 9 g/dL) in the selective iron group at recruitment was 36% ($n = 140$) according to HemoCue. By the standard measurement (Lovibond®; The Tintometer Limited, UK) it was 0.5%. Of the 134 deliveries, 78% ($n = 104$) took place in the health centre, 17% ($n = 23$) in the referral hospital (Mavalane) and 5% ($n = 7$) at home. Home deliveries were recorded in the maternity delivery register at the health centre when the women came with their newborn to have vaccinations and to receive the baby card.

The changes made to the trial protocol included a slight modification to the data collection forms and dropping the aim of collecting data on malaria

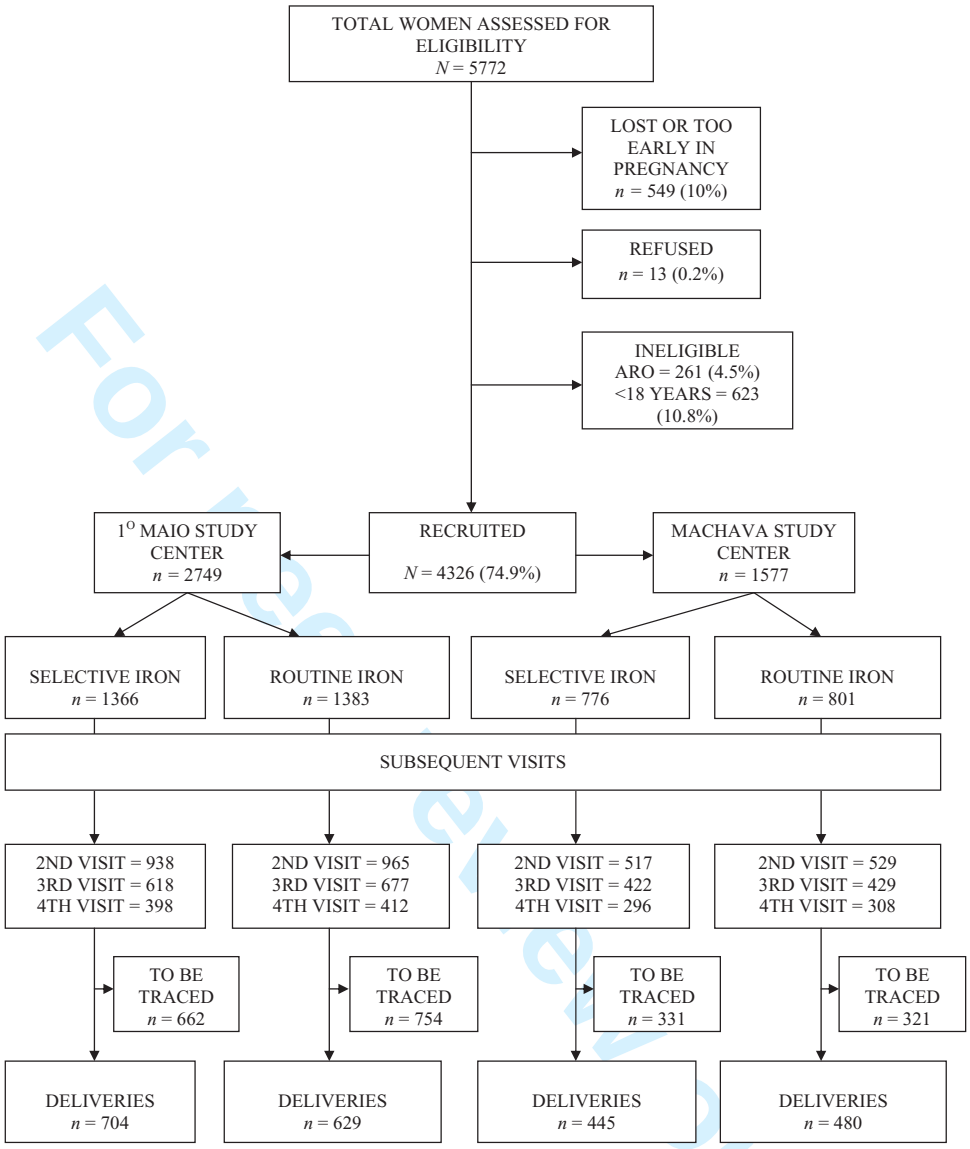

Fig. 1. PROFEG Trial flow diagram.

activation, which had proved unfeasible. The main procedures were not modified.

Statistical analysis

The data were analysed by basically computing descriptive results (means and proportions) of the differences between the iron groups.

Results

Recruitment and exclusions

Figure 1 presents the flow of women in the study. Of the 5778 women present at recruitment (3942 in the 1° de Maio health centre and 1836 in the Machava health centre), the final sample size was 4326 (75%) women, randomised into the two study groups (2184

to the Routine iron and 2142 to the Selective iron group).

Excluded women were: women not attending the study nurse at all (usually did not find their way) and those who were too early in pregnancy (<12 weeks) ($n = 549$), refusals ($n = 13$), those with high-risk pregnancy ($n = 261$), and those less than 18 years of age ($n = 623$). We aimed to collect background information on women who refused, but this did not happen systematically.

Randomisation

Women in both study groups were similar to each other as well as in the two study centres, indicating a successful randomisation (Table 2). Thirty-three per cent of the women in the Selective iron group had low haemoglobin at enrolment, and were given iron as planned (Table 2).

Follow-up visits and deliveries

The number of visits varied, but was similar in the two groups (Table 3). Most women had only two subsequent visits. The maximum number of visits was seven (about 0.3% of women). For simplicity, the number of subsequent visits were grouped from 1 to 5+ (Table 3).

Table 4 shows the timing of the subsequent visits. At each subsequent visit, most women were between 24 and 36 weeks gestation, and this was similar in both study groups. Consequently, it is possible that most of the women might have delivered before the next visit (Table 4); however, the reasons for absence in subsequent visits and whether the women had delivered or not were not adequately ascertained.

Thus far, we have not obtained information on all deliveries; currently, we are in the process of locating further delivery data by various methods. Even by excluding estimated late miscarriages (5%), early stillbirths (3%) and estimated home births (10%), we would have expected to obtain data for 3547 women (82%) of the 4326 women who participated in the trial. In the event, we obtained data on only 2258 (64% of 3547) women. We were alerted to these problems too late to be able make adjustments. As delivery cards for our study women were found in all four assumed delivery places, we did not realise the numbers were fewer than expected until the time at which most deliveries would have been expected to have occurred.

Compliance

The compliance of the study nurses is illustrated in Table 5: assessing how many of the women coming for subsequent visits had had their haemoglobin measured and were given iron and folic acid tablets. At each subsequent visit, almost all of the Selective iron women were measured for haemoglobin using the recommended HemoCue method. Almost all women in the Routine iron group were given iron tablets at each subsequent visit. About one-third of the Selective iron women received iron tablets because of low haemoglobin, while the other two-thirds received folic acid only (Table 5). HemoCue was also used to measure women in the Routine iron group at the beginning of the trial. Although it was planned not to test the women in this group again in the trial, a misunderstanding meant it was sometimes used later, although for a small number of women.

Table 6 shows compliance with taking iron and folic acid tablets during the week previous to each visit based on self-report. Most women reported taking the tablets regularly as advised and this was similar in the Selective and Routine iron groups in both study centres.

Discussion

This paper described the rationale, design, and success of a pragmatic RCT on iron prophylaxis during pregnancy in Maputo, Mozambique. Recruitment and randomisation in the study were done well. Follow-up visits during the study were similar in both trial arms. However, collecting delivery data posed a challenge, and an estimated 36% of institutionalised births were missed. The missed births are now being traced by matching the women to admissions data in the study health centres and referral hospitals. The compliance of the study personnel (with regards to measurement of women's haemoglobin) and the women to the

Table 2. Characteristics of women at recruitment, by group and centre

Characteristic	All		1º de Maio study centre		Machava study centre	
	n = 4326		*n* = 2749		*n* = 1577	
	Selective iron*	Routine iron†	Selective iron*	Routine iron†	Selective iron*	Routine iron†
	n = 2142	*n* = 2184	*n* = 1366	*n* = 1383	*n* = 776	*n* = 801
Maternal age (years), mean (SD)‡	24.8 (5.5)	24.7 (5.5)	24.5 (5.5)	24.2 (5.4)	25.2 (5.4)	25.4 (5.5)
Maternal age, *n* (%)						
< 20	357 (17)	390 (18)	247 (18)	270 (20)	110 (14)	120 (15)
20–24	859 (40)	842 (39)	566 (41)	559 (40)	293 (38)	283 (35)
25–29	502 (23)	510 (23)	297 (22)	301 (22)	205 (26)	209 (26)
30–34	257 (12)	296 (13)	145 (11)	167 (12)	112 (14)	129 (16)
≥35	146 (7)	132 (6)	98 (7)	73 (5)	48 (7)	59 (8)
Missing	21 (1)	14 (1)	13 (1)	13 (1)	8 (1)	1 (0)
Gestational age (weeks), mean (SD)‡	10.4 (6.0)	10.2 (5.9)	9.9 (5.8)	9.6 (5.6)	11.2 (6.1)	11.2 (6.4)
Measurement of gestational age, *n* (%)						
Last menstruation	1850 (86)	1900 (87)	1138 (83)	1165 (84)	712 (92)	735 (92)
Uterine height	284 (13)	272 (12)	222 (16)	211 (15)	62 (8)	61 (7)
Missing	8 (1)	12 (1)	6 (1)	7 (1)	2 (0)	5 (1)
Previous abortions, *n* (%)						
Yes	274 (13)	298 (14)	165 (12)	180 (13)	109 (14)	118 (15)
No	1861 (87)	1881 (86)	1197 (88)	1201 (87)	664 (86)	680 (85)
Missing	7 (0)	5 (0)	4 (0)	2 (0)	3 (0)	3 (0)
Previous stillbirths, *n* (%)						
Yes	172 (8)	176 (8)	79 (6)	94 (7)	93 (12)	82 (10)
No	1962 (92)	2002 (92)	1284 (94)	1287 (93)	678 (87)	715 (89)
Missing	8 (0)	6 (0)	3 (0)	2 (0)	5 (1)	4 (1)
Number of previous deliveries, *n* (%)						
None	631 (30)	699 (32)	429 (31)	476 (34)	202 (26)	223 (28)
One	671 (31)	658 (30)	434 (32)	436 (32)	237 (31)	222 (28)
Two	399 (19)	397 (18)	237 (17)	236 (17)	162 (21)	161 (20)
Three or more	435 (20)	425 (20)	262 (19)	233 (17)	173 (22)	192 (24)
Missing	6 (0)	5 (0)	4 (1)	2 (0)	2 (0)	3 (0)
Number of previous live births, *n* (%)						
None	652 (30)	719 (33)	443 (32)	493 (36)	209 (27)	226 (28)
One	665 (31)	658 (30)	430 (32)	434 (31)	235 (30)	224 (28)
Two	402 (19)	391 (18)	240 (18)	234 (17)	162 (21)	157 (20)
Three or more	418 (20)	411 (19)	249 (18)	220 (16)	169 (22)	191 (24)
Missing	5 (0)	5 (0)	4 (0)	2 (0)	1 (0)	3 (0)
HIV status, *n* (%)						
Positive	446 (21)	428 (20)	271 (20)	251 (18)	175 (23)	177 (22)
Negative	1696 (79)	1756 (80)	1095 (80)	1132 (82)	601 (77)	624 (78)
Haemoglobin by HemoCue (g/dL), mean (SD)‡	9.6 (1.7)		9.6 (1.7)		9.7 (1.7)	
Haemoglobin by HemoCue (g/dL), *n* (%)						
< 7.0	141 (7)		102 (8)		39 (5)	
7.0–8.90	535 (25)		343 (25)		192 (25)	
9.0–9.90	512 (24)		336 (25)		176 (23)	
10.0–10-90	462 (22)		294 (22)		168 (22)	
11.0–11.90	298 (14)		168 (12)		130 (17)	
≥12.0	174 (8)		110 (8)		64 (8)	
Not measured	20 (1)		13 (1)		7 (1)	
Iron + folic acid given, *n* (%)						
Yes	708 (33)	2164 (99)	464 (34)	1368 (99)	244 (32)	796 (99)
No	1421 (66)	14 (1)	892 (65)	11 (1)	529 (68)	3 (0)
Missing	13 (1)	6 (0)	10 (1)	4 (0)	3 (0)	2 (0)
Only folic acid given, *n* (%)						
Yes	1426 (67)	15 (1)	894 (65)	10 (1)	532 (69)	5 (1)
No	701 (33)	2159 (99)	460 (34)	1367 (99)	241 (31)	792 (99)
Missing	15 (0)	10 (0)	12 (1)	6 (0)	3 (0)	4 (0)
Fever during current pregnancy, *n* (%)						
Yes	550 (26)	527 (24)	405 (30)	377 (27)	145 (19)	150 (19)
Headache during current pregnancy, *n* (%)						
Yes	917 (43)	944 (43)	634 (46)	682 (49)	283 (37)	262 (33)
Cold/chills during current pregnancy, *n* (%)						
Yes	412 (19)	406 (19)	299 (22)	298 (22)	113 (15)	108 (14)
Vomit/nausea during current pregnancy, *n* (%)						
Yes	612 (29)	605 (28)	434 (32)	444 (32)	178 (23)	161 (20)
Body aches during current pregnancy, *n* (%)						
Yes	487 (23)	488 (22)	361 (26)	379 (27)	126 (16)	109 (14)
Malaria prophylaxis during current pregnancy, *n* (%)						
Yes	856 (40)	923 (42)	481 (35)	499 (36)	375 (48)	424 (53)
Malaria during current pregnancy, *n* (%)						
Yes	125 (6)	135 (6)	84 (6)	86 (6)	41 (5)	49 (6)
Had malaria test, *n* (%)						
Yes	159 (7)	164 (8)	107 (8)	107 (8)	52 (7)	57 (7)

*Policy 2: daily intake of 400 µg of folic acid and received iron (120 mg) if their haemoglobin was <9 g/dL. †Policy 1: daily intake of 60 mg ferrous sulphate plus 400 µg of folic acid. ‡Missing data excluded when calculating the mean and standard deviation: maternal age (Selective *n* = 21, Routine *n* = 14); gestational age (Selective *n* = 8, Routine *n* = 12); haemoglobin (Selective *n* = 20).

Table 3. Number of prenatal visits by study group and centre of study

Number of visits	All		1º de Maio study centre[§]		Machava study centre[§]	
	n = 4326		*n* = 2749		*n* = 1577	
	Selective iron*	Routine iron[†]	Selective iron*	Routine iron[†]	Selective iron[†]	Routine iron[†]
	n = 2142	*n* = 2184	*n* = 1366	*n* = 1383	*n* = 776	*n* = 801
One, *n* (%)[‡]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Two, *n* (%)[‡]	633 (30)	595 (27)	424 (31)	396 (29)	209 (27)	199 (25)
Three, *n* (%)[‡]	462 (22)	513 (23)	287 (21)	319 (23)	175 (23)	194 (24)
Four, *n* (%)[‡]	299 (14)	321 (15)	177 (13)	192 (14)	122 (16)	129 (16)
Five+, *n* (%)[‡]	247 (12)	247 (11)	146 (11)	151 (11)	101 (13)	96 (12)

*Daily intake of 400 µg of folic acid and received iron (120 mg) if their haemoglobin was <9 g/dL. [†]Daily intake of 60 mg ferrous sulphate plus 400 µg of folic acid. [‡]The denominator is the number of subjects in each trial arm. [§]There were no significant differences between the iron groups and within each study centre.

Table 4. Pregnancy week at subsequent visits after enrolment by study group and centre of study

Pregnancy week at subsequent visits	All		1º de Maio study centre		Machava study centre	
	Selective iron*	Routine iron[†]	Selective iron*	Routine iron[†]	Selective iron*	Routine iron[†]
	n = 2142	*n* = 2184	*n* = 1366	*n* = 1383	*n* = 776	*n* = 801
First subsequent visit, *n* (%)[‡]	1455 (68)	1494 (68)	938 (69)	965 (70)	517 (67)	529 (66)
<30 week[§]	991 (68)	1011 (68)	635 (68)	659 (68)	356 (69)	352 (66)
30–34 week[§]	293 (20)	309 (21)	171 (18)	179 (19)	122 (24)	130 (25)
≥35 week[§]	83 (6)	78 (5)	52 (6)	40 (4)	31 (6)	38 (7)
Missing[§]	88 (6)	96 (6)	80 (8)	87 (9)	8 (1)	9 (2)
Second subsequent visit, *n* (%)[‡]	1040 (49)	1106 (51)	618 (45)	677 (49)	422 (54)	429 (54)
<30 week[§]	523 (50)	515 (47)	329 (53)	356 (53)	194 (46)	159 (37)
30–34 week[§]	311 (30)	377 (34)	168 (27)	204 (30)	143 (34)	173 (40)
≥35 week[§]	163 (16)	176 (16)	82 (14)	81 (12)	81 (19)	95 (22)
Missing[§]	43 (4)	38 (3)	39 (6)	36 (5)	4 (1)	2 (1)
Third subsequent visit, *n* (%)[‡]	694 (33)	720 (33)	398 (29)	412 (30)	296 (38)	308 (38)
<30 week[§]	186 (27)	173 (24)	129 (32)	123 (30)	57 (20)	50 (16)
30–34 week[§]	265 (38)	304 (42)	140 (35)	180 (44)	125 (42)	124 (40)
≥35 week[§]	208 (30)	219 (31)	98 (25)	85 (20)	110 (37)	134 (44)
Missing[§]	35 (5)	24 (3)	31 (8)	24 (6)	4 (1)	0 (0)

*Daily intake of 400 µg of folic acid and received iron (120 mg) if their haemoglobin was <9 g/dL. [†]Daily intake of 60 mg of ferrous sulphate plus 400 µg of folic acid. [‡]Frequency and percentage of women at recruitment who attended subsequent visits. [§]The denominator is the number of those who attended at each subsequent visit.

study protocol (uptake of the recommended tablets) was good. Several administrative and practical challenges were encountered during the course of the trial, from planning through to the process of implementation. A pilot trial carried out in the study contexts before the actual trial highlighted areas that needed to be resolved prior to the main trial.

Despite the widespread recommendation of prophylactic iron supplementation during pregnancy, the data available provide insufficient evidence on its benefits to maternal and child health in low-income settings. In malaria-prone settings, the small sample sizes, exclusion by outcomes and large dropouts that have characterised previous trials has meant the

Table 5. Measure of compliance by the personnel assessed by frequency of measuring haemoglobin (Selective iron group) and given iron and folic acid (Routine iron group) during subsequent visits, by group and centre

Subsequent visit after enrolment	All		1º de Maio study centre		Machava study centre	
	n = 4326		*n* = 2749		*n* = 1577	
	Selective iron*	Routine iron†	Selective iron*	Routine iron†	Selective iron*	Routine iron†
	n = 2142	*n* = 2184	*n* = 1366	*n* = 1383	*n* = 776	*n* = 801
First subsequent visit, *n* (%)‡	1455 (68)	1494 (68)	938 (69)	965 (70)	517 (67)	529 (66)
Haemoglobin measured, *n* (%)§						
HemoCue	1416 (97)	156 (10)	919 (98)	142 (15)	497 (96)	14 (3)
Iron given, *n* (%)§	493 (34)	1460 (98)	320 (34)	948 (98)	173 (33)	512 (97)
Folic acid only given, *n* (%)§	946 (65)	29 (2)	603 (64)	11 (1)	343 (66)	18 (3)
Second subsequent visit, *n* (%)‡	1040 (49)	1106 (51)	618 (45)	677 (49)	422 (54)	429 (54)
Haemoglobin measured, *n* (%)§						
HemoCue	1000 (96)	66 (6)	618 (100)	52 (8)	396 (94)	14 (3)
Iron given, *n* (%)§	350 (34)	1090 (99)	210 (34)	670 (99)	140 (33)	420 (98)
Folic acid only given, *n* (%)§	671 (65)	16 (1)	392 (63)	7 (1)	279 (66)	9 (2)
Third subsequent visit, *n* (%)‡	694 (33)	720 (33)	398 (29)	412 (30)	296 (38)	308 (38)
Haemoglobin measured, *n* (%)§						
HemoCue	673 (97)	16 (2)	389 (98)	9 (2)	284 (96)	7 (2)
Iron given, *n* (%)§	185 (27)	707 (98)	110 (28)	405 (98)	75 (25)	302 (98)
Folic acid only given, *n* (%)§	495 (71)	13 (2)	277 (70)	6 (1)	218 (74)	7 (2)

*Daily intake of 400 μg of folic acid and received iron (120 mg) if their haemoglobin was <9 g/dL. †Daily intake of 60 mg of ferrous sulphate plus 400 μg of folic acid. ‡Frequency and percentage of women at recruitment who attended subsequent visits. §The denominator is the number of those who attended at each subsequent visit.

Table 6. Measure of compliance by women's reports, by group and centre

Subsequent visit after enrolment	All		1º de Maio study centre		Machava study centre	
	n = 4326		*n* = 2749		*n* = 1577	
	Selective iron*	Routine iron†	Selective iron*	Routine iron†	Selective iron*	Routine iron†
	n = 2142	*n* = 2184	*n* = 1366	*n* = 1383	*n* = 776	*n* = 801
First subsequent visit, *n* (%)‡	1455 (68)	1494 (68)	938 (69)	965 (70)	517 (67)	529 (66)
Tablets taken during the past week, *n* (%)§						
Regularly	1344 (92)	1380 (92)	883 (94)	912 (95)	461 (89)	468 (88)
Sometimes	90 (6)	88 (6)	42 (4)	35 (4)	48 (9)	53 (10)
No	15 (1)	20 (1)	7 (1)	14 (1)	8 (2)	6 (1)
Second subsequent visit, *n* (%)‡	1040 (49)	1106 (51)	618 (45)	677 (49)	422 (54)	429 (54)
Tablets taken during the past week, *n* (%)§						
Regularly	962 (93)	1031 (93)	588 (95)	652 (96)	374 (87)	379 (88)
Sometimes	70 (7)	60 (5)	24 (4)	15 (2)	46 (11)	45 (10)
No	5 (0.5)	8 (0.7)	4 (0.6)	7 (1)	1 (0.2)	1 (0.2)
Third subsequent visit, *n* (%)‡	694 (33)	720 (33)	398 (29)	412 (30)	296 (38)	308 (38)
Tablets taken during the past week, *n* (%)§						
Regularly	647 (93)	670 (93)	381 (96)	402 (98)	266 (90)	268 (87)
Sometimes	43 (6)	46 (6)	15 (4)	7 (2)	28 (9)	39 (13)
No	3 (0.4)	3 (0.4)	2 (0.5)	2 (0.5)	1 (0.3)	1 (0.3)

*Daily intake of 400 μg of folic acid and received iron (120 mg) if their haemoglobin was <9 g/dL. †Daily intake of 60 mg of ferrous sulphate plus 400 μg of folic acid. ‡Frequency and percentage of women at recruitment who attended subsequent visits. §The denominator is the number of those who attended at each subsequent visit.

evidence falls short in clarifying the advantages and disadvantages of prophylactic iron supplementation during pregnancy [APHA, Atlanta, 2001 (T. Juncker *et al*., unpublished observations)] (Fleming *et al*. 1986; Menendez *et al*. 1994; Preziosi *et al*. 1997; Ndyomugyenyi & Magnussen 2000). Although the trials from non-malarial areas generally had large sample sizes and better designs, their results are conflicting (Zeng *et al*. 2008; Christian *et al*. 2003a,b, 2008, 2009a,b, 2010), while the results from non-malarial areas may not be applicable in malarial settings.

Unlike previous studies that employed explanatory designs to test the efficacy of prenatal iron prophylaxis, we utilised the pragmatic trial design so as to compare the effectiveness of two policies for prophylactic iron administration. Pragmatic trials are more suitable to study effects in normal clinical practice; they have the basic aim of informing choice between treatments (Roland & Torgerson 1998; MacPherson 2004). A pragmatic trial design was useful to compare two policies of iron supplementation in a real-life situation. In these types of trials, placebo and blinding are not customary (Roland & Torgerson 1998; MacPherson 2004). Although several calls have been made to increase the use of pragmatic trials to address clinical questions, they are rarely used and researchers are less experienced with them (Zwarenstein *et al*. 2008). For this reason, tutoring was necessary for the local research team prior to the trial starting.

As a cut-off to define anaemia, we used haemoglobin values lower than 9 g/dL. The WHO's haemoglobin cut-off level for determining anaemia during pregnancy is 11 g/dL (WHO 1972). Thus, our cut-off value can be questioned. However, the WHO recommended value is based on haemoglobin levels of women in developed countries. For developing countries, clinical signs of symptoms of anaemia usually appear when the haemoglobin level is below 7 g/dL (van den Broek *et al*. 1999). At the planning stage, we asked health care providers in Maputo about the acceptable cut-off point for haemoglobin level requiring iron treatment. Their opinions varied between 8 and 11 g/dL. Based on this feedback, we concluded that using a 9 g/dL cut-off level for treatment with iron would not endanger the woman's or fetus's health, and would also enable us to answer the research questions. An earlier study on anaemia during pregnancy in Mozambique found that 5–15% of pregnant women had haemoglobin values below 9 g/dL and that 58% had levels below 11 g/dL (Liljestrand *et al*. 1986).

Although the study health centres had previously conducted RCTs in relation to HIV, it nevertheless remained a delicate issue at the time of the study, both at the grass roots level and among higher authorities. Despite this and the voluntary nature of HIV testing, almost all the women (99%) underwent the test. This facilitated our study.

Carrying out this trial was challenging. One key challenge was the sluggishness of administrative and financial procedures. Planning the trial took a long time as did obtaining authorisations from the ethics committees and the local authorities. There was no research infrastructure in the local health care and the existing tradition did not value accurate record keeping. In addition, university facilities were modest and money transfers were cumbersome.

Finding qualified research assistants for the study posed another challenge. We chose retired nurses, as having older and experienced nurses created an environment of trust among the women. However, it took time to train them in the study procedures. Another problem centred on the study nurses receiving a higher salary than MCH nurses, which may have undermined support for the study among MCH nurses. We gave small monthly incentives to the MCH nurses for their collaboration, but that may not have been enough. MCH nurses may not have always informed the women that they needed to see the study nurses. This issue was more likely at the 1° de Maio health centre where the study location was different to that of usual prenatal care consultation.

The number of women participating declined with each subsequent visit. The reasons could not be ascertained, but they may have been due to having no further prenatal visits, women visiting other health centres, or data not being collected. However, considering that a majority of the women were at 30–34 weeks of gestation at their final visit, it is possible that most of them might have delivered already. We are investigating the potential reasons for why women missed subsequent visits. Our data suggest that the

compliance of both the study nurses and the women was good. Women were given their tablets at each visit. To assess whether they complied, they were asked in each visit about the frequency of taking their tablets. We cannot be sure how reliably women answered as they might have been intimated by the nurses. The nurses, however, were instructed to encourage the women to tell the truth and they were informed that any answers they provided were acceptable.

The greatest challenge in the trial was gathering birth data by the planned method. This led to changes in the data collection protocol and presently, we are tracing the women's birth data from health centres, hospitals and death registers. This has prolonged the outcome data collection and increased the study costs. Possible reasons for losing birth data include: a miscarriage or home birth with no notification sent to the study health centre; the mother dying or moving from the area before birth; the mother delivering outside the study locations (self-referrals); delivery nurses not putting the study cards aside; and mothers not having their study cards at delivery.

Conclusions

The planned trial proved feasible in an ordinary health care setting in Maputo. The loss of mothers' delivery data might have been avoided by better surveillance of the process during the trial and better knowledge of the actual patient flow patterns in Maputo.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

BIN, SP, EH designed, analysed and prepared the present paper. EH designed and is responsible for the conception of the PROFEG Trial. SP, FA, GS, BC, OA, JC, MD and MN participated in the planning of the PROFEG Trial, made substantial contribution in its execution and participated in the interpretation of results and the critical review of the paper. OA and ER were responsible for data acquisition and preparation, interpretation of results and for the critical review of the paper.

References

Adetifa I. & Okomo U. (2009) Iron supplementation for reducing morbidity and mortality in children with HIV. *Cochrane Database of Systematic Reviews* (1), CD006736.

Boelaert J.R., Weinberg G.A. & Weinberg E.D. (1996) Altered iron metabolism in HIV infection: mechanisms, possible consequences, and proposals for management. *Infectious Agents and Disease* **5**, 36–46.

Brabin B.J., Hakimi M. & Pelletier D. (2001) An analysis of anemia and pregnancy-related maternal mortality. *Journal of Nutrition* **131**, 604S–615S.

van den Broek N.R., Ntonya C., Mhango E. & White S.A. (1999) Diagnosing anaemia in pregnancy in rural clinics: assessing the potential of the haemoglobin colour scale. *Bulletin of World Health Organization* **77**, 15–21.

Christian P., Khatry S.K., Katz J., Pradhan E.K., LeClerg S.C., Shrestha S.R. *et al.* (2003a) Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal: a double blind randomized community trial. *British Medical Journal* **326**, 571.

Christian P., West K.P., Khatry S.K., LeClerg S.C., Pradhan E.K., Katz J. *et al.* (2003b) Effects of maternal micronutrient supplementation on fetal loss and infant mortality: a cluster-randomized trial in Nepal. *American Journal of Clinical Nutrition* **78**, 1194–1202.

Christian P., Darmstadt G.L., Wu L., Khatry S.K., Leclerg S.C., Katz J. *et al.* (2008) The effect of maternal micronutrient supplementation on early neonatal morbidity in rural Nepal: a randomized, control, community trial. *Archives Disease in Childhood* **93**, 660–664.

Christian P., Khatry S.K., LeClerq S.C. & Dali S.M. (2009a) Effects of prenatal micronutrient supplementation on complications of labor and delivery and puerperal morbidity in rural Nepal. *International Journal of Gynaecology and Obstetrics* **106**, 3–7.

Christian P., Stewart C.P., LeClerq S.C., Wu L., Katz J., West K.P. *et al*. (2009b) Antenatal and postnatal iron supplementation and childhood mortality in rural Neap: a prospective follow-up in a randomized, controlled community trial. *American Journal of Epidemiology* **170**, 1127–1136.

Christian P., Murray-Kolb L.E., Khatry S.K., Katz J., Schaefer B.A., Cole P.M. *et al*. (2010) Prenatal micronutrient supplementation and intellectual and motor function in early school-aged children in Nepal. *Journal of American Medical Association* **304**, 2716–2723.

Fall C.H., Fisher D.J., Osmond C., Margetts B.M. & Maternal Micronutrient Supplementation Study Group (2009) Multiple micronutrient supplementation during pregnancy in low-income countries: a meta-analysis of effects on birth size and length of gestation. *Food and Nutrition Bulletin* **30**, S533–S546.

Fleming A.F., Ghatoura G.B.S., Harrison K.A., Briggs N.D. & Dunn D.T. (1986) The prevention of anaemia in pregnancy in primigravidae in the guinea savanna of Nigeria. *Annals of Tropical Medicine and Parasitology* **80**, 211–233.

Gera T. & Sachdev H.P.S. (2002) Effect of iron supplementation on incidence of infectious illness in children: systematic review. *British Medical Journal* **325**, 1142.

Gordeuk V.R., Delanghe J.R., Langlois M.R. & Boelaert J.R. (2001) Iron status and the outcome of HIV infection: an overview. *Journal of Clinical Virology* **20**, 111–115.

Hemminki E. & Rimpelä U. (1991) A randomized comparison of routine versus selective iron supplementation during pregnancy. *Journal of the American College of Nutrition* **10**, 3–10.

Idemyor V. (2007) Human immunodeficiency virus (HIV) and malaria interaction in sub-Saharan Africa: the collision of two titans. *HIV Clinical Trials* **8**, 246–253.

Kabyemela E.R., Fried M., Kurtis J.D., Mutabingwa T.K. & Duffy P.E. (2008) Decreased susceptibility to *Plasmodium falciparum* infection in pregnant women with iron deficiency. *Journal of Infectious Diseases* **198**, 163–166.

Lao T.T., Tam K.F. & Chan L.Y. (2000) Third trimester iron status and pregnancy outcome in non-anaemic women: pregnancy unfavourably affected by maternal iron excess. *Human Reproduction* **15**, 1843–1848.

Lawn J.E., Cousens S., Zupan J. & Lancet Neonatal Survival Steering Team (2005) 4 million neonatal deaths: when? Where? Why? *Lancet* **365**, 891–900.

Liljestrand J., Bergström S. & Birgergård G. (1986) Anaemia of pregnancy in Mozambique. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 249–255.

Lindlöw M., Ward P. & Zorzi N. (2004) *Primary Health Care in Mozambique: Service Delivery in a Complex Hierarchy*. The World Bank: Washington, DC.

MacPherson H. (2004) Pragmatic clinical trials. *Complementary Therapies in Medicine* **12**, 136–140.

McLean E., Cogswell M., Egli I., Wojdyla D. & de Benoist B. (2009) Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. *Public Health Nutrition.* **12**, 444–454.

Measure Demographic and Health Surveys (2009) Mozambique: Standard AIDS Indicator Survey.

Menendez C., Todd J., Alonso P.L., Francis N., Lulat S., Ceesay S. *et al*. (1994) The effects of iron supplementation during pregnancy, given by traditional birth attendants, on the prevalence of anaemia and malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 590–593.

Mozambiqan Ministry of Health (May 2002) [Guide to the implementation of the programme; Prevention of Mother to Child Transmission of HIV/AIDS] (Portuguese).

Müngen E. (2003) Iron supplementation in pregnancy. *Journal of Perinatal Medicine* **31**, 420–426.

Ndyomugyenyi R. & Magnussen P. (2000) Chloroquine prophylaxis, iron/folic-acid supplementation or case management of malaria attacks in primigravidae in western Uganda: effects on congenital malaria and infant haemoglobin concentrations. *Annals of Tropical Medicine and Parasitology* **94**, 759–770.

Ojukwu J.U., Okebe J.U., Yahav D. & Paul M. (2009) Oral iron supplementation for preventing or treating anaemia among children in malaria-endemic areas. *Cochrane Database Systematic Reviews* (3), CD006589.

Oppenheimer S.J. (2001) Iron and its relation to immunity and infectious disease. *Journal of Nutrition* **131**, 616S–633S.

Parkkali S., Abacassamo F., Salomé G., Augusto O. & Nikula M. (2008) Routine iron prophylaxis during pregnancy: effects on maternal and child health in Maputo City and the urban part of Maputo Province Mozambique (PROFEG). Stakes Discussion Papers 1/2008. Helsinki: STAKES.

Peña-Rosas J.P. & Viteri F.E. (2009) Effects and safety of preventive oral iron or iron + folic acid supplementation for women during pregnancy. *Cochrane Database Systematic Reviews* (4), CD004736.

Prentice A.M. (2008) Iron metabolism, malaria, and other infections: what is all the fuss about? *Journal of Nutrition* **138**, 2537–2541.

Preziosi P., Prual A., Galan P., Daouda H., Boureima H. & Hercberg S. (1997) Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. *American Journal of Clinical Nutrition* **66**, 1178–1182.

Roland M. & Torgerson D.J. (1998) Understanding controlled trials: what are pragmatic trials? *British Medical Journal* **316**, 285.

Sazawal S., Black R.E., Ramsan M., Chwaya H.M., Stoltzfus R.J., Dutta A. *et al*. (2006) Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomized, placebo-controlled trial. *Lancet* **367**, 133–143.

Stoltzfus R.J. (2001) Iron-deficiency anemia: reexamining the nature and magnitude of the public health problem. Summary: implications for research and programs. *Journal of Nutrition* **131**, 697S–700S.

Stoltzfus R.J., Mullanny L. & Black R.E. (2004) Iron deficiency anaemia. In: *Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributable to Selected Major Risk Factors* (eds M. Ezzati, A.D. Lopez, A. Rodgers & C.J.L. Murray), Vol. 1, pp 163–209. World Health Organization: Geneva, Switzerland.

The World Bank (2011) *Country Indicators, Mozambique*. Available at: http://data.worldbank.org/indicator (Accessed 7 March 2011).

Villar J., Merialdi M., Gülmezoglu M., Abalos E., Carroli G., Kulier R. *et al*. (2003) Nutritional interventions during pregnancy for the prevention or treatment of maternal mortality and preterm delivery: an overview of randomized controlled trials. *Journal of Nutrition* **133**, 1606S–1625S.

WHO (1972) *Nutritional anaemias*. Report of a WHO Group of Experts. (WHO Technical Report Series, No. 503). World Health Organization: Geneva.

WHO (2011) *International Travel and Health 2011*. Available at: http://www.who.int/ith/chapters/ith2011chap7.pdf (Accessed 20 February 2011).

Yip R. (2000) Significance of an abnormally low or high hemoglobin concentration during pregnancy: special consideration of iron nutrition. *American Journal of Clinical Nutrition* **72**, 272S–279S.

Zeng L., Cheng Y., Dang S., Chang S., Kong L. & Yan H. (2008) Impact of micronutrient supplementation during pregnancy on birth weight, duration of gestation, and perinatal mortality in rural western China: double blind cluster randomized controlled trial. *British Medical Journal* **337**, a2001.

Zwarenstein M., Treweek S., Gagnier J.J., Altman D.G., Tunis S., Haynes B. *et al*. (2008) Improving the reporting of pragmatic trials: an extension of the CONSORT statement. *British Medical Journal* **337**, a2390.

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Comparison of routine prenatal iron prophylaxis and screening and treatment for anemia: pregnancy results and preliminary birth results from a pragmatic randomized controlled trial (PROFEG) in Maputo, Mozambique

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Comparison of routine prenatal iron prophylaxis and screening and treatment for anemia: pregnancy results and preliminary birth results from a pragmatic randomized controlled trial (PROFEG) in Maputo, Mozambique

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ABSTRACT

Objective: To present the pregnancy results and interim birth results of a pragmatic randomized controlled trial comparing routine iron prophylaxis with screening and treatment for anemia during pregnancy in a setting of endemic malaria and HIV.

Design: A pragmatic randomized controlled trial

Setting: Two health centers (1º de Maio and Machava) in Maputo, Mozambique, a setting of endemic malaria and high prevalence of HIV.

Participants: Pregnant women (~~≥12 wk gestation;~~ ≥18 years old; non-high-risk pregnancy, *N*=4326) attending prenatal care consultation at the two health centers were recruited to the trial

Interventions: The women were randomly allocated to either Routine iron (n=2184; 60 mg ferrous sulphate plus 400 µg of folic acid daily throughout pregnancy) or Selective iron (n=2142; screening and treatment for anemia and daily intake of 1 mg of folic acid).

Outcome measures: The primary outcomes were preterm delivery (delivery <37 weeks of gestation) and low birth weight (<2500 grams). The secondary outcomes were symptoms suggestive of malaria and self-reported malaria during pregnancy; birth length; cesarean section; maternal and child health status after delivery.

Results: The number of follow-up visits was similar in the two groups. Between the first and fifth visits, the two groups were similar regarding the occurrence of fever, headache, cold/chills, nausea/vomiting, and body aches. There was a suggestion of increased incidence of self-reported malaria during pregnancy (odds ratio 1.37, 95% confidence interval 0.98-1.92) in the Routine iron group. Birth data were available for 1109 (51%) in the Routine iron and for 1149 (54%) in the Selective iron groups. The birth outcomes were relatively similar in the two groups. However, there was a suggestion (statistically non-significant) of poorer outcomes in the Routine iron group with regard to long hospital stay after birth (relative risk [RR] 1.43, 95% CI 0.97-1.26; risk difference

[RD] 0.02, 95% CI -0.00-0.03) and unavailability of delivery data (RR 1.06, 95% CI 1.00-1.13; RD 0.03, 95% CI -0.01-0.07).

Conclusions: These interim results suggest that routine iron prophylaxis during pregnancy did not confer advantage over screening and treatment for anemia regarding maternal and child health. Complete data on birth outcomes are being collected for firmer conclusions**.**

Trial registration: The trial is registered at ClinicalTrials.gov, number NCT00488579 (June 2007). The first women were randomized to the trial proper April 2007- March 2008. The pilot was November 2006-March 2008. The 3-month lag was due to technical difficulties in completing trial registration.

Funding: The study was funded by two grants from the Academy of Finland (2004: 210631; 2010: 139191).

Keywords: iron, pregnancy, birth, malaria, HIV, pragmatic trial, Mozambique

ARTICLE SUMMARY:

Article focus:

- The benefits of iron prophylaxis during pregnancy on maternal and child health in developing country settings with endemic malaria and high prevalence of HIV is unclear.
- Iron has been linked to increased risk of infections.
- Among children less than three years, there are indications of harm of universal iron prophylaxis.

Key messages:

- Routine iron prophylaxis during pregnancy did not suggest better maternal and child health outcomes than screening and treatment for anemia in a setting of endemic malaria and HIV.

Strengths and limitations of this study

- So far, this represents the largest trial investigating the benefits of prophylactic iron during pregnancy on maternal and child health in malaria-endemic settings.
- The compliance of the study nurses with the trial protocol and that of the women with regards to uptake of the iron and folic acid tablets was good, as was the follow-up during pregnancy.
- The collection of delivery data was challenging, resulting in up to an estimated 40% of missing birth data, which are now been traced ~~with~~ using various methods.

INTRODUCTION

Despite widespread recommendation of routine iron prophylaxis during pregnancy, its benefits and risks for the mother and child, beyond the reduction of the risk of anemia, remain unclear, particularly in low-income settings. Reviews of randomized controlled trials (RTCs) done for the Cochrane Collaboration and the World Health Organization (WHO) have failed to conclude on the effects of ~~whether~~ routine iron prophylaxis during pregnancy ~~is beneficial or harmful to~~on pregnancy and birth outcomes.[1,2] There is some evidence that high hemoglobin concentration in late pregnancy may be associated with adverse effects on pregnancy.[3,4] Based on evidence from non-pregnant populations, ~~I~~it has ~~also~~ been suggested that iron ~~increases the risk~~may advance the rate of infections.[5-7] The host requires iron for biochemical functioning, but iron may as well promote the replication of infectious agents.[6] For developing country settings which are still plagued by infectious diseases, such as malaria and HIV, the possible ~~tential~~ ~~of~~ association between iron ~~to increase the risk of~~ and infections raises serious public health concerns.[8,9]

Previous trials conducted in malarial developing country settings that have evaluated the effects of iron supplementation during pregnancy on maternal and child outcomes have been hampered by small samples, large dropouts, and several outcome-related exclusions.[10-14] The findings from the trials were conflicting on the role of prophylactic iron supplementation on birth weight, prematurity, perinatal mortality, incidence of malaria, and other pregnancy and birth outcomes. Consequently, the evidence they provide is insufficient in addressing the question of the advantages and disadvantages of prenatal prophylactic iron. The results of studies from non-malarial areas[15-21], although of better quality, may not be relevant due to different settings.[15-21] Although, results were also conflicting in a number of outcomes, the main findings included slightly longer birth length, longer gestational age, and reduced risk of preterm delivery, intrapartum hemorrhage, low birth weight, and infant and child mortality in the iron-folic acid group ~~(Nwaru et al *Submitted*)~~.[22]

This limited evidence and the importance of iron prophylaxis in prenatal programs call for further investigation on the benefits of prenatal iron supplementation in areas of endemic malaria and high prevalence of HIV. Using a pragmatic randomized controlled trial, we investigated the effects of routine iron prophylaxis throughout pregnancy compared to screening and treatment for anemia on maternal and child health in Maputo, Mozambique. The present paper presents the pregnancy results and interim birth results. About 40% of births were missed by the original data collection method ~~(Nwaru et al *Submitted*)~~[22], and missing birth data are currently being retrieved with various complementary methods. The completed birth results will be presented later.

MATERIALS AND METHODS

Study design and population

The details of the PROFEG trial have been described elsewhere ~~(Nwaru et al *Submitted*)~~[22] and only the main features are given here. The trial was a pragmatic randomized controlled trial to compare two iron administration policies (routine iron prophylaxis versus screening and treatment for anemia during pregnancy) on maternal and child health in Maputo, Mozambique. The trial was carried out in two health centers, 1o de Maio in Maputo City, the capital (November 2006 - October 2008) and Machava 2, in Maputo Province (June 2007 - October 2008), Mozambique. ~~1~~º ~~de Maio in Maputo City (the capital) Machava 2, in Maputo Province, in 2007-2008;~~ ~~t~~The completion of collection of birth data continued until 2012. The health center of Machava 2 in Maputo province is close to Maputo city. The population is urban and semi urban and malaria is endemic in both areas. Seasonal increase of malaria is usually observed towards the end of the rainy season (February to April).[23]

In the study area all woman are eligible to attend prenatal care. The usual care recommendations at the time of the trial included daily prophylactic iron-folate supplementation (60 mg+400 µg) throughout pregnancy; one dose of mebendazol 500 mg for intestinal parasite; three doeses of sulfadoxine pyrimethamine for malaria prophylaxis (started around 20 weeks gestation, or when quickening occurs, or when the foetal heart is heard); hemoglobin measurement (Lovibond ® is routinely used) and syphilis screening at the first prenatal visit; and three doses of tetanus vaccine (at the 5th and 7th month and at delivery). If malaria was suspected during prenatal consultations, it was diagnosed by laboratory tests and clinical signs. In most health centers, including our study centers, HIV testing was offered.[22] Antiretroviral (ARV) drugs were provided by various international organizations, but we do not have information of how many women received treatment during pregnancy. The recommendation was to give ARV (Nevirapine) at delivery to prevent mother-child transmission. ~~Women in Mozambique were given ARVs (Nevirapine) by the health centers linked with prenatal care prevention for mother to child transmission at labor and after delivery. We had no information if the women received ARV for their own illness, because at the time of the trial ARVs in Maputo were administered by different centers and not by the normal health centers.~~

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Recruitment of study participants

Pregnant women attending their first prenatal visit were the target group. During the routine early morning health education sessions, all women who came for their first prenatal visit were given general information about the study. Recruitment into the study occurred during individual consultations and was carried out by study nurses who were employed and trained by the project. ~~They carried a recruitment book in which they entered the information of the recruited women.~~ In

1º de Maio health center, the women visited the study nurses after their routine prenatal care consultations with the maternal and child health (MCH) nurses. In Machava, the study nurse and the routine MCH nurse saw the women in the same room. The study nurses checked for women's eligibility to participate in the study. ~~All pregnant women attending their first prenatal visit were the target group.~~ The exclusion criteria were: ~~woman missed attending a visit with the study nurses; too early in pregnancy (< 12 weeks);~~ women with high obstetric risk ~~;~~ and those aged less than 18 years. If eligible, the nurses asked the women to join the study ~~if they did not meet any of the exclusion criteria~~. Oral and written informed consent was obtained. Three types of women were missed from the study: women whom MCH nurses sent back home because of too early pregnancy, women who did not go to the study nurse, and women who refused the study.

Randomization

The ~~participating~~ women were randomized into either the Routine iron group (i.e., routine iron prophylaxis from the first to the last prenatal visit) or the Selective iron group group (i.e. regular screening for hemoglobin level and treatment for anemia). ~~Researchers (OA) used t~~TResearcher (OA) use the STATA statistical software (StataCorp LP, Texas, USA) was used to generate sequential random numbers separately for the two centers and the women were assigned to either of the groups with a probability of 50%. The codes for the groups were put into sealed and numbered opaque envelopes; the number was the woman's study number and was repeated in the documents in the envelope. The envelope contained a study identification card (yellow for the Routine iron group and pink for the Selective, 10 x 20cm) and the informed consent form.

Sample size

We did not have up-to-date reliable baseline data of pregnant women's and newborns' health in Maputo ~~before~~ or of the effects of iron on pregnancy and birth outcomes. ~~Because of lack of prior reliable data on the baseline rates of the impact of iron,~~Thus , we used different estimates of the baseline values for preterm delivery, low birth weight, clinical malaria, and perinatal mortality to calculate the sample size ~~was calculated with various assumptions of the base line rates~~, with power (85% and 90%), significance level of 5%, and the size of the difference to be detected (20% and 30%) ~~for pre term delivery, low birth weight, clinical malaria, and perinatal mortality~~. Based on these calculations and the expected feasibility, ~~we decided a targeta~~we decided a sample size of 2000 women in each ~~of the two~~ group~~s~~ to be enough to measure ~~showed to be the safe and could provide~~ clinically meaningful effects. The STATA statistical software was used to estimate the sample size.

Interventions

On each prenatal visit, ~~W~~women in the Routine iron group received 30 tablets (supply of one month) of 60 mg ferrous sulphate plus 400 µg of folic acid per day combined in one tablet. In the Selective group, women's hemoglobin levels were measured at each visit by the study nurses using a rapid hemoglobin measure, HemoCue® Hb 201+, (Hemocue AB, Ängelholm, Sweden). If the hemoglobin was 9g/dl or more, they received 30 tablets of 1 mg of folic acid per day. If their hemoglobin was below the cut-off of <9g/dl Hb, they received a monthly double dose of iron (60 mg + 60 mg) for the treatment of anemia. ~~Women in the Selective iron were given 30 tablets of 1 mg of folic acid per day.~~ Folic acid 1 mg tablets were used because at the time of the trial pure folic acid was not licensed in Mozambique in 400 µg tablets. ~~In the Selective group, at each visit, the nurses measured the hemoglobin using a rapid hemoglobin measure, HemoCue® Hb 201+,~~

~~(Hemocue AB, Ängelholm, Sweden). If their hemoglobin was below the cut-off of <9g/l Hb, they received a monthly double dose of iron (60 mg + 60 mg) for the treatment of anemia.~~ The tablets were given in a plastic bag having the drug's name and dosage~~e~~ on it.

Data collection and follow-up

Data were collected on standard study data forms by three methods: 1) study nurses abstracted prenatal data from mothers' maternity cards, 2) study nurses asked women additional questions at the time of the prenatal visits, and 3) study nurses or researchers afterwards collected birth data from hospital birth records. Delivery nurses were informed of the study and asked to put the delivery cards into a separate study box. The study women were to be identified by the color of the identification card stapled to their maternity card. However, this did not succeed very well. By excluding estimated late miscarriages (5%), early stillbirths (3 %) and home births (10%), we should have received delivery data for 3547 women (82%) of the 4326 women who participated in the trial. We received birth data for only 2258 (64% of the estimated 3547) women.

Outcome measures

The primary outcomes were preterm delivery (delivery <37 weeks of gestation and low birth weight (<2500 grams); data on weight came from the birth records; for gestation weeks various routine data sources were used (see below). Originally, we had malaria activation as a primary outcome, but the pilot showed that it was not feasible. Secondary outcomes were perinatal mortality (as available from our data collection forms; unlikely to cover early stillbirths or neonatal deaths occurring at home); complications during pregnancy and labor; symptoms suggestive of malaria (fever, headache, cold/chills, nausea/vomiting, and body aches); and self-reported malaria during pregnancy (the woman was asked for diagnosed malaria since her last visit).

~~Calculation of g~~Gestational ~~age~~weeks ~~at birth~~

In the prenatal visits routine MCH nurses determined gestational weeks in various ways, even though all ~~information~~ways were~~as~~ not systematically ~~given~~noted down. In the first prenatal visits, the date of last menstrual period, uterine fundal height, assumed date of delivery and length of gestation (best estimate) were~~as~~ noted. The study nurses abstracted all this information and the best estimate was used in this paper. In birth records, the last menstrual period, date of fertilization, assumed date of fertilization, and length of gestation were to be given by delivery nurses. However, these data were very poorly filled and ~~O~~only 681 (30%) of the women with delivery data had their gestational ~~age~~ weeks recorded at birth. Thus the gestational ~~age~~ weeks for women without that information was estimated from dates using the following algorithm: gestational ~~age~~ weeks at first visit in days + days between the first visit and delivery; the days were then transformed into weeks. For some women (n = 196), the date of delivery was not available. In these cases, date of discharge from the hospital after delivery (minus the length of stay at the hospital) (n = 22) or the date of admission to the hospital (n = 60 women who did not have the date of discharge) was used.

Adherence

The women were instructed and encouraged at each visit to take the tablets they were given. Women allocated to the Routine iron group could refuse to take the iron tablets and they were classified as non-compliant with the intervention. Women who belonged in the Selective iron group and who wanted iron (even if their hemoglobin level was not below the cut-off level) were given iron; they were classified as non-compliant with the intervention. The following questions were asked on each visit: “Was hemoglobin measured?”; “Was iron/folic acid given to the woman?”; “Number of iron/folic acid tablets given?”; “Did the woman take the tablets during the past week?” At each subsequent visit, almost all of the Selective iron women (98%) were measured for

hemoglobin using the recommended HemoCue® method and the same proportion of women in the Routine iron group were given iron tablets at each subsequent visit.

Ethical approval

Ethical approval for the study was obtained from the Mozambique Ministry of Health Ethics Committee (CNBS [Ref. 84/CNBS/06]). A positive statement was obtained from the National Research and Development Centre for Welfare and Health (STAKES) (now the National Institute for Health and Welfare), Helsinki, Finland (Dno 2571/501/2007). The trial is registered at ClinicalTrials.gov, number NCT00488579.

Statistical analysis

All analyses were done on an intention-to-treat basis. Twin pregnancies (n = 48 pairs) were included in the analysis because their numbers were similar in the two groups and their exclusion did not alter the results. For pregnancy outcomes, all women (n = 4326), and for birth outcomes, women with birth data (n = 2258) were included. Differences in health indicators (fever, headache, cold/chills, nausea/vomiting, body aches, malaria) between the two iron groups at each subsequent visit (up to the 5th visit) during pregnancy were analyzed by using binomial generalized estimating equations (GEE) with an exchangeable correlation structure. GEE takes into account the within person correlation in the setting of repeated measures.

Differences in continuously distributed birth outcomes (birth weight, duration of gestation, length of hospital stay) were analyzed by using the two sample Student's t-test. Categorical outcomes were analyzed by using Pearson Chi-square test or Fisher's exact test (in the case of cells with less than 5 cases). To estimate the risk ratios of the effect of iron, the binary birth outcomes (low birth weight [< 2500 g], preterm birth [< 37 weeks], cesarean section delivery, child and maternal ill-health or

death at birth, negative fetal heart beat, delivery in a reference health center, long hospital stay after birth [≥ 2 days], and unavailability of delivery data) were analyzed by generalized linear models. The result estimates are presented with 95% confidence intervals (95% CI). Statistical significance was set at $P < 0.05$. STATA 11 statistical software was used for the analyses.

RESULTS

Of the 4326 women recruited to the trial, 2184 were randomly allocated to the Routine iron group and 2142 to the Selective iron group (Figure 1). The total number of prenatal visits varied but the maximum number of visits was seven. The number of follow-up visits was similar in the two groups (Figure 1). About 40% of delivery data were missed when using the original data collection method and the interim birth data were available for 1109 (51%) in the Routine iron group and for 1149 (54% of women) in the Selective.

Table 1 compares maternal background characteristics between the groups by the availability of birth data. Mean hemoglobin for the Selective group was similar between those with and without delivery data. The occurrence of symptoms suggestive of malaria (fever, headache, cold/chills, nausea/vomiting, and body aches) and self-reported malaria during the current pregnancy prior to the first prenatal visit were similar between the Routine and Selective iron groups. The women in the two groups with and without birth data were comparable.

Between the first and fifth visits, the two groups were similar regarding the occurrence of fever, headache, cold/chills, nausea/vomiting, and body aches (Table 2). There was a suggestion of increased incidence of self-reported malaria during pregnancy (odds ratio 1.37, 95% confidence interval 0.98-1.92) in the Routine iron group (Table 2). Table 2 presents the data for the second and third follow-up visits, but this was the case also in subsequent visits (data not shown).

Tables 3 presents the distribution of birth data by intervention group and Table 4 gives the estimates of the effect sizes on the birth outcomes. The birth outcomes were similar in the two groups. However, there was a suggestion (statistically non-significant) that the Routine iron group had worse outcomes in regard to babies with negative heartbeat at admission, and longer mother's hospital stay after birth (Table 3). The effect of iron on the primary outcomes was similar in the two groups. The groups were also relatively similar concerning most other outcomes. However, there was a suggestion of more babies with negative fetal heartbeat at admission, longer mother's hospital stay after birth and unavailability of delivery data in the Routine iron group (Table 4). By excluding births by cesarean section, the estimates for longer mother's hospital stay remained the same (data not shown).

DISCUSSION

The results from this trial indicate that routine iron prophylaxis during pregnancy was not advantageous over the policy of screening and treatment for anemia with regard to pregnancy and birth outcomes. If anything, screening and treatment for anemia appeared to be better. Among all the trial women there was a suggestion of an increased risk of self-reported malaria during pregnancy seen in the Routine iron group. The interim birth data suggested longer hospital stay after birth and higher risk of negative fetal heart beat in the Routine iron group. However, all these differences were statistically non-significant and the complete birth data are needed to conclude any putative effects of iron on birth outcomes.

One of the strengths of our trial is its large sample. So far, this represents the largest trial investigating the benefits of prophylactic iron during pregnancy on maternal and child health in

malaria-endemic settings. The compliance of the study nurses with the trial protocol and that of the women with regards to uptake of the iron and folic acid tablets was good, as was the follow-up during pregnancy ~~(Nwaru et al *Submitted*)~~.[22] However, during pregnancy, we lacked objective measures of malaria; hence, our results may not reflect the putative effect of iron on clinical malaria. The collection of delivery data was challenging, resulting in up to an estimated 40% of missing birth data. We did not realize the extent of the problem until most deliveries had occurred. We are currently tracing the birth data using various methods (abstracting hospital records and death register data and calling women), with results to be reported separately after finalization.

A comparison of our findings with previous studies conducted in malaria endemic areas is problematic because of key differences: the previous studies have compared iron versus no iron and our study compares two policies of iron administration: routine prophylaxis versus screening and treatment. Nevertheless, the studies from Nigeria[11] and The Gambia[12] found no significant effect of iron prophylaxis on malaria; they had used a more reliable measure of malaria (clinical and parasitological analysis). A Ugandan study[14] did not observe any effect of iron supplementation on the incidence of congenital malaria in the offspring. A Bangladeshi study[10] found a difference in preterm delivery (less in the non-iron group), but no association was seen with other outcomes examined, similar to the Nigerian study[11], including abortion, hypertension, eclampsia, postnatal complications, birth weight, Apgar scores, prematurity, development of diarrhea at 6 weeks, and perinatal mortality. Other benefits reported with iron prophylaxis include increased mean birth weight[12,14], reduced incidence of prematurity[12], and increased birth length and Apgar score.[13]

Although more complete birth data are needed to reach firm conclusions, we can speculate that the potential for higher incidence of unavailable delivery data in the Routine iron group may indicate that these women had more adverse outcomes, such as miscarriage and stillbirths, and consequently did not deliver in the expected health centers. Similarly, the higher likelihood of longer mother's hospital stay after birth in the Routine iron group may also be indicative of more problems at birth. Delivery by cesarean section did not explain the longer hospital stay as the estimate remained the same after excluding the births that occurred by cesarean section.

Anemia has been associated with maternal and child health risks[2-4], and the association between iron and increased risk of infections[5-7] calls for more definitive evidence on the benefits of iron prophylaxis during pregnancy in settings with increased infectious diseases where infections remain a major cause of maternal and child mortality.[8,9] Our trial in Maputo, Mozambique, is an attempt to investigate whether routine iron prophylaxis during pregnancy is more effective than screening and treatment for anemia in improving maternal and child health in an area of endemic malaria and HIV.

CONCLUSIONS

These interim results from this pragmatic randomized controlled trial indicate that routine iron prophylaxis during pregnancy did not suggest better maternal and child health outcomes than the policy of screening and treatment for anemia. If anything, screening and treatment for anemia appeared to be better. The complete birth data are needed for a firm conclusion. Which of the two methods, Routine or Selective iron prophylaxis, is more feasible, will be discussed in later publications.

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AUTHORS' CONTRIBUTION

Designed, analyzed, and wrote the paper (BIN, EH, ER, and SP). Designed and responsible for the conception of the PROFEG Trial (EH). Participated in the planning of the PROFEG Trial and made substantial contribution in its execution and participated in interpretation of results and critically reviewing the manuscript (BC, CS, EH, FA, GS, JC, MD, MN, OA, SP). Responsible for data preparation and cleaning (ER, OA).

COMPETING INTEREST

None declared.

DATA SHARING

Statistical codes and dataset are available from the corresponding author bright.nwaru@uta.fi. Informed consent was not obtained for data sharing but the presented data are anonymised and risk of identification is low.

REFERENCES

1. Peña-Rosas JP, Viteri FE. Effects and safety of preventive oral iron or iron+folic acid supplementation for women during pregnancy. Cochrane Database of Systematic Reviews 2009, Issue 4, art. No.: CD004736.
2. Villar J. Merialdi M, Gulmezoglu AM, Abalos E, Carroli G, Kulier R, et al. Nutritional interventions during pregnancy for the prevention or treatment of maternal mortality and preterm delivery: an overview of randomized controlled trials. *J Nutr* 2003; 133:1606-25.
3. Lao TT, Tam KF, Chan LY. Third trimester iron status and pregnancy outcome in non-anaemic women: pregnancy unfavourably affected by maternal iron excess. *Hum Reprod* 2000; 15: 1843-48.
4. Yip R. Significance of an abnormally low or high hemoglobin concentration during pregnancy: special consideration of iron nutrition. *Am J Clin Nutr* 2000 72: 272S-9S.
5. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. *J Nutr* 2001; 131: 616S-35S.
6. Prentice AM. Iron metabolism, malaria, and other infections: what is all the fuss about? *J Nutr* 2008; 138: 2537-41.
7. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. *BMJ* 2002; 325: 1142.
8. Lawn JE, Cousens S, Zupan J, Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? *Lancet* 2005; 365: 891-900.
9. Idemyor V. Human Immunodeficiency Virus (HIV) and Malaria Interaction in Sub-Saharan Africa: The Collision of Two Titans. *HIV Clin Trials* 2007; 8: 246-53.

10. Juncker T, Ameer S, Mmaum A, et al. A trial of iron and folate supplementation during pregnancy in Bangladesh. Abstract presented at the American Public Health Association, 2001, Atlanta, USA.

11. Fleming AF, Ghatoura GB, Harrison KA, Briggs ND, Dunn DT. The prevention of anaemia in pregnancy in primigravidae in the guinea savanna of Nigeria. *Ann Trop Med Parasitol* 1986; 80: 211-33.

12. Menendez C, Todd J, Alonso PL, Francis N, Lulat S, Ceesay S, et al The effects of iron supplementation during pregnancy, given by traditional birth attendants, on the prevalence of anaemia and malaria. *Trans R Soc Trop Med Hyg* 1994; 88: 590-3.

13. Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. *Am J Clin Nutr* 1997; 66: 1178-82.

14. Ndyomugyenyi R, Magnussen P. Chloroquine prophylaxis, iron/folic-acid supplementation or case management of malaria attacks in primigravidae in western Uganda: effects on congenital malaria and infant haemoglobin concentrations. *Ann Trop Med Parasitol* 2000; 94: 759-70.

15. Christian P, Khatry SK, Katz J, Pradhan EK, LeClerg SC, Shrestha SR, et al. Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal: a double blind randomized community trial. *BMJ* 2003; 326: 571.

16. Christian P, West KP, Khatry SK, LeClerg SC, Pradhan EK, Katz J, et al. Effects of maternal micronutrient supplementation on fetal loss and infant mortality: a cluster-randomized trial in Nepal. *Am J Clin Nutr* 2003; 78: 1194-1202.

17. Christian P, Darmstadt GL, Wu L, Khatry SK, LeClerg SC, Katz J, et al The effect of maternal micronutrient supplementation on early neonatal morbidity in rural Nepal: a randomized, control, community trial. *Arch Dis Child* 2008; 93: 660-4.

18. Christian P, Khatry SK, LeClerq SC, Dali SM. Effects of prenatal micronutrient supplementation on complications of labor and delivery and puerperal morbidity in rural Nepal. *Int J Gynaecol Obstet* 2009; 106: 3-7.

19. Christian P, Stewart CP, LeClerq SC, Wu L, Katz J, West KP Jr, et al. Antenatal and postnatal iron supplementation and childhood mortality in rural Neap: a prospective follow-up in a randomized, controlled community trial. *Am J Epidemiol* 2009; 170: 1127-36.

20. Christian P, Murray-Kolb LE, Khatry SK, Katz J, Schaefer BA, Cole PM, et al. Prenatal micronutrient supplementation and intellectual and motor function in early school-aged children in Nepal. *JAMA* 2010; 304: 2716-23.

21. Zeng L, Dibley MJ, Cheng Y, Dang S, Chang S, Kong L, et al. Impact of micronutrient supplementation during pregnancy on birth weight, duration of gestation, and perinatal mortality in rural western China: double blind cluster randomized controlled trial. *BMJ* 2008; 337: a2001.

22. Nwaru BI, Parkkali S, Abacassamo F, Salomé G, Chilundo B, Augusto O, et al. A pragmatic randomised controlled trial on routine iron prophylaxis during pregnancy in Maputo, Mozambique (PROFEG): rationale, desgin, and success. *Matern Child Nutr*. In press.

~~21.~~23. Dgedge M. Implementation of an insecticide Bednet programme for malaria prevention through the primary health care system in Mozambique. Doctoral thesis. London School of Hygiene and Tropical Medicine, 2000.

~~22.~~24. Müngen E. Iron supplementation in pregnancy. *J Perinat. Med* 2003; 31: 420-6.

~~23.~~25. Brabin BJ, Hakimi M, Pelletier D. An analysis of anemia and pregnancy-related maternal mortality. *J Nutr*. 2001; 131(suppl): 604S-15S.

~~24.~~26. Stoltzfus RJ, Mullanny L, Black RE. Iron deficiency anaemia. In: Ezzati M, Lopez AD, Rodgers A. et al, eds. Comparative Quantification of Health Risks: Global and

Regional Burden of Disease Attributable to Selected Major Risk Factors. Vol 1. Geneva, Switzerland: World Health Organization; 2004: 163-209.

Table 1. Characteristics of women at recruitment by availability of delivery data and group allocation, proportions % (numbers)

Characteristics	Delivery data, *N* = 2258		No delivery data, *N* = 2068	
	Selective iron (1149) % (*n*)	Routine iron (1109) % (*n*)	Selective iron (993) % (*n*)	Routine iron (1075) % (*n*)
Maternal age, mean (SD) years	24.6 (5.4)	24.7 (5.3)	25.0 (5.6)	24.6 (5.6)
Maternal age (categorized)				
< 20 years	17.5 (201)	16.5 (183)	15.7 (156)	19.3 (207)
20-24 years	41.1 (472)	39.9 (443)	39.0 (387)	37.1 (399)
25-29 years	23.1 (265)	23.3 (258)	23.9 (237)	23.4 (252)
30-34 years	11.3 (130)	13.8 (153)	12.9 (128)	13.3 (143)
≥ 35 years	6.3 (72)	5.6 (62)	7.4 (74)	6.5 (70)
Missing	0.8 (9)	0.9 (10)	1.1 (11)	0.4 (4)
Hemoglobin by HemoCue® (g/dl), mean (SD)	9.6 (1.7)		9.6 (1.7)	
Hemoglobin by HemoCue® (g/dl), n (%)				
< 7.0	6.9 (79)		6.2 (62)	
7.0-8.90	24.6 (283)		25.4 (252)	
9.0-9.90	23.5 (270)		24.4 (242)	
10.0-10-90	21.9 (252)		21.1 (210)	
11.0-11.90	14.1 (162)		13.7 (136)	
≥ 12.0	7.9 (91)		8.4 (83)	
Not measured	1.0 (12)		0.8 (8)	
Previous abortions				
No	87.6 (1007)	86.8 (963)	86.0 (854)	85.4 (918)
Yes	12.1 (139)	12.8 (142)	13.6 (135)	14.5 (156)
Missing	0.3 (3)	0.4 (4)	0.4 (4)	0.1 (1)
Gestational age, mean (SD) weeks	21.6 (5.9)	21.7 (5.6)	21.0 (5.9)	21.3 (5.8)

Gestational age (categorized)				
< 16	19.2 (221)	16.4 (182)	21.5 (213)	20.1 (216)
17-20	21.8 (250)	24.2 (268)	22.5 (223)	21.7 (233)
21-26	34.3 (394)	32.6 (361)	31.3 (311)	33.7 (362)
> 27	19.7 (226)	19.7 (219)	17.5 (174)	18.2 (196)
No information	58 (5.0)	7.1 (79)	7.3 (72)	6.3 (68)
Previous stillbirths				
No	91.5 (1052)	92.3 (1024)	91.6 (910)	91.0 (978)
Yes	8.2 (94)	7.2 (80)	7.9 (78)	8.9 (96)
Missing	0.3 (3)	0.5 (5)	0.5 (5)	0.1 (1)
Previous deliveries				
None	29.7 (341)	30.3 (336)	29.2 (290)	33.8 (363)
One	31.9 (367)	31.7 (352)	30.6 (304)	28.5 (306)
Two	19.2 (221)	17.8 (197)	17.9 (178)	18.6 (200)
Three or more	18.8(216)	19.8 (220)	22.0 (218)	19.0 (205)
Missing	0.4 (4)	0.4 (4)	0.3 (3)	0.1 (1)
HIV status				
Negative	81.2 (934)	81.8 (907)	76.7 (762)	79.0 (849)
Positive	18.8 (215)	18.2 (202)	23.3 (231)	21.0 (226)
Twin pregnancy				
No	98.7 (1134)	98.6 (1093)	99.2 (985)	99.2 (1066)
Yes	1.3 (15)	1.4 (16)	0.8 (8)	0.8 (9)
Symptoms during current pregnancy before first prenatal visit				
Fever				

Yes	22.9 (264)	24.4 (271)	28.8 (286)	23.8 (256)
Headache				
Yes	41.5 (477)	43.5 (482)	44.3 (440)	43.0 (462)
Cold/chills				
Yes	18.0 (207)	18.4 (204)	20.6 (205)	18.8 (202)
Vomit/nausea				
Yes	27.5 (316)	26.9 (298)	29.8 (296)	28.6 (307)
Body aches				
Yes	21.8 (251)	21.3 (237)	23.8 (236)	23.3 (251)
Self-reported malaria				
Yes	5.7 (66)	6.0 (67)	5.9 (59)	6.3 (68)
Had malaria test				
Yes	7.0 (80)	7.1 (79)	8.0 (79)	7.9 (85)

Table 2. Proportions (%) of women (numbers) with outcomes suggesting malaria during pregnancy, and odds ratios (OR) and 95% confidence intervals (95% CI) for group effect, $n = 4326$

Outcomes	Second visit[1] % (n)		Third visit[2] % (n)		Between the first and fifth visit[3] OR (95% CI)		
	Selective iron $n = 1455$	Routine iron $n = 1494$	Selective iron $n = 1040$	Routine iron $n = 1106$	Selective iron	Routine iron	P-value
Fever	11.5 (168)	10.0 (150)	11.3 (117)	12.1 (134)	1.00	0.95 (0.81-1.11)	0.523
Headache	24.9 (363)	24.3 (363)	24.9 (259)	25.1 (278)	1.00	0.98 (0.87-1.10)	0.738
Cold/chills	8.2 (120)	7.0 (104)	6.7 (70)	7.8 (86)	1.00	0.92 (0.76-1.11)	0.361
Vomit/nausea	9.1 (133)	10.2 (153)	8.5 (88)	9.6 (109)	1.00	1.09 (0.92-1.31)	0.323
Body aches	10.1 (147)	9.2 (138)	10.9 (113)	9.8 (108)	1.00	0.89 (0.75-1.06)	0.180
Self-reported malaria	2.4 (35)	3.0 (45)	1.5 (16)	2.2 (24)	1.00	1.37 (0.98-1.92)	0.068

[1]Betetween first and second visit

[2]Between second and third visit

[3]The effect estimates calculated by binomial generalized estimating equations (with exchangeable correlation structure) to account for the repeated measures of the outcomes.

Table 3. Birth outcomes by group allocation, percentages, % (numbers) of women or babies or means (SD).

Outcomes	Selective iron *n* = 1149	Routine iron *n* =1109	*P*-value[1]
Birth weight, mean (SD) grams	2996.3 (508.4)	2989.4 (514.9)	0.752
Birth weight, % (*n*)			0.443
< 2500 g	11.8 (136)	12.8 (142)	
2500-2999 g	30.6 (351)	31.1 (345)	
3000-3499 g	40.5 (465)	37.8 (419)	
3500-3999 g	12.7 (146)	13.8 (153)	
≥ 4000 g	3.0 (34)	2.1 (23)	
No information	1.5 (17)	2.4 (27)	
Duration of gestation, mean (SD) weeks	38.3 (4.2)	38.4 (4.0)	0.689
Duration of gestation, % (*n*)			0.056
< 37 weeks	28.8 (331)	27.0 (299)	
≥ 37 weeks	67.2 (772)	66.9 (742)	
No information	4.0 (46)	6.1 (68)	
Mode of delivery, % (*n*)			0.235
Normal	87.6 (1007)	89.4 (991)	
Cesarean section	1.3 (15)	2.0 (22)	
No information	11.1 (127)	8.7 (96)	
Child health status at birth, % (*n*)			0.685
Well	94.0 (1080)	92.1 (1022)	
Ill	0.7 (8)	1.0 (11)	
Dead	1.8 (21)	2.0 (22)	
No information	3.5 (40)	5.0 (55)	

Still birth, % (*n*)			0.558
No	81.2 (933)	79.7 (884)	
Yes	2.5 (29)	2.9 (32)	
No information	16.3 (187)	17.4 (193)	
Fetal heart beat at admission, % (*n*)			0.085
Negative	1.6 (18)	2.6 (29)	
Positive	85.6 (984)	85.2 (945)	
No information	12.8 (147)	12.2 (135)	
Mother's health status at birth, % (*n*)			0.895
Well	95.6 (1098)	94.9 (1052)	
Ill	0.4 (4)	0.4 (4)	
Dead	0.1 (1)	0.2 (2)	
No information	4.0 (46)	4.6 (51)	
Length of hospital stay, mean (SD) days	1.33 (1.21)	1.63 (1.30)	0.075
Length of hospital stay after birth, % (*n*)			0.103
≤ 1 day	65.1 (748)	60.7 (673)	
2 days	23.5 (270)	24.2 (268)	
≥ 3 days	4.0 (46)	5.6 (62)	
No information	7.4 (85)	9.6 (106)	
Place of delivery, % (*n*)			0.652
1o de Maio (health center)	44.4 (510)	42.6 (472)	
Machava (health center)	35.1 (403)	38.2 (424)	
Jose Macamo (hospital)	3.7 (43)	3.6 (40)	
Mavalane (hospital)	14.3 (164)	12.8 (142)	

Central Hospital	0.3 (3)	0.3 (3)
At home	1.1 (13)	1.3 (14)
On the way to hospital	0.0 (0)	0.2 (2)
No information	1.1 (13)	1.1 (12)

[1]Based on T-test for continuous outcomes, Pearson Chi-square test or Fisher's exact test for categorical outcomes. Subjects with no information were not included in the tests.

Table 4. Numbers, proportions (%), and risk ratios (RR, 95% confidence intervals CI) of birth outcomes by iron groups

Outcomes	Selective iron *n*	Routine iron *n*	Selective iron *%*	Routine iron %	Selective iron	Routine iron RR (95% CI)[2]	*P*-value
Primary health outcomes							
Low birth weight (<2500 grams)	136	142	11.8	12.8	1.00	1.09 (0.88-1.36)	0.431
Preterm delivery (<37 weeks)	331	299	28.8	27.0	1.00	0.96 (0.84-1.09)	0.185
Secondary health outcomes							
Cesarean section delivery	15	22	1.3	2.0	1.00	1.48 (0.77-2.84)	0.238
Negative fetal heart beat at admission	18	29	1.6	2.6	1.00	1.66 (0.93-2.96)	0.089
Child ill or dead at birth	29	33	2.5	3.0	1.00	1.20 (0.73-1.96)	0.473
Mother ill or dead at birth	5	6	0.4	0.5	1.00	1.25 (0.38-4.09)	0.711
Other outcomes							
Delivery in reference center[1]	210	185	18.3	16.7	1.00	0.91 (0.76-1.09)	0.316
Long hospital stay after delivery (≥ 3 days)	46	62	4.0	5.6	1.00	1.43 (0.97-1.26)	0.059
No delivery data	993	1075	46.4	49.2	1.00	1.06 (1.00-1.13)	0.060

[1]Jose Macamo or Mavalane or Central Hospital
[2]The estimates were not adjusted for any baseline characteristic because the two groups did not differ from each other at baseline

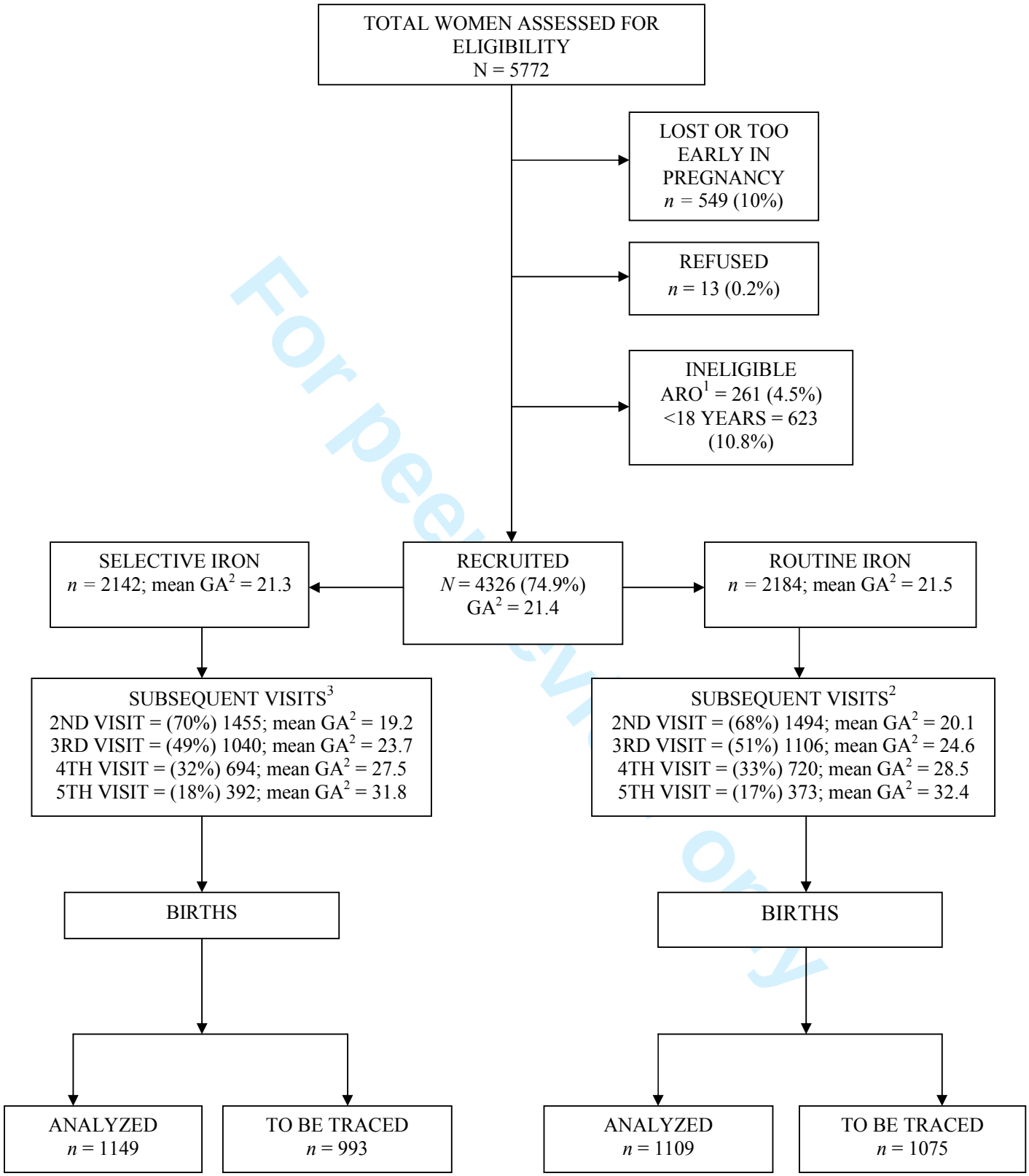

Figure 1. PROFEG Trial Flow Diagram

[1]ARO = high risk pregnancy; [2]GA = gestational age in weeks; [3]After recruitment, % were calculated from recruited, *n* = 4326

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2, 3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5,6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6,7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	None
Participants	4a	Eligibility criteria for participants	7,8
	4b	Settings and locations where the data were collected	6,7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9,10
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10
	6b	Any changes to trial outcomes after the trial commenced, with reasons	None
Sample size	7a	How sample size was determined	8,9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	Not done.

		assessing outcomes) and how	Pragmatic trial design
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11,12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Not done
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	13, Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	13, Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6,7
	14b	Why the trial ended or was stopped	Ended as planned
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	13, Tables 2-4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	13,14, Tables 2-4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Table 4
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	None
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA (benefits and harms not distinguished)
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14,15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	15, 16
Other information			
Registration	23	Registration number and name of trial registry	3
Protocol	24	Where the full trial protocol can be accessed, if available	From Authors
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	3, 17

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Comparison of routine prenatal iron prophylaxis and screening and treatment for anemia: pregnancy results and preliminary birth results from a pragmatic randomized controlled trial (PROFEG) in Maputo, Mozambique

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Comparison of routine prenatal iron prophylaxis and screening and treatment for anemia: pregnancy results and preliminary birth results from a pragmatic randomized controlled trial (PROFEG) in Maputo, Mozambique

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ABSTRACT

Objective: To present the pregnancy results and interim birth results of a pragmatic randomized controlled trial comparing routine iron prophylaxis with screening and treatment for anemia during pregnancy in a setting of endemic malaria and HIV.

Design: A pragmatic randomized controlled trial

Setting: Two health centers (1º de Maio and Machava) in Maputo, Mozambique, a setting of endemic malaria and high prevalence of HIV.

Participants: Pregnant women (≥18 years old; non-high-risk pregnancy, *N*=4326) attending prenatal care consultation at the two health centers were recruited to the trial

Interventions: The women were randomly allocated to either Routine iron (n=2184; 60 mg ferrous sulphate plus 400 µg of folic acid daily throughout pregnancy) or Selective iron (n=2142; screening and treatment for anemia and daily intake of 1 mg of folic acid).

Outcome measures: The primary outcomes were preterm delivery (delivery <37 weeks of gestation) and low birth weight (<2500 grams). The secondary outcomes were symptoms suggestive of malaria and self-reported malaria during pregnancy; birth length; cesarean section; maternal and child health status after delivery.

Results: The number of follow-up visits was similar in the two groups. Between the first and fifth visits, the two groups were similar regarding the occurrence of fever, headache, cold/chills, nausea/vomiting, and body aches. There was a suggestion of increased incidence of self-reported malaria during pregnancy (odds ratio 1.37, 95% confidence interval 0.98-1.92) in the Routine iron group. Birth data were available for 1109 (51%) in the Routine iron and for 1149 (54%) in the Selective iron groups. The birth outcomes were relatively similar in the two groups. However, there was a suggestion (statistically non-significant) of poorer outcomes in the Routine iron group with regard to long hospital stay after birth (relative risk [RR] 1.43, 95% CI 0.97-1.26; risk difference

[RD] 0.02, 95% CI -0.00-0.03) and unavailability of delivery data (RR 1.06, 95% CI 1.00-1.13; RD 0.03, 95% CI -0.01-0.07).

Conclusions: These interim results suggest that routine iron prophylaxis during pregnancy did not confer advantage over screening and treatment for anemia regarding maternal and child health. Complete data on birth outcomes are being collected for firmer conclusions**.**

Trial registration: The trial is registered at ClinicalTrials.gov, number NCT00488579 (June 2007). The first women were randomized to the trial proper April 2007- March 2008. The pilot was November 2006-March 2008. The 3-month lag was due to technical difficulties in completing trial registration.

Funding: The study was funded by two grants from the Academy of Finland (2004: 210631; 2010: 139191).

Keywords: iron, pregnancy, birth, malaria, HIV, pragmatic trial, Mozambique

ARTICLE SUMMARY:

Article focus:

- The benefits of iron prophylaxis during pregnancy on maternal and child health in developing country settings with endemic malaria and high prevalence of HIV is unclear.
- Iron has been linked to increased risk of infections.
- Among children less than three years, there are indications of harm of universal iron prophylaxis.

Key messages:

- Routine iron prophylaxis during pregnancy did not suggest better maternal and child health outcomes than screening and treatment for anemia in a setting of endemic malaria and HIV.

Strengths and limitations of this study

- So far, this represents the largest trial investigating the benefits of prophylactic iron during pregnancy on maternal and child health in malaria-endemic settings.
- The compliance of the study nurses with the trial protocol and that of the women with regards to uptake of the iron and folic acid tablets was good, as was the follow-up during pregnancy.
- The collection of delivery data was challenging, resulting in up to an estimated 40% of missing birth data, which are now been traced using various methods.

INTRODUCTION

Despite widespread recommendation of routine iron prophylaxis during pregnancy, its benefits and risks for the mother and child, beyond the reduction of the risk of anemia, remain unclear, particularly in low-income settings. Reviews of randomized controlled trials (RTCs) done for the Cochrane Collaboration and the World Health Organization (WHO) have failed to conclude on the effects of routine iron prophylaxis during pregnancy on pregnancy and birth outcomes.[1,2] There is some evidence that high hemoglobin concentration in late pregnancy may be associated with adverse effects on pregnancy.[3,4] Based on evidence from non-pregnant populations, it has been suggested that iron may advance the rate of infections.[5-7] The host requires iron for biochemical functioning, but iron may as well promote the replication of infectious agents.[6] For developing country settings which are still plagued by infectious diseases, such as malaria and HIV, the possible association between iron and infections raises serious public health concerns.[8,9]

Previous trials conducted in malarial developing country settings that have evaluated the effects of iron supplementation during pregnancy on maternal and child outcomes have been hampered by small samples, large dropouts, and several outcome-related exclusions.[10-14] The findings from the trials were conflicting on the role of prophylactic iron supplementation on birth weight, prematurity, perinatal mortality, incidence of malaria, and other pregnancy and birth outcomes. Consequently, the evidence they provide is insufficient in addressing the question of the advantages and disadvantages of prenatal prophylactic iron. The results of studies from non-malarial areas[15-21], although of better quality, may not be relevant due to different settings.[15-21] Although, results were also conflicting in a number of outcomes, the main findings included slightly longer birth length,

longer gestational age, and reduced risk of preterm delivery, intrapartum hemorrhage, low birth weight, and infant and child mortality in the iron-folic acid group.[22]

This limited evidence and the importance of iron prophylaxis in prenatal programs call for further investigation on the benefits of prenatal iron supplementation in areas of endemic malaria and high prevalence of HIV. Using a pragmatic randomized controlled trial, we investigated the effects of routine iron prophylaxis throughout pregnancy compared to screening and treatment for anemia on maternal and child health in Maputo, Mozambique. The present paper presents the pregnancy results and interim birth results. About 40% of births were missed by the original data collection method[22], and missing birth data are currently being retrieved with various complementary methods. The completed birth results will be presented later.

MATERIALS AND METHODS

Study design and population

The details of the PROFEG trial have been described elsewhere[22] and only the main features are given here. The trial was a pragmatic randomized controlled trial to compare two iron administration policies (routine iron prophylaxis versus screening and treatment for anemia during pregnancy) on maternal and child health in Maputo, Mozambique. The trial was carried out in two health centers, 1o de Maio in Maputo City, the capital (November 2006 - October 2008) and Machava 2, in Maputo Province (June 2007 - October 2008), Mozambique. The completion of collection of birth data continued until 2012. The health center of Machava 2 in Maputo province is close to Maputo city. The population is urban and semi urban and malaria is endemic in both areas. Seasonal increase of malaria is usually observed towards the end of the rainy season (February to April).[23]

In the study area all woman are eligible to attend prenatal care. The usual care recommendations at the time of the trial included daily prophylactic iron-folate supplementation (60 mg+400 µg) throughout pregnancy; one dose of mebendazol 500 mg for intestinal parasite; three doeses of sulfadoxine pyrimethamine for malaria prophylaxis (started around 20 weeks gestation, or when quickening occurs, or when the foetal heart is heard); hemoglobin measurement (Lovibond ® is routinely used) and syphilis screening at the first prenatal visit; and three doses of tetanus vaccine (at the 5th and 7th month and at delivery). If malaria was suspected during prenatal consultations, it was diagnosed by laboratory tests and clinical signs. In most health centers, including our study centers, HIV testing was offered.[22] Antiretroviral (ARV) drugs were provided by various international organizations, but we do not have information of how many women received treatment during pregnancy. The recommendation was to give ARV (Nevirapine) at delivery to prevent mother-child transmission.

Recruitment of study participants

Pregnant women attending their first prenatal visit were the target group. During the routine early morning health education sessions, all women who came for their first prenatal visit were given general information about the study. Recruitment into the study occurred during individual consultations and was carried out by study nurses who were employed and trained by the project. In 1^{o} de Maio health center, the women visited the study nurses after their routine prenatal care consultations with the maternal and child health (MCH) nurses. In Machava, the study nurse and the routine MCH nurse saw the women in the same room. The study nurses checked for women's eligibility to participate in the study. The exclusion criteria were: women with high obstetric risk and those aged less than 18 years. If eligible, the nurses asked the women to join the study. Oral and

written informed consent was obtained. Three types of women were missed from the study: women whom MCH nurses sent back home because of too early pregnancy, women who did not go to the study nurse, and women who refused the study.

Randomization

The women were randomized into either the Routine iron group (i.e., routine iron prophylaxis from the first to the last prenatal visit) or the Selective iron group group (i.e. regular screening for hemoglobin level and treatment for anemia). Researcher (OA) use the STATA statistical software (StataCorp LP, Texas, USA) was used to generate sequential random numbers separately for the two centers and the women were assigned to either of the groups with a probability of 50%. The codes for the groups were put into sealed and numbered opaque envelopes; the number was the woman's study number and was repeated in the documents in the envelope. The envelope contained a study identification card (yellow for the Routine iron group and pink for the Selective, 10 x 20cm) and the informed consent form.

Sample size

We did not have up-to-date reliable baseline data of pregnant women's and newborns' health in Maputo or of the effects of iron on pregnancy and birth outcomes. Thus , we used different estimates of the baseline values for preterm delivery, low birth weight, clinical malaria, and perinatal mortality to calculate the sample size, with power (85% and 90%), significance level of 5%, and the size of the difference to be detected (20% and 30%). Based on these calculations and the expected feasibility, we decided a sample size of 2000 women in each group to be enough to measure clinically meaningful effects. The STATA statistical software was used to estimate the

sample size. A table showing the various baseline assumptions used for power calculation and in estimating the sample size for the study is included as Appendix 1.

Interventions

On each prenatal visit, women in the Routine iron group received 30 tablets (supply of one month) of 60 mg ferrous sulphate plus 400 µg of folic acid per day combined in one tablet. In the Selective group, women's hemoglobin levels were measured at each visit by the study nurses using a rapid hemoglobin measure, HemoCue® Hb 201+, (Hemocue AB, Ängelholm, Sweden). If the hemoglobin was 9g/dl or more, they received 30 tablets of 1 mg of folic acid per day. If their hemoglobin was below the cut-off of <9g/dl Hb, they received a monthly double dose of iron (60 mg + 60 mg) for the treatment of anemia. Folic acid 1 mg tablets were used because at the time of the trial pure folic acid was not licensed in Mozambique in 400 µg tablets. The tablets were given in a plastic bag having the drug's name and dosage on it.

Data collection and follow-up

Data were collected on standard study data forms by three methods: 1) study nurses abstracted prenatal data from mothers' maternity cards, 2) study nurses asked women additional questions at the time of the prenatal visits, and 3) study nurses or researchers afterwards collected birth data from hospital birth records. Delivery nurses were informed of the study and asked to put the delivery cards into a separate study box. The study women were to be identified by the color of the identification card stapled to their maternity card. However, this did not succeed very well. By

excluding estimated late miscarriages (5%), early stillbirths (3 %) and home births (10%), we should have received delivery data for 3547 women (82%) of the 4326 women who participated in the trial. We received birth data for only 2258 (64% of the estimated 3547) women.

Outcome measures

The primary outcomes were preterm delivery (delivery <37 weeks of gestation and low birth weight (<2500 grams); data on weight came from the birth records; for gestation weeks various routine data sources were used (see below). Originally, we had malaria activation as a primary outcome, but the pilot showed that it was not feasible. Secondary outcomes were perinatal mortality (as available from our data collection forms; unlikely to cover early stillbirths or neonatal deaths occurring at home); complications during pregnancy and labor; symptoms suggestive of malaria (fever, headache, cold/chills, nausea/vomiting, and body aches); and self-reported malaria during pregnancy (the woman was asked for diagnosed malaria since her last visit).

Gestational weeks

In the prenatal visits routine MCH nurses determined gestational weeks in various ways, even though all ways were not systematically noted down. In the first prenatal visits, the date of last menstrual period, uterine fundal height, assumed date of delivery and length of gestation (best estimate) were noted. The study nurses abstracted all this information and the best estimate was used in this paper. In birth records, the last menstrual period, date of fertilization, assumed date of fertilization, and length of gestation were to be given by delivery nurses. However, these data were very poorly filled and only 681 (30%) of the women with delivery data had their gestational weeks recorded at birth. Thus the gestational weeks for women without that information was estimated from dates using the following algorithm: gestational weeks at first visit in days + days between the

first visit and delivery; the days were then transformed into weeks. For some women (n = 196), the date of delivery was not available. In these cases, date of discharge from the hospital after delivery (minus the length of stay at the hospital) (n = 22) or the date of admission to the hospital (n = 60 women who did not have the date of discharge) was used.

Adherence

The women were instructed and encouraged at each visit to take the tablets they were given. Women allocated to the Routine iron group could refuse to take the iron tablets and they were classified as non-compliant with the intervention. Women who belonged in the Selective iron group and who wanted iron (even if their hemoglobin level was not below the cut-off level) were given iron; they were classified as non-compliant with the intervention. The following questions were asked on each visit: "Was hemoglobin measured?"; "Was iron/folic acid given to the woman?"; "Number of iron/folic acid tablets given?"; "Did the woman take the tablets during the past week?" At each subsequent visit, almost all of the Selective iron women (98%) were measured for hemoglobin using the recommended HemoCue® method and the same proportion of women in the Routine iron group were given iron tablets at each subsequent visit.

Ethical approval

Ethical approval for the study was obtained from the Mozambique Ministry of Health Ethics Committee (CNBS [Ref. 84/CNBS/06]). A positive statement was obtained from the National Research and Development Centre for Welfare and Health (STAKES) (now the National Institute for Health and Welfare), Helsinki, Finland (Dno 2571/501/2007). The trial is registered at ClinicalTrials.gov, number NCT00488579.

Statistical analysis

All analyses were done on an intention-to-treat basis. Twin pregnancies (n = 48 pairs) were included in the analysis because their numbers were similar in the two groups and their exclusion did not alter the results. For pregnancy outcomes, all women (n = 4326), and for birth outcomes, women with birth data (n = 2258) were included. Differences in health indicators (fever, headache, cold/chills, nausea/vomiting, body aches, malaria) between the two iron groups at each subsequent visit (up to the 5th visit) during pregnancy were analyzed by using binomial generalized estimating equations (GEE) with an exchangeable correlation structure. GEE takes into account the within person correlation in the setting of repeated measures.

Differences in continuously distributed birth outcomes (birth weight, duration of gestation, length of hospital stay) were analyzed by using the two sample Student's t-test. Categorical outcomes were analyzed by using Pearson Chi-square test or Fisher's exact test (in the case of cells with less than 5 cases). To estimate the risk ratios of the effect of iron, the binary birth outcomes (low birth weight [< 2500 g], preterm birth [< 37 weeks], cesarean section delivery, child and maternal ill-health or death at birth, negative fetal heart beat, delivery in a reference health center, long hospital stay after birth [≥ 2 days], and unavailability of delivery data) were analyzed by generalized linear models. The result estimates are presented with 95% confidence intervals (95% CI). Statistical significance was set at $P < 0.05$. STATA 11 statistical software was used for the analyses.

RESULTS

Of the 4326 women recruited to the trial, 2184 were randomly allocated to the Routine iron group and 2142 to the Selective iron group (Figure 1). The total number of prenatal visits varied but the maximum number of visits was seven. The number of follow-up visits was similar in the two groups (Figure 1). About 40% of delivery data were missed when using the original data collection

method and the interim birth data were available for 1109 (51%) in the Routine iron group and for 1149 (54% of women) in the Selective.

Table 1 compares maternal background characteristics between the groups by the availability of birth data. Mean hemoglobin for the Selective group was similar between those with and without delivery data. The occurrence of symptoms suggestive of malaria (fever, headache, cold/chills, nausea/vomiting, and body aches) and self-reported malaria during the current pregnancy prior to the first prenatal visit were similar between the Routine and Selective iron groups. The women in the two groups with and without birth data were comparable.

Between the first and fifth visits, the two groups were similar regarding the occurrence of fever, headache, cold/chills, nausea/vomiting, and body aches (Table 2). There was a suggestion of increased incidence of self-reported malaria during pregnancy (odds ratio 1.37, 95% confidence interval 0.98-1.92) in the Routine iron group (Table 2). Table 2 presents the data for the second and third follow-up visits, but this was the case also in subsequent visits (data not shown).

Tables 3 presents the distribution of birth data by intervention group and Table 4 gives the estimates of the effect sizes on the birth outcomes. The birth outcomes were similar in the two groups. However, there was a suggestion (statistically non-significant) that the Routine iron group had worse outcomes in regard to babies with negative heartbeat at admission, and longer mother's hospital stay after birth (Table 3). The effect of iron on the primary outcomes was similar in the two groups. The groups were also relatively similar concerning most other outcomes. However, there was a suggestion of more babies with negative fetal heartbeat at admission, longer mother's hospital stay after birth and unavailability of delivery data in the Routine iron group (Table 4). By excluding

births by cesarean section, the estimates for longer mother's hospital stay remained the same (data not shown).

DISCUSSION

The results from this trial indicate that routine iron prophylaxis during pregnancy was not advantageous over the policy of screening and treatment for anemia with regard to pregnancy and birth outcomes. If anything, screening and treatment for anemia appeared to be better. Among all the trial women there was a suggestion of an increased risk of self-reported malaria during pregnancy seen in the Routine iron group. The interim birth data suggested longer hospital stay after birth and higher risk of negative fetal heart beat in the Routine iron group. However, all these differences were statistically non-significant and the complete birth data are needed to conclude any putative effects of iron on birth outcomes.

One of the strengths of our trial is its large sample. So far, this represents the largest trial investigating the benefits of prophylactic iron during pregnancy on maternal and child health in malaria-endemic settings. The compliance of the study nurses with the trial protocol and that of the women with regards to uptake of the iron and folic acid tablets was good, as was the follow-up during pregnancy.[22] However, during pregnancy, we lacked objective measures of malaria; hence, our results may not reflect the putative effect of iron on clinical malaria. The collection of delivery data was challenging, resulting in up to an estimated 40% of missing birth data. We did not realize the extent of the problem until most deliveries had occurred. We are currently tracing the birth data using various methods (abstracting hospital records and death register data and calling women), with results to be reported separately after finalization.

A comparison of our findings with previous studies conducted in malaria endemic areas is problematic because of key differences: the previous studies have compared iron versus no iron and our study compares two policies of iron administration: routine prophylaxis versus screening and treatment. Nevertheless, the studies from Nigeria[11] and The Gambia[12] found no significant effect of iron prophylaxis on malaria; they had used a more reliable measure of malaria (clinical and parasitological analysis). A Ugandan study[14] did not observe any effect of iron supplementation on the incidence of congenital malaria in the offspring. A Bangladeshi study[10] found a difference in preterm delivery (less in the non-iron group), but no association was seen with other outcomes examined, similar to the Nigerian study[11], including abortion, hypertension, eclampsia, postnatal complications, birth weight, Apgar scores, prematurity, development of diarrhea at 6 weeks, and perinatal mortality. Other benefits reported with iron prophylaxis include increased mean birth weight[12,14], reduced incidence of prematurity[12], and increased birth length and Apgar score.[13]

Although more complete birth data are needed to reach firm conclusions, we can speculate that the potential for higher incidence of unavailable delivery data in the Routine iron group may indicate that these women had more adverse outcomes, such as miscarriage and stillbirths, and consequently did not deliver in the expected health centers. Similarly, the higher likelihood of longer mother's hospital stay after birth in the Routine iron group may also be indicative of more problems at birth. Delivery by cesarean section did not explain the longer hospital stay as the estimate remained the same after excluding the births that occurred by cesarean section.

Anemia has been associated with maternal and child health risks[24-26], and the association between iron and increased risk of infections[5-7]calls for more definitive evidence on the benefits of iron

prophylaxis during pregnancy in settings with increased infectious diseases where infections remain a major cause of maternal and child mortality.[8,9] Our trial in Maputo, Mozambique, is an attempt to investigate whether routine iron prophylaxis during pregnancy is more effective than screening and treatment for anemia in improving maternal and child health in an area of endemic malaria and HIV.

CONCLUSIONS

These interim results from this pragmatic randomized controlled trial indicate that routine iron prophylaxis during pregnancy did not suggest better maternal and child health outcomes than the policy of screening and treatment for anemia. If anything, screening and treatment for anemia appeared to be better. The complete birth data are needed for a firm conclusion. Which of the two methods, Routine or Selective iron prophylaxis, is more feasible, will be discussed in later publications.

ACKNOWLEDGMENTS

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AUTHORS' CONTRIBUTION

Designed, analyzed, and wrote the paper (BIN, EH, ER, and SP). Designed and responsible for the conception of the PROFEG Trial (EH). Participated in the planning of the PROFEG Trial and made substantial contribution in its execution and participated in interpretation of results and critically

reviewing the manuscript (BC, CS, EH, FA, GS, JC, MD, MN, OA, SP). Responsible for data preparation and cleaning (ER, OA).

COMPETING INTEREST

None declared.

DATA SHARING

Statistical codes and dataset are available from the corresponding author bright.nwaru@uta.fi. Informed consent was not obtained for data sharing but the presented data are anonymised and risk of identification is low.

REFERENCES

1. Peña-Rosas JP, Viteri FE. Effects and safety of preventive oral iron or iron+folic acid supplementation for women during pregnancy. Cochrane Database of Systematic Reviews 2009, Issue 4, art. No.: CD004736.
2. Villar J, Merialdi M, Gulmezoglu AM, et al. Nutritional interventions during pregnancy for the prevention or treatment of maternal mortality and preterm delivery: an overview of randomized controlled trials. *J Nutr* 2003; 133:1606-25.

3. Lao TT, Tam KF, Chan LY. Third trimester iron status and pregnancy outcome in non-anaemic women: pregnancy unfavourably affected by maternal iron excess. *Hum Reprod* 2000; 15: 1843-48.

4. Yip R. Significance of an abnormally low or high hemoglobin concentration during pregnancy: special consideration of iron nutrition. *Am J Clin Nutr* 2000 72: 272S-9S.

5. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. *J Nutr* 2001; 131: 616S-35S.

6. Prentice AM. Iron metabolism, malaria, and other infections: what is all the fuss about? *J Nutr* 2008; 138: 2537-41.

7. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. *BMJ* 2002; 325: 1142.

8. Lawn JE, Cousens S, Zupan J, Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? *Lancet* 2005; 365: 891-900.

9. Idemyor V. Human Immunodeficiency Virus (HIV) and Malaria Interaction in Sub-Saharan Africa: The Collision of Two Titans. *HIV Clin Trials* 2007; 8: 246-53.

10. Juncker T, Ameer S, Mmaum A, et al. A trial of iron and folate supplementation during pregnancy in Bangladesh. Abstract presented at the American Public Health Association, 2001, Atlanta, USA.

11. Fleming AF, Ghatoura GB, Harrison KA, et al. The prevention of anaemia in pregnancy in primigravidae in the guinea savanna of Nigeria. *Ann Trop Med Parasitol* 1986; 80: 211-33.

12. Menendez C, Todd J, Alonso PL, et al The effects of iron supplementation during pregnancy, given by traditional birth attendants, on the prevalence of anaemia and malaria. *Trans R Soc Trop Med Hyg* 1994; 88: 590-3.

13. Preziosi P, Prual A, Galan P, et al. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. *Am J Clin Nutr* 1997; 66: 1178-82.

14. Ndyomugyenyi R, Magnussen P. Chloroquine prophylaxis, iron/folic-acid supplementation or case management of malaria attacks in primigravidae in western Uganda: effects on congenital malaria and infant haemoglobin concentrations. *Ann Trop Med Parasitol* 2000; 94: 759-70.

15. Christian P, Khatry SK, Katz J, et al. Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal: a double blind randomized community trial. *BMJ* 2003; 326: 571.

16. Christian P, West KP, Khatry SK, et al. Effects of maternal micronutrient supplementation on fetal loss and infant mortality: a cluster-randomized trial in Nepal. *Am J Clin Nutr* 2003; 78: 1194-1202.

17. Christian P, Darmstadt GL, Wu L, et al The effect of maternal micronutrient supplementation on early neonatal morbidity in rural Nepal: a randomized, control, community trial. *Arch Dis Child* 2008; 93: 660-4.

18. Christian P, Khatry SK, LeClerq SC, et al. Effects of prenatal micronutrient supplementation on complications of labor and delivery and puerperal morbidity in rural Nepal. *Int J Gynaecol Obstet* 2009; 106: 3-7.

19. Christian P, Stewart CP, LeClerq SC, et al. Antenatal and postnatal iron supplementation and childhood mortality in rural Neap: a prospective follow-up in a randomized, controlled community trial. *Am J Epidemiol* 2009; 170: 1127-36.

20. Christian P, Murray-Kolb LE, Khatry SK, et al. Prenatal micronutrient supplementation and intellectual and motor function in early school-aged children in Nepal. *JAMA* 2010; 304: 2716-23.

21. Zeng L, Dibley MJ, Cheng Y, et al. Impact of micronutrient supplementation during pregnancy on birth weight, duration of gestation, and perinatal mortality in rural western China: double blind cluster randomized controlled trial. *BMJ* 2008; 337: a2001.

22. Nwaru BI, Parkkali S, Abacassamo F, et al. A pragmatic randomised controlled trial on routine iron prophylaxis during pregnancy in Maputo, Mozambique (PROFEG): rationale, desgin, and success. *Matern Child Nutr*. In press.

23. Dgedge M. Implementation of an insecticide Bednet programme for malaria prevention through the primary health care system in Mozambique. Doctoral thesis. London School of Hygiene and Tropical Medicine, 2000.

24. Müngen E. Iron supplementation in pregnancy. *J Perinat. Med* 2003; 31: 420-6.

25. Brabin BJ, Hakimi M, Pelletier D. An analysis of anemia and pregnancy-related maternal mortality. *J Nutr*. 2001; 131(suppl): 604S-15S.

26. Stoltzfus RJ, Mullanny L, Black RE. Iron deficiency anaemia. In: Ezzati M, Lopez AD, Rodgers A. et al, eds. Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributable to Selected Major Risk Factors. Vol 1. Geneva, Switzerland: World Health Organization; 2004: 163-209.

Table 1. Characteristics of women at recruitment by availability of delivery data and group allocation, proportions % (numbers)

Characteristics	Delivery data, *N* = 2258		No delivery data, *N* = 2068	
	Selective iron (1149) % (*n*)	Routine iron (1109) % (*n*)	Selective iron (993) % (*n*)	Routine iron (1075) % (*n*)
Maternal age, mean (SD) years	24.6 (5.4)	24.7 (5.3)	25.0 (5.6)	24.6 (5.6)
Maternal age (categorized)				
< 20 years	17.5 (201)	16.5 (183)	15.7 (156)	19.3 (207)
20-24 years	41.1 (472)	39.9 (443)	39.0 (387)	37.1 (399)
25-29 years	23.1 (265)	23.3 (258)	23.9 (237)	23.4 (252)
30-34 years	11.3 (130)	13.8 (153)	12.9 (128)	13.3 (143)
≥ 35 years	6.3 (72)	5.6 (62)	7.4 (74)	6.5 (70)
Missing	0.8 (9)	0.9 (10)	1.1 (11)	0.4 (4)
Hemoglobin by HemoCue® (g/dl), mean (SD)	9.6 (1.7)		9.6 (1.7)	
Hemoglobin by HemoCue® (g/dl), n (%)				
< 7.0	6.9 (79)		6.2 (62)	
7.0-8.90	24.6 (283)		25.4 (252)	
9.0-9.90	23.5 (270)		24.4 (242)	
10.0-10-90	21.9 (252)		21.1 (210)	
11.0-11.90	14.1 (162)		13.7 (136)	
≥ 12.0	7.9 (91)		8.4 (83)	
Not measured	1.0 (12)		0.8 (8)	
Previous abortions				
No	87.6 (1007)	86.8 (963)	86.0 (854)	85.4 (918)
Yes	12.1 (139)	12.8 (142)	13.6 (135)	14.5 (156)
Missing	0.3 (3)	0.4 (4)	0.4 (4)	0.1 (1)
Gestational age, mean (SD) weeks	21.6 (5.9)	21.7 (5.6)	21.0 (5.9)	21.3 (5.8)

Gestational age (categorized)				
< 16	19.2 (221)	16.4 (182)	21.5 (213)	20.1 (216)
17-20	21.8 (250)	24.2 (268)	22.5 (223)	21.7 (233)
21-26	34.3 (394)	32.6 (361)	31.3 (311)	33.7 (362)
> 27	19.7 (226)	19.7 (219)	17.5 (174)	18.2 (196)
No information	58 (5.0)	7.1 (79)	7.3 (72)	6.3 (68)
Previous stillbirths				
No	91.5 (1052)	92.3 (1024)	91.6 (910)	91.0 (978)
Yes	8.2 (94)	7.2 (80)	7.9 (78)	8.9 (96)
Missing	0.3 (3)	0.5 (5)	0.5 (5)	0.1 (1)
Previous deliveries				
None	29.7 (341)	30.3 (336)	29.2 (290)	33.8 (363)
One	31.9 (367)	31.7 (352)	30.6 (304)	28.5 (306)
Two	19.2 (221)	17.8 (197)	17.9 (178)	18.6 (200)
Three or more	18.8(216)	19.8 (220)	22.0 (218)	19.0 (205)
Missing	0.4 (4)	0.4 (4)	0.3 (3)	0.1 (1)
HIV status				
Negative	81.2 (934)	81.8 (907)	76.7 (762)	79.0 (849)
Positive	18.8 (215)	18.2 (202)	23.3 (231)	21.0 (226)
Twin pregnancy				
No	98.7 (1134)	98.6 (1093)	99.2 (985)	99.2 (1066)
Yes	1.3 (15)	1.4 (16)	0.8 (8)	0.8 (9)
Symptoms during current pregnancy before first prenatal visit				
Fever				

Yes	22.9 (264)	24.4 (271)	28.8 (286)	23.8 (256)
Headache				
Yes	41.5 (477)	43.5 (482)	44.3 (440)	43.0 (462)
Cold/chills				
Yes	18.0 (207)	18.4 (204)	20.6 (205)	18.8 (202)
Vomit/nausea				
Yes	27.5 (316)	26.9 (298)	29.8 (296)	28.6 (307)
Body aches				
Yes	21.8 (251)	21.3 (237)	23.8 (236)	23.3 (251)
Self-reported malaria				
Yes	5.7 (66)	6.0 (67)	5.9 (59)	6.3 (68)
Had malaria test				
Yes	7.0 (80)	7.1 (79)	8.0 (79)	7.9 (85)

Table 2. Proportions (%) of women (numbers) with outcomes suggesting malaria during pregnancy, and odds ratios (OR) and 95% confidence intervals (95% CI) for group effect, $n = 4326$

Outcomes	Second visit[1] % (n)		Third visit[2] % (n)		Between the first and fifth visit[3] OR (95% CI)		
	Selective iron $n = 1455$	Routine iron $n = 1494$	Selective iron $n = 1040$	Routine iron $n = 1106$	Selective iron	Routine iron	P-value
Fever	11.5 (168)	10.0 (150)	11.3 (117)	12.1 (134)	1.00	0.95 (0.81-1.11)	0.523
Headache	24.9 (363)	24.3 (363)	24.9 (259)	25.1 (278)	1.00	0.98 (0.87-1.10)	0.738
Cold/chills	8.2 (120)	7.0 (104)	6.7 (70)	7.8 (86)	1.00	0.92 (0.76-1.11)	0.361
Vomit/nausea	9.1 (133)	10.2 (153)	8.5 (88)	9.6 (109)	1.00	1.09 (0.92-1.31)	0.323
Body aches	10.1 (147)	9.2 (138)	10.9 (113)	9.8 (108)	1.00	0.89 (0.75-1.06)	0.180
Self-reported malaria	2.4 (35)	3.0 (45)	1.5 (16)	2.2 (24)	1.00	1.37 (0.98-1.92)	0.068

[1]Betetween first and second visit

[2]Between second and third visit

[3]The effect estimates calculated by binomial generalized estimating equations (with exchangeable correlation structure) to account for the repeated measures of the outcomes.

Table 3. Birth outcomes by group allocation, percentages, % (numbers) of women or babies or means (SD).

Outcomes	Selective iron *n* = 1149	Routine iron *n* =1109	*P*-value[1]
Birth weight, mean (SD) grams	2996.3 (508.4)	2989.4 (514.9)	0.752
Birth weight, % (*n*)			0.443
< 2500 g	11.8 (136)	12.8 (142)	
2500-2999 g	30.6 (351)	31.1 (345)	
3000-3499 g	40.5 (465)	37.8 (419)	
3500-3999 g	12.7 (146)	13.8 (153)	
≥ 4000 g	3.0 (34)	2.1 (23)	
No information	1.5 (17)	2.4 (27)	
Duration of gestation, mean (SD) weeks	38.3 (4.2)	38.4 (4.0)	0.689
Duration of gestation, % (*n*)			0.056
< 37 weeks	28.8 (331)	27.0 (299)	
≥ 37 weeks	67.2 (772)	66.9 (742)	
No information	4.0 (46)	6.1 (68)	
Mode of delivery, % (*n*)			0.235
Normal	87.6 (1007)	89.4 (991)	
Cesarean section	1.3 (15)	2.0 (22)	
No information	11.1 (127)	8.7 (96)	
Child health status at birth, % (*n*)			0.685
Well	94.0 (1080)	92.1 (1022)	
Ill	0.7 (8)	1.0 (11)	
Dead	1.8 (21)	2.0 (22)	
No information	3.5 (40)	5.0 (55)	

Still birth, % (*n*)			0.558
No	81.2 (933)	79.7 (884)	
Yes	2.5 (29)	2.9 (32)	
No information	16.3 (187)	17.4 (193)	
Fetal heart beat at admission, % (*n*)			0.085
Negative	1.6 (18)	2.6 (29)	
Positive	85.6 (984)	85.2 (945)	
No information	12.8 (147)	12.2 (135)	
Mother's health status at birth, % (*n*)			0.895
Well	95.6 (1098)	94.9 (1052)	
Ill	0.4 (4)	0.4 (4)	
Dead	0.1 (1)	0.2 (2)	
No information	4.0 (46)	4.6 (51)	
Length of hospital stay, mean (SD) days	1.33 (1.21)	1.63 (1.30)	0.075
Length of hospital stay after birth, % (*n*)			0.103
≤ 1 day	65.1 (748)	60.7 (673)	
2 days	23.5 (270)	24.2 (268)	
≥ 3 days	4.0 (46)	5.6 (62)	
No information	7.4 (85)	9.6 (106)	
Place of delivery, % (*n*)			0.652
1o de Maio (health center)	44.4 (510)	42.6 (472)	
Machava (health center)	35.1 (403)	38.2 (424)	
Jose Macamo (hospital)	3.7 (43)	3.6 (40)	
Mavalane (hospital)	14.3 (164)	12.8 (142)	

Central Hospital	0.3 (3)	0.3 (3)
At home	1.1 (13)	1.3 (14)
On the way to hospital	0.0 (0)	0.2 (2)
No information	1.1 (13)	1.1 (12)

[1]Based on T-test for continuous outcomes, Pearson Chi-square test or Fisher's exact test for categorical outcomes. Subjects with no information were not included in the tests.

Table 4. Numbers, proportions (%), and risk ratios (RR, 95% confidence intervals CI) of birth outcomes by iron groups

Outcomes	Selective iron *n*	Routine iron *n*	Selective iron %	Routine iron %	Selective iron	Routine iron RR (95% CI)[2]	*P*-value
Primary health outcomes							
Low birth weight (<2500 grams)	136	142	11.8	12.8	1.00	1.09 (0.88-1.36)	0.431
Preterm delivery (<37 weeks)	331	299	28.8	27.0	1.00	0.96 (0.84-1.09)	0.185
Secondary health outcomes							
Cesarean section delivery	15	22	1.3	2.0	1.00	1.48 (0.77-2.84)	0.238
Negative fetal heart beat at admission	18	29	1.6	2.6	1.00	1.66 (0.93-2.96)	0.089
Child ill or dead at birth	29	33	2.5	3.0	1.00	1.20 (0.73-1.96)	0.473
Mother ill or dead at birth	5	6	0.4	0.5	1.00	1.25 (0.38-4.09)	0.711
Other outcomes							
Delivery in reference center[1]	210	185	18.3	16.7	1.00	0.91 (0.76-1.09)	0.316
Long hospital stay after delivery (≥ 3 days)	46	62	4.0	5.6	1.00	1.43 (0.97-1.26)	0.059
No delivery data	993	1075	46.4	49.2	1.00	1.06 (1.00-1.13)	0.060

[1]Jose Macamo or Mavalane or Central Hospital

[2]The estimates were not adjusted for any baseline characteristic because the two groups did not differ from each other at baseline

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2, 3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5,6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6,7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	None
Participants	4a	Eligibility criteria for participants	7,8
	4b	Settings and locations where the data were collected	6,7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9,10
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10
	6b	Any changes to trial outcomes after the trial commenced, with reasons	None
Sample size	7a	How sample size was determined	8,9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	Not done.

		assessing outcomes) and how	Pragmatic trial design
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11,12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Not done
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	13, Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	13, Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6,7
	14b	Why the trial ended or was stopped	Ended as planned
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	13, Tables 2-4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	13,14, Tables 2-4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Table 4
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	None
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA (benefits and harms not distinguished)
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14,15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	15, 16
Other information			
Registration	23	Registration number and name of trial registry	3
Protocol	24	Where the full trial protocol can be accessed, if available	From Authors
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	3, 17

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

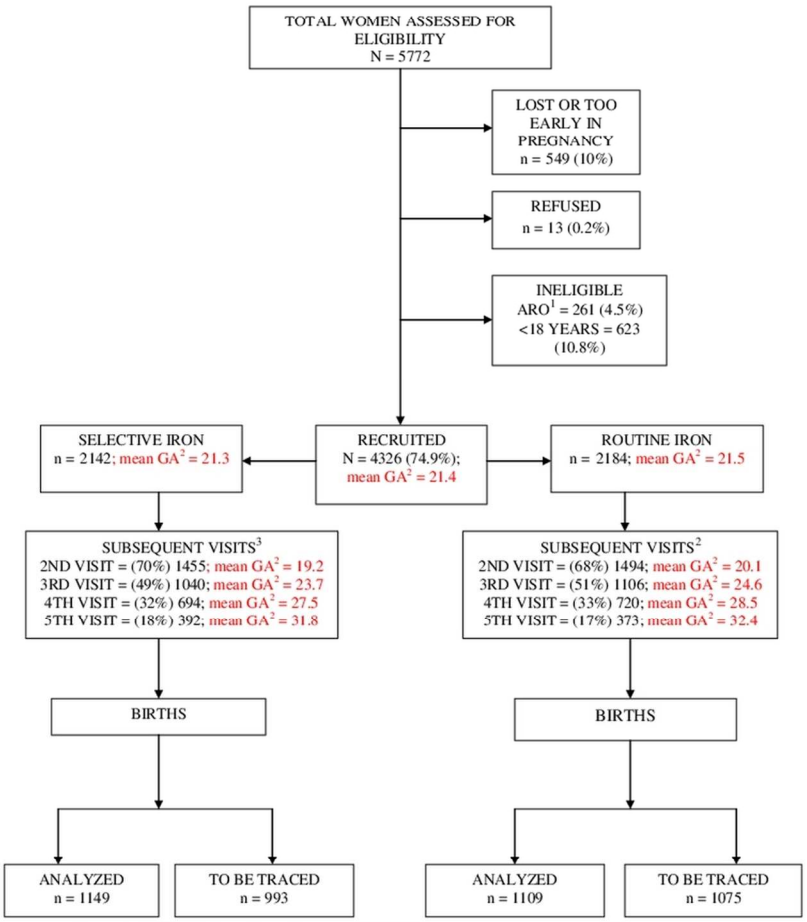

Figure 1. PROFEG Trial Flow Diagram

[1]ARO = high risk pregnancy; [2]GA = gestational age in weeks; [3]After recruitment, % were calculated from recruited, n = 4326

90x127mm (300 x 300 DPI)

Appendix 1: Baseline assumptions used for power calculations and sample size

The sample size was estimated using various outcomes (see Table), expected reduction (20 and 30%) and power (85 and 90%) and significance level of 5%; the base-line rates were estimated on the basis of literature. Based on these calculations we decided that two thousand women were needed in each trial arm (4000 in total). Example calculations are shown in the table below.

Power	85%	85%	90%	90%
Expected reduction (with intervention)	20%	30%	20%	30%
Preterm delivery (iron group = 18%)	805	341	2197	931
Low birth weight rate (iron group = 14%)	1079	457	2947	1246
Malaria reactivation (estimated incidence in iron group = 20%)	709	301	1935	821
Perinatal mortality (iron group = 3.4%)	353	152	926	413

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2, 3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5,6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	None
Participants	4a	Eligibility criteria for participants	7
	4b	Settings and locations where the data were collected	6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	None
Sample size	7a	How sample size was determined	7,8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6,7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	Not done.

		assessing outcomes) and how	Pragmatic trial design
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	10,11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Not done
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	11, Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	11, Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6
	14b	Why the trial ended or was stopped	Ended as planned
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	11, Tables 2-4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	11,12, Tables 2-4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Table 4
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	None
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA (benefits and harms not distinguished)
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13, 14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13, 14
Other information			
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