

https://dspace.org/

Birth outcomes and associated risk factors of anaemia in early pregnancy in a nulliparous cohort

Masukume, Gwinyai

2015-09-08
http://hdl.handle.net/10539/18544

Downloaded from a DSpace repository. https://dspace.org/

Birth outcomes and associated risk factors of anaemia in early pregnancy in a nulliparous cohort

Gwinyai Masukume

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Science in Epidemiology & Biostatistics.

Johannesburg, February 2015

DECLARATION

I, Gwinyai Masukume, am submitting my research report in partial fulfillment of the requirements of the MSc in the field of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of the Witwatersrand. This report has not been submitted previously for any degree or examination at this or any other university. I hereby declare that this research report is my own work. Where I have used the thoughts or ideas of others, the required referencing conventions have been adhered to.

Signed:



Date : 5 February 2015

DEDICATION

I dedicate this research report to my late mum.

ACKNOWLEDGMENTS

A project of this nature cannot be a one person show. First, I acknowledge Prof. Philip Baker who has changed the course of my career to focus more on obstetrics; knowing him has been such a great honour. Many thanks to Prof. Louise Kenny and Dr. Ali Khashan for their invaluable time and advice. To Prof. Gill Nelson who helped keep me together during the ups and downs of research report writing, thank you.

I would also like to express my profound gratitude to my dad and sisters who provided unwavering support and encouragement.

Last, but no means least, I appreciate the participants who volunteered to make pregnancy and child birth even safer.

ABSTRACT

Background

Anaemia in pregnancy is a major public health and economic problem worldwide, that contributes to both maternal and fetal morbidity and mortality. Clinical manifestations of anaemia in pregnancy include fetal growth restriction, preterm delivery, low birth weight, impaired lactation, poor maternal/infant behavioural interactions and post partum depression.

Objective

The aim of the study was to calculate the prevalence of anaemia in early pregnancy in a cohort of 'low risk' women participating in a large international multicentre prospective study (n = 5 609), to identify the modifiable risk factors for anaemia in pregnancy in this cohort, and to compare the birth outcomes between pregnancies with and without anaemia in early gestation.

Methods

The study is an analysis of data that were collected prospectively during the Screening for Pregnancy Endpoints (SCOPE) study. Anaemia was defined according to the World Health Organization's definition of anaemia in pregnancy (haemoglobin < 11g/dL). Binary logistic regression with adjustment for potential confounders (country, maternal age, having a marital partner, ethnic origin, years of schooling, and having paid work) was the main method of analysis.

Results

The hallmark findings were the low prevalence of anaemia (2.2%), that having no marital partner was an independent risk factor for having anaemia (OR 1.34, 95% CI 1.01-1.78), and that there was no statistically significant effect of anaemia on adverse pregnancy outcomes (small for gestational age, pre-tem birth, mode of delivery, low birth weight, APGAR score < 7 at one and five minutes). Adverse pregnancy outcomes were however more common in those with anaemia than in those without.

Conclusion

The absence of a marital partner is an important non-modifiable factor that should be added to the conceptual framework of anaemia's determinants. Although not statistically significant, clinically, a trend towards a higher risk of adverse pregnancy outcomes was observed in women that were anaemic in early pregnancy.

TABLE OF CONTENTS

DECLARATION i
DEDICATION ii
ACKNOWLEDGEMENTS iii
ABSTRACT iv
TABLE OF CONTENTS v
LIST OF FIGURES vii
LI ST OF TABLES viii
ABBREVIATIONS AND DEFINITION OF TERMS ix
Chapter 1: INTRODUCTION 1
1.1 Background 1
1.2 Statement of the problem 2
1.3 Justification for the study 2
1.4 Literature review 2
1.4.1 Epidemiology 2
1.4.2 Aetio-pathogenesis 3
1.5 The Screening for Pregnancy Endpoints study 5
1.6 Research question 6
1.7 Study objectives 6
Chapter 2: METHODS 7
2.1 Study design 7
2.2 Study setting 7
2.3 Study population 7
2.4 Power of study 7
2.5 Data management 8
2.5.1 Measurement 8
2.5.2 Study variables 8
2.5.3 Data quality control 10
2.6 Statistical analyses 10
2.6.1 Descriptive statistics 10

2.6.2 Inferential statistics 11
2.6.3 Missing data 11
2.7 Ethical considerations 12
Chapter 3: RESULTS 13
3.1 Cohort description 13
3.2 Adjusting the measured haemoglobin for cigarette smoking 13
3.3 Bivariable analysis 15
3.4 Survival analysis 17
3.5 Multivariable analysis 18
3.5.1 Anaemia as outcome 18
3.5.2 Anaemia as risk factor 19
3.6 Structural equation model 21
Chapter 4: DISCUSSION 23
4.1 Comparison with other studies 23
4.2 Strengths 25
4.3 Limitations 25
4.4 Conclusion 25
References 27
Appendix 1 - Tests for normality 31
Appendix 2 - Correlation between haemoglobin and birth weight 32
Appendix 3 – Model goodness of fit 33
Appendix 4 - Ethics clearance certificate 34

LIST OF FIGURES

Figure 1.1. Conceptual model of the determinants of anaemia 4
Figure 3.1. Participant flow chart 14
Figure 3.2. Kaplan-Meier plot estimates of anaemia status (not anaemic or anaemic) and gestational age at delivery 17
Figure 3.3. Kaplan-Meier plot estimates of anaemia status (not anaemic, mildly or moderately anaemic) and gestational age at delivery 18
Figure 3.4. Structural equation model 22

LIST OF TABLES

Table 2.1. Criteria for the adjustment of haemoglobin due to smoking cigarettes 9
Table 2.2. Adjustment of the haemoglobin for smoking for a hypothetical participant 9
Table 3.1. Number of participants by anaemia status after adjusting the haemoglobin concentration for the effect of smoking cigarettes during a particular period 14
Table 3.2. Comparison of participants without and with anaemia 15
Table 3.3. Factors associated with anaemia in early pregnancy 19
Table 3.4. Sensitivity analysis: assuming the 19 participants with missing haemoglobin were all anaemic 20
Table 3.5. Pregnancy outcomes and anaemia status 21

ABBREVIATIONS

BMI	Body mass index
CDC	The United States Centers for Disease Control and Prevention
Hb	Haemoglobin
HIV	Human immunodeficiency virus
IQR	Interquartile range
KM	Kaplan-Meier
SCOPE	SCreening fOr Pregnancy Endpoints
SD	Standard deviation
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
WHO	World Health Organization

DEFINITION OF TERMS

Booking haemoglobin - Participant's first haemoglobin measurement at the first antenatal visit (g/dL) before 20 weeks gestation (typically measured during the first trimester)

High green leafy vegetables consumption - ≥ 3 times per day

High fruit consumption - ≥ 3 times per day

Low birth weight - birth weight < 2 500g

Low, middle and high income country - according to the World Bank’s classification of economies by GNI (gross national income) per capita

Nulliparous - no previous pregnancy continuing beyond 19 weeks and 6 days gestation

Preterm delivery – delivery < 37 completed weeks

Socioeconomic index - using the New Zealand Socioeconomic Index guide (Galbraith C, Jenkin G, Davis P, Coope P, New Zealand Social Economic Index 1996 Users Guide, Statistics New Zealand, Wellington, New Zealand)

CHAPTER 1 – INTRODUCTION

This chapter commences with the definition of anaemia in pregnancy, taking several factors into account, as it is difficult to study that which is not clearly defined. The statement of the problem and the study justification follow; these are bolstered by a review of the contemporary literature. A background on the primary study, from where the data for this secondary analysis originated, is given. The chapter ends with the research question and objectives of the study.

1.1 Background

Anaemia is a state where the delivery of oxygen to the tissues is impaired because of a quantitative or qualitative deficiency of haemoglobin (Hb) or red blood cells [1]. In pregnancy, according to the World Health Organization (WHO), anaemia occurs when, at sea level, the haemoglobin is < 11 g/dL or the haematocrit is < 33%, regardless of gestation [2,3]. Haemoglobin < 7 g/dL is defined by the WHO as severe anaemia and haemoglobin < 4 g/dL, as very severe anaemia.

The United States Centers for Disease Control and Prevention's (CDC) definition of anaemia in pregnancy varies, depending on the gestation. In the first and third trimesters, haemoglobin < 11 g/dL and haematocrit < 33% is defined as anaemia; in the second trimester, the corresponding values are 10.5 g/dL and 32% [4].

Despite the above definitions, anaemia in pregnancy is not quite as clear-cut as alluded to above, because of the physiological changes that occur, which also involve the haematologic system [5].

Overall, in pregnancy, plasma volume increases to a greater extent than the increase in red cell mass, causing a relative decrease in the haemoglobin and haematocrit (physiologic/ dilutional anaemia) [5]. Some authorities maintain that additional variables of altitude, cigarette smoking and ethnicity may alter the definition of anaemia in individuals [6,7]. Others are of the opinion that altitude should not modify the definition of anaemia in pregnancy [8] and that there is insufficient information to alter the definition of anaemia based on ethnicity [3].

These issues regarding the definition of anaemia in pregnancy are important as they have implications on how other variables should be taken into account when dealing with anaemia in pregnancy and for comparison with other studies which may use different definitions.

1.2 Statement of the problem

Studies have shown that anaemia in pregnancy has several risk factors and is associated with adverse maternal and peri-natal outcomes (this is discussed in detail in the literature review – section 1.4). Some of the serious adverse outcomes include maternal and peri-natal death; other adverse outcomes include low birth weight, preterm labour, etc.

1.3 Justification for the study

Given the serious problems caused by anaemia in pregnancy, it is important to continue characterising and quantifying the birth outcomes and identifying potentially modifiable risk factors associated with anaemia in pregnancy.

1.4 Literature review

1.4.1 *Epidemiology*

According to a 2013 study, approximately 38% of pregnant women worldwide are anaemic [9]. The estimated prevalence of anaemia in pregnancy, according to a 2009 study, differs widely between continents, being highest in Africa (55.8%) and Asia (41.6 %), and lowest in Europe (18.7%) and North America (6.1%) [10]. The prevalence in other regions falls between these limits. In general, as pregnancy progresses, the prevalence of anaemia increases [11].

Anaemia in pregnancy is a major public health and economic problem worldwide, which contributes to both maternal and fetal morbidity and mortality; anaemia in pregnancy can also have profound short-term and far-reaching sequelae for the newborn [12-14]. Anaemia, even in early pregnancy, has been associated with adverse pregnancy outcomes [15]. Clinical manifestations of anaemia in pregnancy include fetal growth restriction, preterm delivery, low birth weight [16], impaired lactation, poor maternal/infant behavioural interactions, post partum depression, and increased fetal and neonatal mortality [12,13]. Iron deficiency anaemia in

particular has been associated with decreased work capability of adults and reduced cognitive function of children that may persist into adulthood; impaired motor development is another manifestation of anaemia. All these factors lead to economic losses [9,17].

1.4.2 *Aetio-pathogenesis*

The risk factors/determinants/causes of anaemia can be found at multiple interacting levels [18]; the proximal causes of anaemia can be considered to be decreased red blood cell/haemoglobin production or increased loss of red blood cells/haemoglobin. Causative factors for this include nutritional, infectious and genetic entities which, in turn, are associated with access to food, health services, education, clean water, sanitation, etc. Ultimately, the political economy is the most distal cause of anaemia as it directly affects the other causes. In short, multiple interacting factors at different levels operate in the aetiology of anaemia, starting from the political economy and culminating in decreased red blood cell/haemoglobin production or increased loss of red blood cells/haemoglobin, and thus anaemia. The aetiology of anaemia in pregnancy must therefore be considered in the broader context of the "Conceptual model of the determinants of anaemia", which is depicted diagrammatically by Balarajan and colleagues, in Figure 1.1.

Besides the physiologic haemodilution of pregnancy, there are many risk factors for anaemia in pregnancy. On a global scale, some of the important risk factors include deficiency of nutrients such as iron (reportedly the most common risk factor), folate and vitamin B_{12}, infections such as human immunodeficiency virus (HIV), malaria and hook worms, and disorders in the structure or production of haemoglobin such as sickle cell disease and the thalassemias [12,13]. Other risk factors include teenage pregnancy, 'low' educational level, 'poor' socioeconomic status, a short inter pregnancy interval, and high parity [18-20]. In aggregate, in high income countries compared to low and middle income countries, nutritional, infectious and genetic risk factors for anaemia are less common [21], and so anaemia in pregnancy occurs at a lower prevalence in high income countries [9].

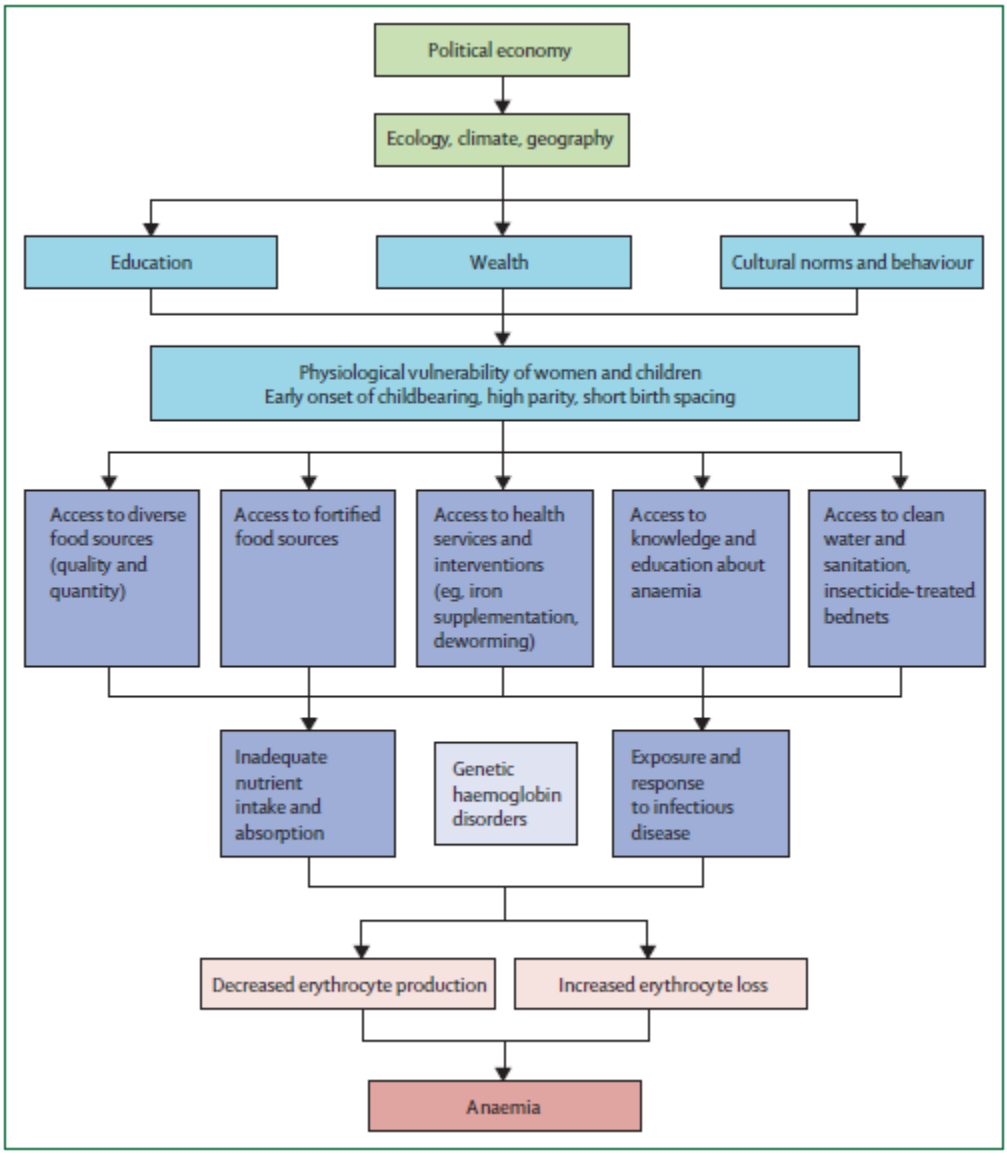


Figure 1.1. Conceptual model of the determinants of anaemia, reproduced with permission from Balarajan *et al*. [18]

Impaired delivery of oxygen to tissues seems to be the central mechanism by which anaemia increases the risk of maternal organ (brain, heart, kidney) injury and mortality [22]. Oxygen delivery to the uterus (and fetus) may therefore be reduced if a pregnant woman has anaemia [23].

1.5 The SCreening fOr Pregnancy Endpoints (SCOPE) study

The SCOPE study is an ongoing international prospective multicentre cohort study of 5 690 'low-risk' nulliparous (no previous birth) women with singleton pregnancies in four high income countries, viz. New Zealand, Australia, England and Ireland (www.scopestudy.net/). The primary aim of the SCOPE study is to develop screening tests to predict pre-eclampsia, fetal growth restriction and spontaneous preterm births in a low risk population. Some of the papers published from the SCOPE study include those on prediction of pre-eclampsia [24,25], fetal growth restriction [26] and spontaneous preterm birth [27].

Details of the SCOPE study methods have been published elsewhere [28] but the methods are described, in part, here. Recruitment of participants into the study started in Auckland, New Zealand in 2004 and finished in Cork, Ireland in 2011. The participants were aged from 16 to 45 years at entry into the cohort. Women's partners, along with their newborn infants, are also included in the study. Women before 15 weeks' gestation were recruited into the study through community midwives, general practitioners, hospital antenatal clinics, obstetricians and self referral. Women were excluded if they: 1) were considered to be at high risk of pre-eclampsia, fetal growth restriction or spontaneous preterm birth due to underlying medical conditions (chronic hypertension, diabetes, renal disease, systemic lupus erythematosus, anti-phospholipid syndrome, sickle cell disease, HIV), previous cervical knife cone biopsy, ≥ 3 previous terminations or ≥ 3 miscarriages, current ruptured membranes; 2) had a major uterine anomaly, a known major fetal anomaly or abnormal karyotype; or 3) received an intervention that could modify pregnancy outcome (e.g. aspirin therapy, cervical suture).

At 14-16 weeks and 19- 21 weeks gestation, the participants were interviewed and examined by a research midwife. Blood and urine tests, and an ultrasound scan, were conducted at 19-21 weeks. Participants were followed prospectively until birth, with pregnancy outcome data and baby measurements collected by research midwives.

1.6 Research question

What are the risk factors for, and birth outcomes of, women with anaemia in pregnancy who participated in a large prospective international multicentre study from 2004 to 2011 (the SCOPE study)?

1.7 Study objectives

1. To calculate the prevalence of anaemia in pregnancy (haemoglobin concentration < 11 g/dL) in a cohort of women participating in the SCOPE study from 2004 to 2011.
2. To identify the modifiable risk factors for anaemia in pregnancy in this cohort of women.
3. To compare the birth outcomes between women with and without anaemia in early pregnancy, in this cohort.

CHAPTER 2 – METHODS

Based on the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [29], more information is provided in this chapter to allow the reader to make an informed assessment of the strengths and weaknesses of this study. Details are presented on the study design and setting, the study population and power of the study, data management and statistical analyses and, last but not least, the ethical considerations taken into account.

2.1 Study design

A retrospective secondary analysis of data that were collected prospectively during the SCOPE study (described in Chapter 1) was conducted.

2.2 Study setting

The cohort members were recruited from specific areas in Auckland, New Zealand; Adelaide, Australia; Leeds, London and Manchester, England; Cork, Ireland. These are all high income countries; two are in the Southern and two are in the Northern hemisphere.

2.3 Study population

A total of 5 609 ‘healthy’ nulliparous women recruited into the SCOPE study from 2004 to 2011, with a booking haemoglobin before 20 weeks of gestation - obtained mainly during the first trimester (personal communication, Philip N Baker, 2013). All 5 609 women were included in the secondary data analysis. The study setting and population of the SCOPE study are described in section 1.5.

2.4 Power of the study

To evaluate the adequacy of the sample size, a retrospective power calculation was done using the two-sample comparison of means method in Stata 13IC (Stata, College Station, TX) .

The mean haemoglobin in the group of 125 participants with anaemia was 10.5 g/dL while in the non-anaemic group of 5 484 participants, the mean was 12.9 g/dL. Assuming a two-sided alpha of 0.05 and one standard deviation for both means, the power of this study to detect a difference

in mean haemoglobin concentration of (2.4 g/dL {12.9 g/dL-10.5 g/dL}) within the aforementioned parameters was 100%.

In conclusion, the sample size was adequate to clearly differentiate between anaemic and non-anaemic participants.

2.5 Data management

2.5.1 *Measurement*

SCOPE study data were entered into a centrally accessed internet database with a complete audit trial (MedSciNet) [24]. The relevant data variables for this secondary data analysis were extracted by the SCOPE gatekeeper.

2.5.2 *Study variables*

The study variables (see below) were selected based on *a priori* knowledge as ascertained through the literature review (see Chapter 1).

Women were classified as anaemic or not-anaemic as follows:
Anaemic: anaemia in early pregnancy – adjusted haemoglobin concentration < 11 g/dL.

- [Maternal haemoglobin] adjusting for cigarette smoking (a new variable)

In the SCOPE study, data on smoking were available for three months pre-pregnancy, the first trimester and at the 14-16 week visit. Separate adjustments to the measured haemoglobin due to smoking were made for each of the three periods as well as in combination because a participant could, for example, smoke more during the first trimester than during three months pre-pregnancy. The adjustment that yielded the maximum reduction in measured haemoglobin for each participant was used. Because smoking cigarettes has effects that can persist even epigenetically [30], and haematological indices can take years to return to normal after smoking cessation [31], an adjustment to the measured haemoglobin was made regardless of when maximal smoking occurred (see Table 2.1).

An adjustment to the haemoglobin for altitude was not made because all the participating SCOPE centres were below 1 000m above sea level.

Not-anaemic: because being anaemic and not-anaemic are mutually exclusive, participants not meeting the criterion for anaemia were classified as not-anaemic.

Table 2.1. Criteria for the adjustment of haemoglobin due to smoking cigarettes [3,6].

Smoking status*	Measured haemoglobin adjustment (g/dL)
Non-smoker	0
< 10 cigarettes	0
≥10-<20 cigarettes	-0.3
≥ 20-<40	-0.5
≥40 cigarettes	-0.7

* Number of cigarettes smoked per day: < 10 cigarettes ~ < ½ packet; ≥10-<20 cigarettes ~ ½-1 packet; ≥ 20-<40 cigarettes~ 1-2 packets; ≥40 cigarettes ~ ≥2 packets

Table 2.2. Adjustment of the haemoglobin for smoking for a hypothetical participant with measured haemoglobin of 11.5 g/dL.

Number of cigarettes smoked per day	Period when smoked	Adjustment (g/dL)	Adjusted haemoglobin (g/dL)
15	Three months pre-pregnancy	-0.3	11.2
45	First trimester	-0.7	10.8*
5	First SCOPE visit	0	11.5

* Adjusted haemoglobin measurement that will be used in analysis: 11.5 g/dL – 0.7 g/dL (from the 45 cigarettes) = 10.8 g/dL ∴ anaemic

Typically, although a participant will smoke the most cigarettes per day during the three months pre-pregnancy, one needs to be able to detect the unusual participant who smokes more later, to avoid misclassification of anaemia status as would have occurred in the hypothetical participant if only three months pre-pregnancy smoking was considered (11.5 g/dL – 0.3 g/dL = 11.2 g/dL ∴ not anaemic).

For each objective, the following variables were analysed:

Objective 1: What is the prevalence of anaemia in pregnancy?

- Anaemia (proportion of participants with anaemia)

Objective 2: What are the modifiable risk factors for anaemia in pregnancy?

- *dependent variable* – Anaemic/Not-anaemic
- *independent variables* - Maternal age, country, marital status, ethnicity, schooling, paid work, body mass index, maternal socioeconomic index , fruit consumption, vegetable consumption, folate intake, iron and mineral intake, alcohol, psychological scales, partner age, paternal socioeconomic index

Objective 3: What are the birth outcomes between women with and without anaemia in pregnancy?

- *independent variable* - Anaemic/Not-anaemic
- *dependent variables* - Small for gestational age, preterm delivery, mode of delivery, low birth weight, APGAR score

2.5.3 *Data quality control*

Range checks were done using histograms and duplicates were also checked for.

2.6 Statistical analyses

2.6.1 *Descriptive statistics*

All statistical analysis was conducted using Stata version 13IC (StataCorp LP College Station, TX). Continuous variables were tested for normality using histograms and inverse normal plots (see Appendix 1), as well as the skewness kurtosis test. The continuous variables were described using the mean (standard deviation - SD) if normally distributed or median (interquartile range - IQR) if not normally distributed.

Frequencies (n) and percentages (%) were used to report categorical variables. To compare categorical variables, Pearson's Chi-squared or Fisher's exact tests were used, where appropriate. For the comparison of normally distributed continuous variables, Student's t-test (two-sample t-test) was used; for non-normally distributed data, the Mann-Whitney test was used.

Kaplan-Meier (KM) curves were plotted, depicting anaemic and non-anaemic participants with regard to their time to delivery. The logrank test was used to ascertain the equality of survivor

functions with p-value < 0.05 being considered statistically significant. Although the median gestational ages could have been compared in each anaemia group, KM (survival) plots give a visual depiction and provide more information than the medians. Two-tailed p-values were reported. Because of multiple testing - 30 statistical tests were planned comparing anaemia status with 30 potential predictors - in order to reduce the chances of a false positive result (type 1 error), Bonferonni's method (0.05 ÷ 30) was used, giving an adjusted significance level of p < 0.002.

2.6.2 *Inferential statistics*

It has been suggested that methods using forward stepwise selection (or backward selection or a combination of both forward and backward selection) based on pre-determined p-value criteria are not optimal [32]. A better approach to determine which variables to include or exclude in the multivariable logistic regression model is by using external clinical judgment, which is the approach that was adopted in this analysis [32]. The model was adjusted for country, maternal age, having a marital partner, ethnic origin, years of schooling, and having paid work.

Participant data may not have been independent of the SCOPE centres. To take account of this, the cluster option in Stata was used.

A sensitivity analysis was done where all the participants with a missing booking haemoglobin were assumed to be anaemic.

In an attempt to understand the relationship between multiple variables using *a priori* knowledge, a structural equation model – taking into account clustering, as previously mentioned - was developed using the observed (manifest) variables in the SCOPE study [33]. Structural equation modeling allows the modeling of mediating variables and is useful for the analysis of conceptual models [34]. As mentioned in Chapter 1, the determinants of anaemia were being considered in the broader context of a conceptual model [18].

2.6.3 *Missing data*

Fourteen variables had missing data. Because 14 statistical tests were done to determine if data were missing more from the anaemic than the non-anaemic participants or vice versa (bias analysis), with a target p-value of < 0.05, Bonferonni's method ($0.05 \div 14 \sim 0.004$) was used to maintain the family wise error rate.

2.7 **Ethical considerations**

In the SCOPE study, ethical approval was obtained from local ethics committees and all women provided written informed consent. For the secondary analysis of the SCOPE study dataset, approval was granted by the Human Research Ethics Committee of the University of the Witwatersrand (approved 30/09/2013, clearance certificate number M130966 – Appendix 4). To protect the confidentially of participants, the dataset was sent in an anonymised format, making it impossible to identify any participant. In addition, the dataset was stored on a password protected device which also has physical security.

CHAPTER 3 – RESULTS

This chapter commences with a description of the study cohort, discusses how the measured haemoglobin was adjusted for cigarette smoking, presents results of the bivariable, survival, and multivariable analyses, and ends with a structural equation model based on the conceptual framework outlined in Chapter 1 (Figure 1.1).

3.1 Cohort description

Figure 3.1 depicts the flow of participants within the study; 5 690 participants were recruited into the SCOPE study at 14-16 weeks. Loss to follow-up was minimal: only 48 participants (0.8%) were lost to follow-up. Fourteen participants (0.2%) were found to be ineligible after recruitment, and a further 19 (0.3%) participants did not have a booking haemoglobin and were excluded, resulting in a study population of 5 609 participants at 14-16 weeks. Of these, 125 (2.2%) were anaemic after adjusting for cigarette smoking according to the WHO criteria outlined in Chapter 2 (see Table 3.1).

3.2 Adjusting the measured haemoglobin for cigarette smoking

Without adjustment to the measured haemoglobin, 103 (1.8%) of the participants were anaemic. Taking into account cigarette smoking during three months pre-pregnancy resulted in 125 (2.2%) anaemic participants, and considering first trimester smoking yielded 112 (2.0%). When adjusting for smoking during the first visit (14-16 weeks), the result was the same as that from the unadjusted analysis, viz. 103 (1.8%) participants were anaemic. When smoking cigarettes during three months pre-pregnancy and the first trimester were considered together to adjust the measured haemoglobin, the result was the same as considering three months pre-pregnancy smoking alone (n = 125); these were the anaemic participants included in the analyses.
There was no correlation between the adjusted haemoglobin and birth weight (see Appendix 2).

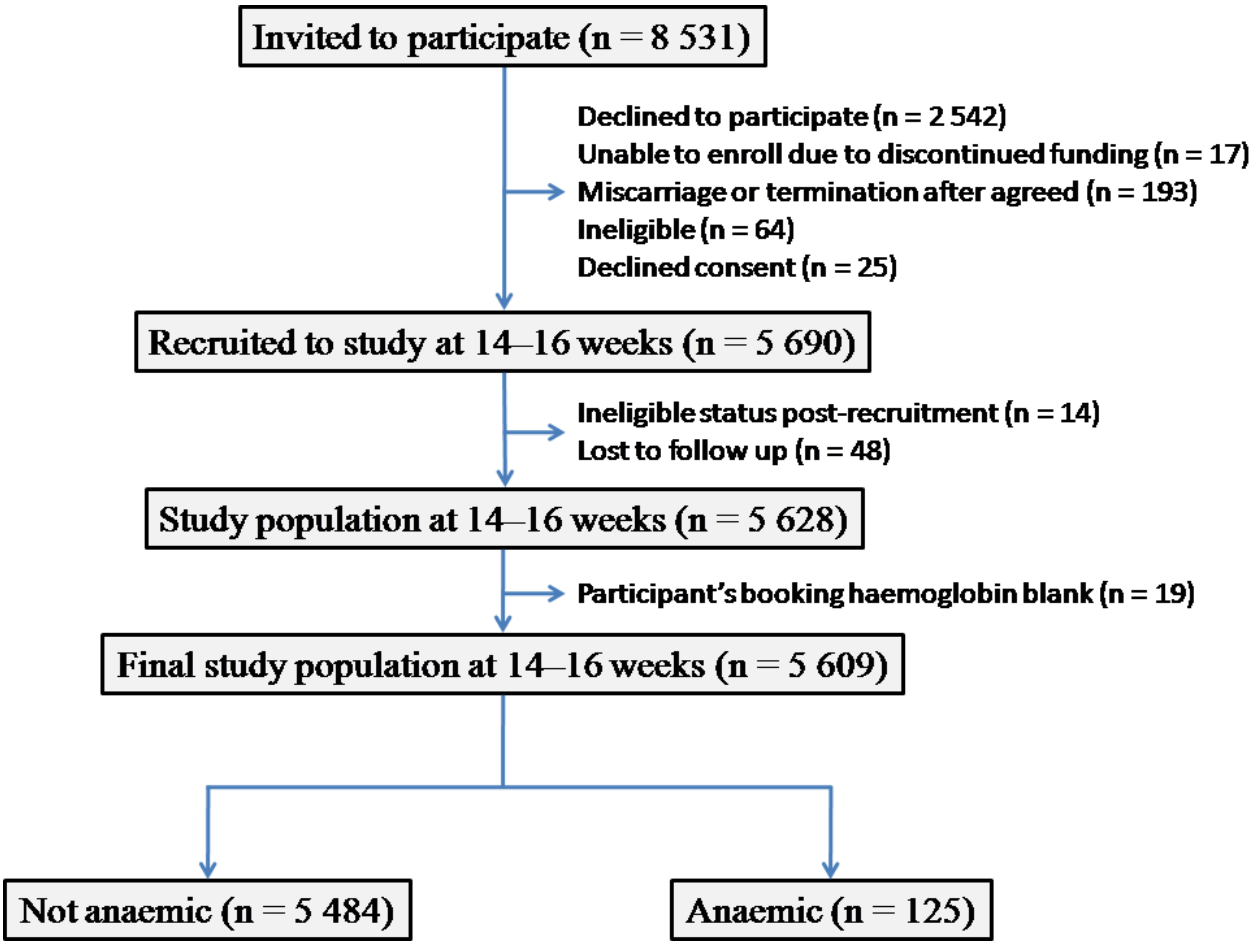


Figure 3.1. Participant flow chart, adapted from McCarthy *et al*. [35].

Table 3.1. Number and proportion of participants by anaemia status after adjusting the haemoglobin concentration for the effect of smoking cigarettes during a particular period (n = 5 609).

Participant's booking haemoglobin (g/dL) before 20 weeks gestation	**Unadjusted**	***3 months pre-pregnancy**	***First trimester**	***First visit (14-16 weeks)**	***#3 months pre-pregnancy and first trimester**
	n(%)	**n(%)**	**n(%)**	**n(%)**	**n(%)**
No anaemia (Hb≥ 11)	5 506 (97.8)	5 484 (97.4)	5 497 (97.7)	5 506 (97.8)	5 484 (97.4)
Anaemia (Hb<11)	103 (1.8)	125 (2.2)	112 (2.0)	103 (1.8)	125 (2.2)
mild (11>Hb≥10)	87 (1.5)	105 (1.9)	95 (1.7)	86 (1.5)	105 (1.9)
moderate (10>Hb≥7)	16 (0.3)	20 (0.4)	17 (0.3)	17 (0.3)	20 (0.4)
severe (Hb<7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

* Adjusted for the number of cigarettes smoked during that period, #adjusted for the period with the highest amount of smoking of the two periods.

3.3 Bivariable analysis

Table 3.2 summarises the comparison of participants with and without anaemia, including the proportion of missing data for each variable. Most of the variables of interest had no missing data and none of the missing data were associated with anaemia. The median haemoglobin for those with and without anaemia was 10.7 g/dL (IQR 10.4-10.8) and 12.8 g/dL (IQR 12.3–13.4), respectively, $p < 0.001$. Factors that were significantly associated with having anaemia were ethnic origin, reporting folate intake before pregnancy and no iron or mineral intake in the first trimester.

Although 76.0% of participants did not have data on serum ferritin, 12 participants (0.88%) were found to have iron deficiency anemia (defined as serum ferritin < 12μg/L and Hb < 11g/dL [15]). Participants with moderate anaemia had a mean and median gestational age at delivery of 39.8 and 40.1 weeks respectively while the corresponding values for mildly anaemic participants were 39.3 and 39.9 weeks.

Table 3.2. Comparison of participants without and with anaemia.

Characteristic	**Not anaemic n = 5 484 n (%)**	**Anaemic[a] n =125 n (%)**	**p-value[b]**
Maternal			
Age (years), median IQR[c]	29 (25–32)	28 (22-33)	0.2359
Teenager	391 (7.1)	14 (11.2)	0.082
Country			
Australia	1 130 (20.6)	30 (24.0)	
Ireland	1 742 (31.8)	28 (22.4)	0.008
New Zealand	1 980 (36.1)	42 (33.6)	
United Kingdom	632 (11.5)	25 (20.0)	
No marital partner	518 (9.5)	20 (16.0)	0.014
Ethnic origin			
European	4 947(90.2)	97 (77.6)	
Asian	160 (2.9)	9 (7.2)	
Indian	125 (2.3)	9 (7.2)	< 0.001*
Polynesian	113 (2.1)	2 (1.6)	
Other (including African)	139 (2.5)	8 (6.4)	
Schooling ≤ 12 years	2 054 (37.4)	63 (50.4)	0.003
No paid work at 15 weeks visit	795 (14.5)	29 (23.2)	0.007
Body mass index (kg/m^2) (ethnicity adjusted,evaluated at 14-16 weeks)			
< 18.5	82 (1.5)	2 (1.6)	0.583
≥ 18.5– < 25	2 998 (54.7)	76 (60.8)	
≥ 25.0 – < 30	1 564 (28.5)	31 (24.8)	
≥ 30	840 (15.3)	16 (12.8)	
Socioeconomic index, median IQR[c]	45 (28-50)	43 (27-50)	0.3058
High fruit intake a month before	1 234 (22.5)	23 (18.4)	0.277

conception (consumption ≥ 3 times per day)			
High green leafy vegetable intake a month before conception (consumption ≥ 3 times per day)	329 (6.0)	7 (5.6)	0.852
Folate			
no intake before pregnancy	2 267 (41.3)	72 (57.6)	< 0.001*
no intake during first trimester	2 19 (4.0)	7 (5.6)	0.366
Iron or mineral			
no intake before conception	3 502 (96.5)	70 (92.1)	0.043
data missing	1 854 (33.8)	49 (39.2)	0.208
no intake during first trimester	3 454 (95.0)	64 (84.2)	< 0.001*
data missing	1 849 (33.7)	49 (39.2)	0.200
Alcohol (units), > 14 units per week			
3 months pre-pregnancy	602 (11.0)	15 (12.0)	0.718
First trimester	342 (6.2)	6 (4.8)	0.510
Psychological scales (evaluated at 14-16 weeks)			
Edinburgh Postnatal Depression Score ≥ 10	1 435 (26.3)	41 (33.6)	0.069
data missing	21 (0.4)	3 (2.4)	0.015
Short form State-Trait Anxiety Inventory Score > 90th centile	433 (7.9)	14 (11.5)	0.154
data missing	27 (0.5)	3 (2.4)	0.028
Perceived Stress Scale Score > 90th centile	483 (8.9)	18 (14.8)	0.024
data missing	30 (0.5)	3 (2.4)	0.036
Paternal			
Age (years), median IQR[c]	31 (27-35)	30 (25-34)	0.0575
data missing	1 215 (22.2)	33 (26.4)	0.259
Socioeconomic index, median IQR[c]	44 (29-50)	44 (29-50.5)	0.8451
data missing	1 215 (22.2)	33 (26.4)	0.259
Pregnancy outcome			
Small for gestational age (<10th percentile for customized birthweight centiles)	617 (11.3)	13 (10.5)	0.778
data missing	20 (0.4)	1 (0.8)	0.378
Preterm delivery (< 37 completed weeks)			
All	347 (6.3)	9 (7.2)	0.698
data missing	14 (0.3)	0 (0)	1.000
Spontaneous	227 (4.2)	7 (5.6)	0.423
data missing	14 (0.3)	0 (0)	1.000
Mode of delivery			
Unassisted vaginal	2 477 (45.3)	47 (37.9)	
Operative vaginal	1 444 (26.4)	35 (28.2)	0.307
Pre-labour Caesarean section	486 (8.9)	11 (8.9)	
Caesarean section in labour	1 058 (19.4)	31 (25.0)	
data missing	19 (0.3)	1 (0)	0.400
Low birth weight (< 2500g)	282 (5.2)	9 (7.3)	0.300
data missing	27 (0.5)	1 (0.8)	0.014
APGAR score at 1 minute < 7	496 (9.2)	16 (12.9)	0.159
data missing	87 (1.6)	1(0.8)	0.484
APGAR score at 5 minutes < 7	60 (1.1)	1 (0.8)	0.754
data missing	88 (1.6)	2 (1.6)	0.997

[a] The presence or absence of anaemia was adjusted for smoking

[b] Pearson's χ^2 test or Fisher's exact test

[c] Mann-Whitney test
* p-value < 0.002 was considered statistically significant, for the missing data analysis p < 0.004 was considered statistically significant

3.4 Survival analysis

There was no significant difference in the median gestational age at delivery for the anaemic and non-anaemic women (38.9 and 40.0 weeks, respectively; logrank test: p > 0.05) (see Figures 3.2 and 3.3).

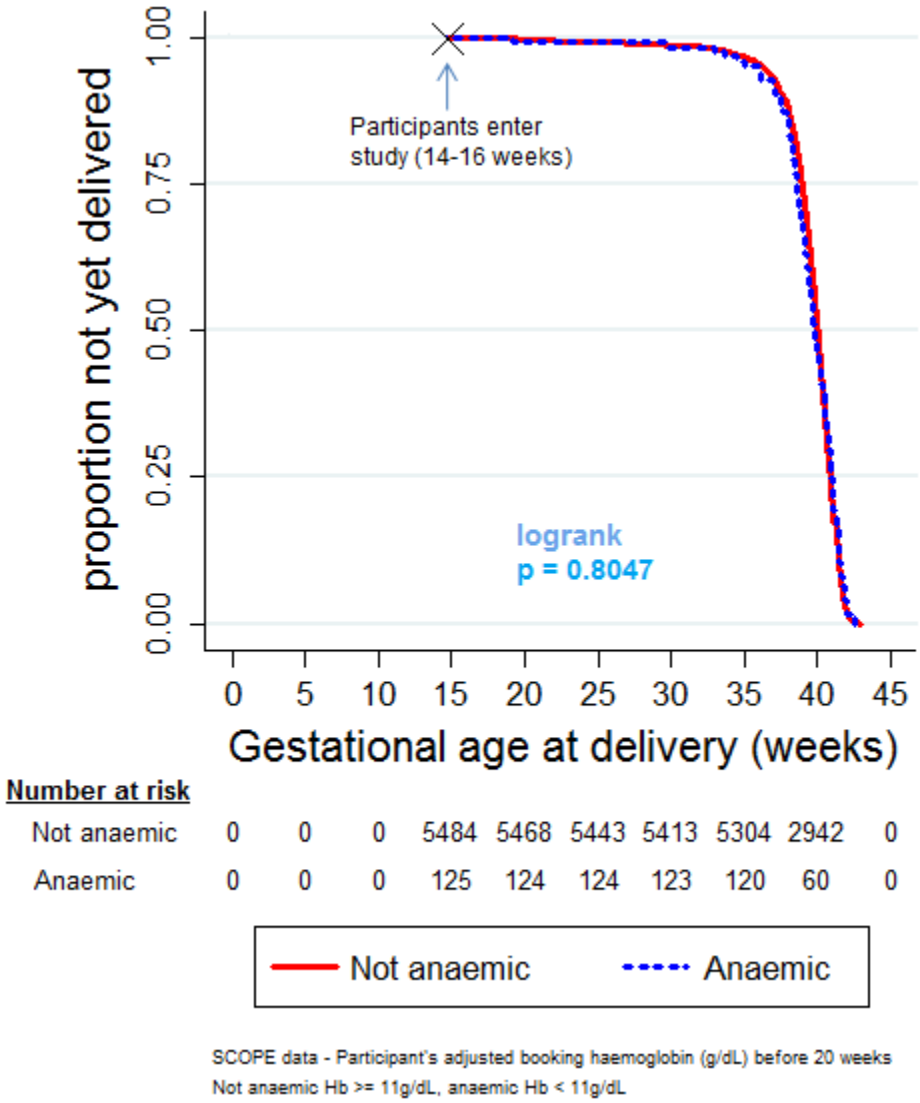


Figure 3.2. Kaplan-Meier plot estimates of anaemia status (not anaemic or anaemic) and gestational age at delivery.

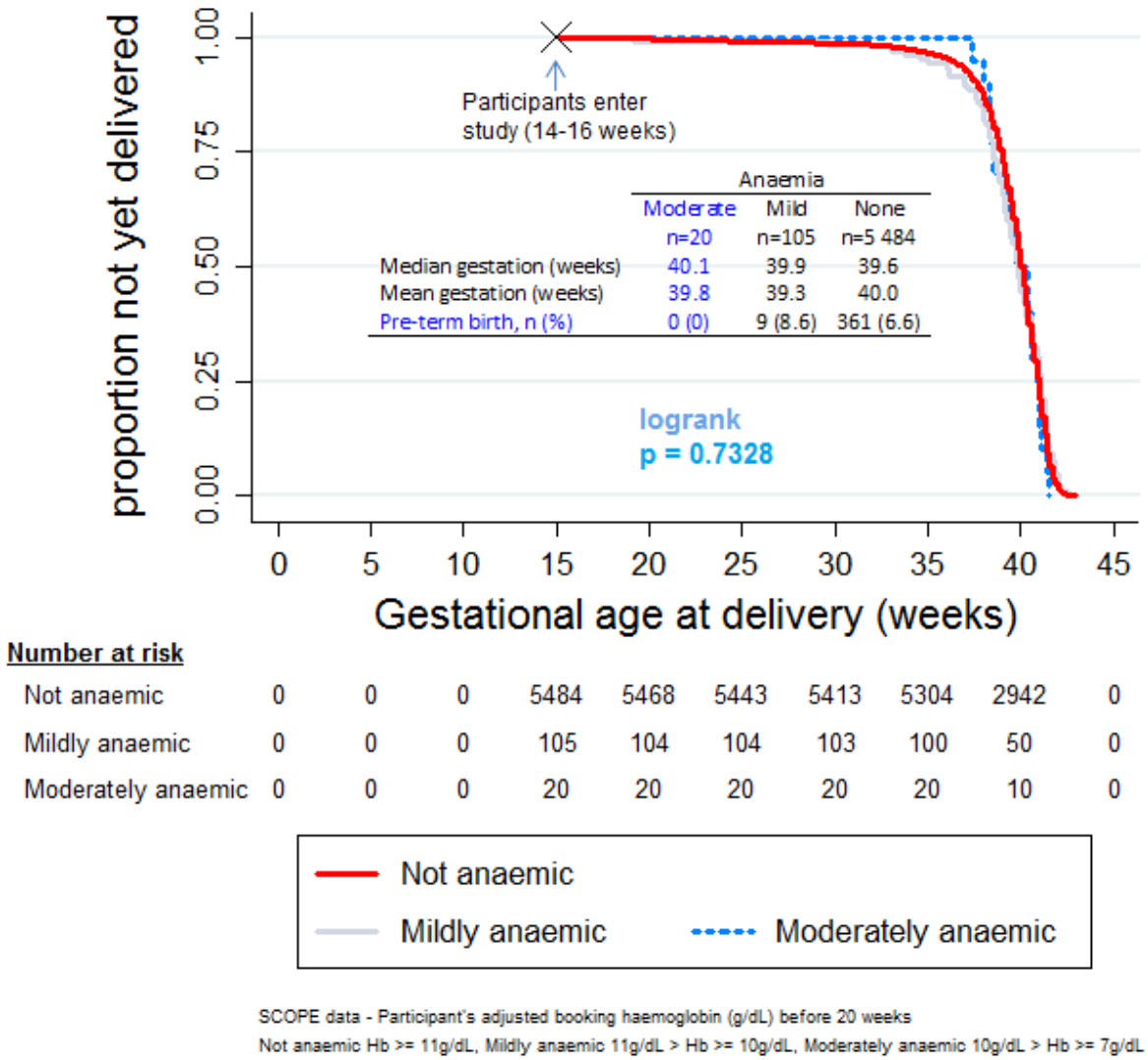


Figure 3.3. Kaplan-Meier plot estimates of anaemia status and gestational age at delivery. (Note the 'curious' behaviour of women with moderate anaemia who have no pre-term birth and are on average – median and mean - delivering later than women with mild anaemia and even those with no anaemia - median.).

3.5 Multivariable analysis

3.5.1 *Anaemia as outcome*

Iron or mineral intake was excluded from the multivariable logistic regression models because 33.8% of the data was missing (this was the only variable omitted because of missing data).

In the final model, which was of good fit (see Appendix 3), the variables that were independently associated with anaemia in early pregnancy were country, ethnic origin and having a marital partner (Table 3.3).

Assuming that the 19 participants without a booking haemoglobin were anaemic lead to having paid work and reporting folate intake prior to pregnancy being protective of anaemia in early pregnancy, there were no other significant changes (see Table 3.4).

3.5.2 *Anaemia as risk factor*

Pregnancy outcomes were similar for anaemic and non-anaemic women in that there was no statistically significant finding (Table 3.5). However, the pregnancy outcome findings suggested a trend towards a higher risk of adverse pregnancy outcomes for anaemic women, for some but not all outcomes.

Table 3.3. Factors associated with anaemia in early pregnancy.

Characteristic	**Unadjusted OR (95% CI)**	**p-value**	**Adjusted OR (95% CI)**	**p-value**
Country				
Australia	Reference		Reference	
Ireland	0.61 (0.61-0.61)	< 0.001	0.83-(0.68-1.01)	0.060
New Zealand	0.80 (0.80-0.80)	< 0.001	0 .93 (0.78-1.10)	0.410
United Kingdom	1.49 (1.08-2.06)	0.016	1.34 (1.06-1.69)	0.016*
Has marital partner	Reference		Reference	
No marital partner	1.83 (1.28-2.61)	0.001	1.34 (1.01-1.78)	0.044*
Ethnic origin				
Asian	Reference		Reference	
European	0.35 (0.20-0.61)	< 0.001	0.44 (0.24-0.84)	0.012*
Other (including African)	1.02 (0.42-2.47)	0.959	0.78 (0.35-1.73)	0.536
Polynesian	0.31 (0.24-0.41)	< 0.001	0.32 (0.18-0.57)	< 0.001*
Indian	1.28 (0.75-2.18)	0.365	1.50 (0.84-2.66)	0.171
Schooling > 12 years	Reference		Reference	
Schooling ≤ 12 years	1.70 (1.39-2.08)	< 0.001	1.26 (0.92-1.72)	0.156
No paid work	Reference		Reference	
Paid work	0.56 (0.43-0.73)	< 0.001	0.73 (0.49-1.07)	0.108
No folate intake before pregnancy	Reference		Reference	
Folate intake before pregnancy	0.52 (0.34-0.80)	0.003	0.64 (0.40-1.03)	0.066

Edinburgh postnatal depression score				
< 10	Reference		Reference	
≥ 10	1.42 (1.09-1.85)	0.009	1.06 (0.82-1.36)	0.654
Perceived stress scale				
≤ 90th centile	Reference		Reference	
> 90th centile	1.78 (0.99-3.21)	0.054	1.47 (0.73-2.96)	0.282
Maternal age (years)	0.97 (0.94-1.00)	0.038	1.02 (0.99-1.05)	0.180

N=5 575 for adjusted model
OR (Odds Ratio), CI (Confidence Interval)
* p-values < 0.05 were considered statistically significant

Table 3.4. Sensitivity analysis: assuming all 19 participants with missing haemoglobin were anaemic.

Characteristic	**Unadjusted OR (95% CI)**	**p-value**	**Adjusted OR (95% CI)**	**p-value**
Country				
Australia	Reference		Reference	
Ireland	0.61 (0.61-0.61)	< 0.001	0.81 (0.66-0.99)	0.041*
New Zealand	0.87 (0.87-0.87)	< 0.001	1.02 (0.85-1.21)	0.860
United Kingdom	1.37 (1.02-1.84)	0.037	1.26 (1.00-1.59)	0.046*
No marital partner	Reference		Reference	
Has marital partner	0.58 (0.42-0.80)	0.001	0.76 (0.59-0.98)	0.036*
Ethnic origin				
Asian	Reference		Reference	
European	0.37 (0.21-0.64)	< 0.001	0.48 (0.25-0.91)	0.025*
Other (including African)	0.92 (0.37-2.28)	0.859	0.74 (0.31-1.77)	0.494
Polynesian	0.42 (0.32-0.57)	< 0.001	0.41 (0.22-0.78)	0.006*
Indian	1.25 (0.65-2.04)	0.627	1.35 (0.68-2.68)	0.386
Schooling > 12 years	Reference		Reference	
Schooling ≤ 12 years	1.58 (1.26-1.99)	< 0.001	1.19 (0.90-1.56)	0.220
No paid work	Reference		Reference	
Paid work	0.55 (0.49-0.61)	< 0.001	0.68 (0.55-0.83)	< 0.001*
No folate intake before pregnancy	Reference		Reference	
Folate intake before pregnancy	0.56 (0.41-0.77)	< 0.001	0.69 (0.52-0.93)	0.014*
Edinburgh postnatal depression score				
< 10	Reference		Reference	
≥ 10	1.36 (1.06-1.75)	0.016	1.03 (0.89-1.20)	0.660
Perceived stress scale				
≤ 90th centile	Reference		Reference	
> 90th centile	1.70 (0.95-3.04)	0.073	1.44 (0.78-2.64)	0.241
Maternal age (years)	0.99 (0.96-1.01)	0.215	1.02 (0.98-1.05)	0.301

N=5 594 for adjusted model
OR (Odds Ratio), CI (Confidence Interval)

* p-values < 0.05 were considered statistically significant

Table 3.5. Pregnancy outcomes and anaemia status.

Characteristic	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)*	p-value
Small for gestational age				
Not anaemic	Reference		Reference	
Anaemic	0.92 (0.53-1.61)	0.752	0.85 (0.50-1.46)	0.560
Pre-term birth (All)				
Not anaemic	Reference		Reference	
Anaemic	1.10 (0.61-2.00)	0.752	1.05 (0.57-1.94)	0.864
Pre-term birth (Spontaneous)				
Not anaemic	Reference		Reference	
Anaemic	1.37 (0.53-3.57)	0.519	1.30 (0.48-3.50)	0.610
Mode of delivery (unassisted vaginal – base outcome)#				
Operative vaginal				
Not anaemic	Reference		Reference	
Anaemic	1.28 (0.69-2.36)	0.434	1.47 (0.75-2.87)	0.259
Pre-labour Caesarean section				
Not anaemic	Reference		Reference	
Anaemic	1.19 (0.68-2.09)	0.536	1.40 (0.74-2.62)	0.299
Caesarean section in labour				
Not anaemic	Reference		Reference	
Anaemic	1.54 (0.81-2.95)	0.189	1.73 (0.86-3.46)	0.123
Low birth weight				
Not anaemic	Reference		Reference	
Anaemic	1.44 (0.67-3.07)	0.351	1.31 (0.62-2.76)	0.485
APGAR score at 1 minute < 7				
Not anaemic	Reference		Reference	
Anaemic	0.68 (0.39-1.19)	0.178	0.71 (0.41-1.24)	0.232
APGAR score at 5 minutes < 7				
Not anaemic	Reference		Reference	
Anaemic	1.37 (0.19-10.02)	0.755	1.47 (0.21-10.3)	0.699

OR (Odds Ratio), CI (Confidence Interval)
Relative risk ratio, multinomial logistic regression
* p-values < 0.05 were considered statistically significant

3.6 Structural equation model

Structural equation modelling (see Figure 3.4) showed that folate intake (f10c_any_folate_prepreg) was indirectly associated with having anaemia in early pregnancy

(adjusted_anemia_status3m). The ‘link’ is marital partner (f5c_marital_partnr). Having paid work (f5c_paidwork) was also indirectly associated with anaemia. Overall, the structural equation model shows the interacting nature of multiple variables.

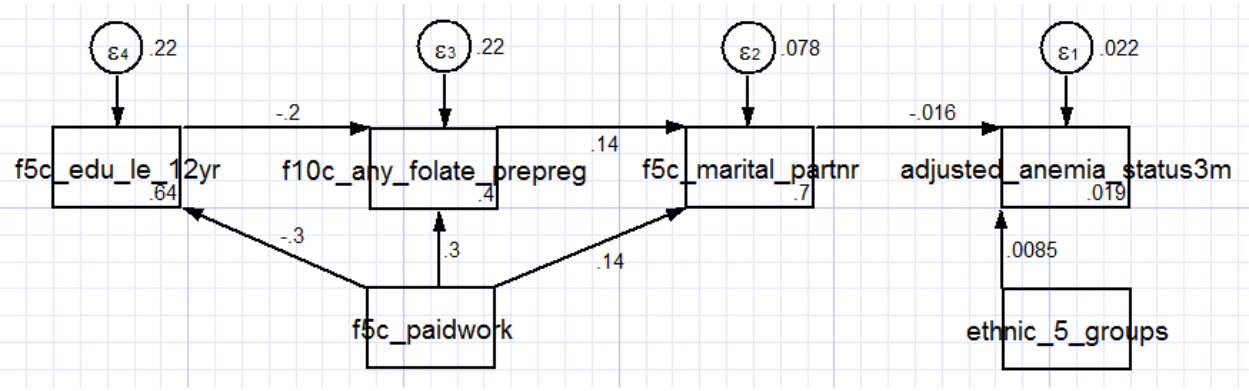


Figure 3.4. Structural equation model. Connected are the statistically significant pathways (direct or indirect). (ε) error terms placed on endogenous variables. Key: f5c_edu_le_12yr "Less than 12 years of schooling", f10c_any_folate_prepreg "Any folate intake prior to pregnancy", f5c_paidwork "Works in a paid job at 15w SCOPE visit", f5c_marital_partnr "Marital Partner", , ethnic_5_groups "Participant's main Ethnicity (5 Groups)", adjusted_anemia_status3m "Anaemia in early pregnancy".

CHAPTER 4 – DISCUSSION

The chapter begins with a comparison of the study's findings with those in previously published literature, followed by the study's strengths and limitations. Finally, the conclusion and policy implications are presented.

4.1 Comparison with other studies

In this contemporary, large multicenter cohort of nulliparous women in their first ongoing pregnancy, we found a very low prevalence of anaemia. The 2.2% prevalence of anaemia in the SCOPE cohort differs sharply with the 22% prevalence reported from high-income regions in recent literature [9]. One possible explanation for this is that SCOPE participants were selected to be 'low risk' and were all nulliparous. It is well recognised that co-morbidities such as high parity and short birth interval can affect anaemic status [18]. Furthermore, despite the multicenter nature of the cohort, the ethnicity of SCOPE participants was homogeneous with 89.9% of European ancestry. We did not adjust our findings for ethnic specific variations in haemoglobin concentration because of the low numbers of non-Caucasian participants and because there are sparse data on how to adjust the haemoglobin for ethnicity [7]. This may also partially explain our findings.

Mandatory folic acid supplementation does not explain the lower incidence of anaemia in this cohort because at the time of patient recruitment none of the participating countries had mandatory folic acid supplementation programmes [36].

In the SCOPE cohort, not having a marital partner was associated with higher odds of having anaemia in early pregnancy. This is not surprising because there is evidence that involvement of fathers during pregnancy is associated with diminished negative maternal behaviours and better neonatal outcomes [37]. In addition, not having a partner suggests that the pregnancy was unintended and therefore women did not take steps to optimize their health prior to pregnancy.

In the adjusted analysis, United Kingdom participants had a lower odds of anaemia in early pregnancy compared to the other countries. It is difficult to untangle the disparate potential contributions of political economy, ecology, geography and climate, all of which are found within the conceptual framework of anaemia's determinants.

Previous studies have shown an association between low education [18,20], the Edinburgh postnatal depression score (depression being linked to folic acid deficiency [38,39]) and teenage pregnancy [19], but we did not find this in our study.

In our study, from a statistical significance viewpoint, anaemia was not associated with adverse pregnancy outcomes. However, adverse pregnancy outcomes tended to be more common in those with anaemia than in those without. Low birth weight and preterm delivery were similar in women with and without anaemia in early pregnancy. This is at odds with findings from a recent comprehensive systematic review and meta-analysis [15]. The low prevalence of anaemia in this study (with small numbers of relevant pregnancy outcomes for anaemic participants), due to the deliberate recruitment of 'low risk' women, could possibly explain the absence of an effect of anaemia on these adverse pregnancy outcomes.

Contrary to the finding that anaemia prevalence is consistently higher in those of lower socioeconomic status and in those with low body weight [40], in this study the prevalence of anaemia was similar across paternal and maternal socioeconomic groups and body weights. Although confirmation of iron deficiency in pregnancy is difficult [13], iron deficiency anaemia is reportedly the most common cause of anaemia in pregnancy. Relatively easy access to iron in fortified cereals and other food products (important sources of iron in industrialized countries) [41], irrespective of socioeconomic status, may partly explain this lack of association.

Although there were few participants with moderate anaemia, they paradoxically delivered later, on average, than participants with mild anaemia (of lesser severity). In fact, none of the participants with moderate anaemia had pre-term labour. This finding could be spurious given the small number of participants. However, the finding is biologically plausible because paradoxical results have been found in transfusion studies where individuals with more severe

anaemia fare better [42, 43]. We are by no means suggesting that women be made to have moderate anaemia as moderate anaemia seems to be associated with a longer pregnancy compared to mild anaemia, but the finding is worth noting because it can generate hypotheses about underlying biological mechanisms.

4.2 Strengths

The strengths of this study include its large multi-country prospective cohort design with excellent-follow up where outcome data were available for approximately 99% of participants. Inclusion of parental infant trios and the availability of a large number of clinical variables further strengthened the study. Stringent real time data monitoring helped to ensure the quality of the data.

4.3 Limitations

The primary study was designed to develop predictive biomarkers for three late pregnancy conditions, and not specifically to answer this study's research question. 'Healthy' nulliparous women with singleton pregnancies recruited into the SCOPE study are not representative of the general pregnant population. The primary study was conducted in high income countries, and other risk factors for anaemia in pregnancy, such as malaria and hook worm infection (which are more common in low and middle income countries), are unlikely to be significant causes of anaemia in this study population. In addition, women with HIV and sickle cell disease, which are known risk factors/causes for anaemia, were excluded from the primary study.

Cigarette smoking was evaluated by self report as is usual in clinical practice. However, underreporting of smoking could be a concern because cotinine levels – a sensitive marker of smoking tobacco – were not measured. Nevertheless, in pregnancy, self-reported tobacco use has been found to be a valid marker of tobacco exposure [44].

4.4 Conclusion

The absence of a marital partner is an important non-modifiable factor that should be added to the conceptual framework of anaemia's determinants. Although not statistically significant,

clinically, a trend towards a higher risk of adverse pregnancy outcomes was observed in women that were anaemic in early pregnancy.

REFERENCES

1. Grewal A. Anaemia and pregnancy: Anaesthetic implications. Indian J Anaesth 2010; 54(5): 380–6.
2. World Health Organization. Iron Deficiency anaemia. Assessment, Prevention, and Control. A guide for programme managers [Internet]. 2001. [cited 30 March 2013]. Available from: http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf.
3. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System [Internet]. 2011. [cited 1 April 2013]. Available from: http://www.who.int/vmnis/indicators/haemoglobin.pdf.
4. Centers for Disease Control and Prevention. Recommendations to prevent and control iron deficiency in the United States. [Internet]. MMWR Recomm Rep 1998; 47(RR-3):1–29. [cited 30 March 2013] Available from: http://www.cdc.gov/mmwr/PDF/rr/rr4703.pdf.
5. Ouzounian JG, Elkayam U. Physiologic changes during normal pregnancy and delivery. Cardiol Clin 2012; 30(3):317-29.
6. Sullivan KM, Mei Z, Grummer-Strawn L, Parvanta I. Haemoglobin adjustments to define anaemia. Trop Med Int Health 2008; (10):1267-71.
7. Harm SK, Yazer MH, Waters JH. Changes in hematologic indices in caucasian and non-caucasian pregnant women in the United States. Korean J Hematol 2012; 47(2):136-41.
8. Gonzales GF, Steenland K, Tapia V. Maternal haemoglobin level and fetal outcome at low and high altitudes. Am J Physiol Regul Integr Comp Physiol 2009; 297(5):R1477-85.
9. Stephens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Francesco Branca, *et al*. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. Lancet Glob Health 2013; 1:e16–25.
10. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005 [Internet]. Public Health Nutr 2009; 12(4):444-54. [cited 9 April 2013] Available from: http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf.

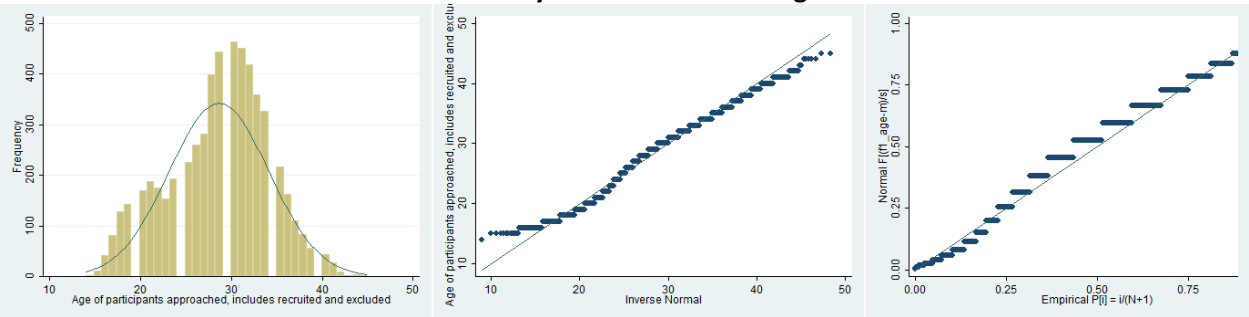
11. Gonzales GF, Tapia V, Fort AL. Maternal and perinatal outcomes in second haemoglobin measurement in nonanemic women at first booking: effect of altitude of residence in peru. ISRN Obstet Gynecol 2012; 2012:368571.
12. Lee AI, Okam MM. Anaemia in pregnancy. Hematol Oncol Clin North Am 2011; 25(2):241-59.
13. Goonewardene M, Shehata M, Hamad A. Anaemia in pregnancy. Best Pract Res Clin Obstet Gynaecol 2012; 26(1):3-24.
14. Chang S, Zeng L, Brouwer ID, Kok FJ, Yan H. Effect of iron deficiency anemia in pregnancy on child mental development in rural China. Pediatrics 2013; 131(3):e755-63.
15. Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, Fawzi WW. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. BMJ 2013; 346:f3443.
16. Levy A, Fraser D, Katz M, Mazor M, Sheiner E. Maternal anaemia during pregnancy is an independent risk factor for low birthweight and preterm delivery. Eur J Obstet Gynecol Reprod Biol 2005; 122(2):182-6.
17. Horton S, Ross J. The economics of iron deficiency. Food Policy 2003; 28:51–75.
18. Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV. Anaemia in low-income and middle-income countries. Lancet 2011; 378(9809):2123-35.
19. Sagili H, Pramya N, Prabhu K, Mascarenhas M, Reddi Rani P. Are teenage pregnancies at high risk? A comparison study in a developing country. Arch Gynecol Obstet 2012; 285(3):573-7.
20. Noranha JA, Al Khasawneh E, Seshan V, Ramasubramaniam S, Raman S. Anaemia in Pregnancy-Consequences and Challenges: A Review of Literature. J South Asian Feder Obst Gynae 2012; 4(1):64-70.
21. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, *et al*. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study. 2010. Lancet 2012; 380(9859):2095-128.
22. Hare GM, Freedman J, David Mazer C. Review article: risks of anaemia and related management strategies: can perioperative blood management improve patient safety? Can J Anaesth 2013; 60(2):168-75.

23. Webster WS, Abela D. The effect of hypoxia in development. Birth Defects Res C Embryo Today 2007; 81(3):215-28.
24. Kenny LC, Broadhurst DI, Dunn W, Brown M, North RA, McCowan L, *et al*. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. Hypertension 2010; 56(4):741-9.
25. North RA, McCowan LM, Dekker GA, Poston L, Chan EH, Stewart AW, *et al*. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. BMJ 2011; 7;342:d1875.
26. McCowan LM, Roberts CT, Dekker GA, Taylor RS, Chan EH, Kenny LC, *et al*. Risk factors for small-for-gestational-age infants by customised birthweight centiles: data from an international prospective cohort study. BJOG 2010; 117(13):1599-607.
27. McCowan LM, Dekker GA, Chan E, Stewart A, Chappell LC, Hunter M, *et al*. Spontaneous preterm birth and small for gestational age infants in women who stop smoking early in pregnancy: prospective cohort study. BMJ 2009; 26;338:b1081.
28. McCowan L, North R, Taylor R. ACTRN12607000551493. Australian New Zealand Clinical Trials Registry [Internet]. 2007. [cited 12 June 2013] Available from: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=82254
29. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Bull World Health Organ 2007 85(11):867-72.
30. Besingi W, Johansson A. Smoke-related DNA methylation changes in the etiology of human disease. Hum Mol Genet 2014; 23(9):2290-7.
31. Van Tiel E, Peeters PH, Smit HA, Nagelkerke NJ, Van Loon AJ, Grobbee DE, *et al*. Quitting smoking may restore hematological characteristics within five years. Ann Epidemiol 2002; 12(6):378-88.
32. Annals of Internal Medicine. Information for Authors - Statistical Guidelines. [Internet]. 2014. [cited 5 December 2014]. Available from: http://annals.org/SS/AuthorInformationStatisticsOnly.aspx%20#multivariable-analysis
33. Amorim LD, Fiaccone RL, Santos CA, Santos TN, Moraes LT, Oliveira NF, *et al*. Structural equation modeling in epidemiology. Cad Saude Publica 2010; 26(12):2251-62.

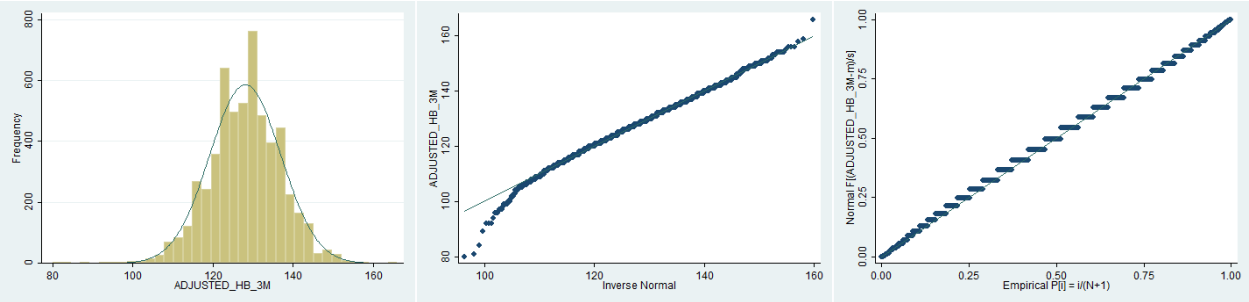
34. Musenge E, Vounatsou P, Collinson M, Tollman S, Kahn K. The contribution of spatial analysis to understanding HIV/TB mortality in children: a structural equation modelling approach. Glob Health Action 2013; 6:19266.
35. McCarthy FP, Khashan AS, North RA, Rahma MB, Walker JJ, Baker PN, *et al*. Pregnancy loss managed by cervical dilatation and curettage increases the risk of spontaneous preterm birth. Hum Reprod 2013; 28(12):3197-206.
36. New Zealand Ministry of Health - Manatū Hauora. Folate/folic acid. Questions and Answers [Internet]. 2014. [cited 12 May 2014]. Available from: http://www.health.govt.nz/our-work/preventative-health-wellness/nutrition/folate-folic-acid.
37. Alio AP, Lewis CA, Scarborough K, Harris K, Fiscella K. A community perspective on the role of fathers during pregnancy: a qualitative study. BMC Pregnancy Childbirth 2013; 13:60.
38. Goshtasebi A, Alizadeh M, Gandevani SB. Association between maternal anaemia and postpartum depression in an urban sample of pregnant women in Iran. J Health Popul Nutr 2013; 31(3):398-402.
39. Lazarou C, Kapsou M. The role of folic acid in prevention and treatment of depression: an overview of existing evidence and implications for practice. Complement Ther Clin Pract 2010; 16(3):161-6.
40. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, *et al*. A systematic analysis of global anemia burden from 1990 to 2010. Blood 2014; 123(5):615-24.
41. Saunders AV, Craig WJ, Baines SK, Posen JS. Iron and vegetarian diets. MJA Open 2012; 1 Suppl 2: 11-16.
42. Hébert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. N Engl J Med 1999; 340(6):409-17.
43. Hébert PC, Carson JL. Transfusion threshold of 7 g per deciliter--the new normal. N Engl J Med 2014; 371(15):1459-61.
44. Kvalvik LG, Nilsen RM, Skjærven R, Vollset SE, Midttun O, Ueland PM, *et al*. Self-reported smoking status and plasma cotinine concentrations among pregnant women in the Norwegian Mother and Child Cohort Study. Pediatr Res 2012; 72(1):101-7.

APPENDIX 1 – tests for normality

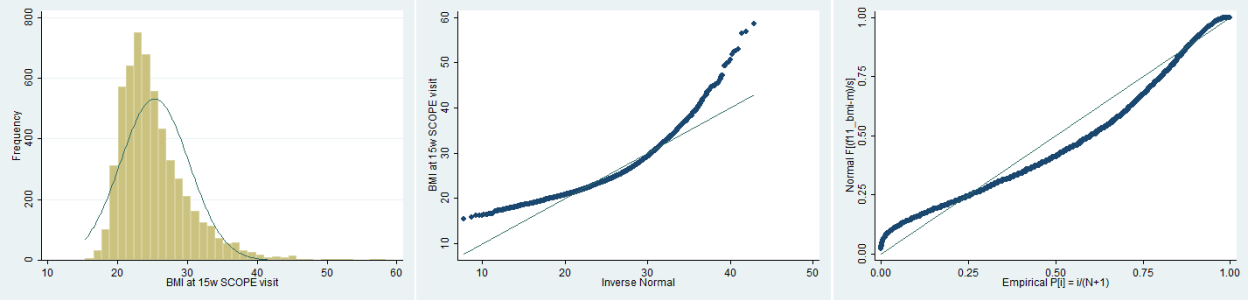
Normality tests for maternal age



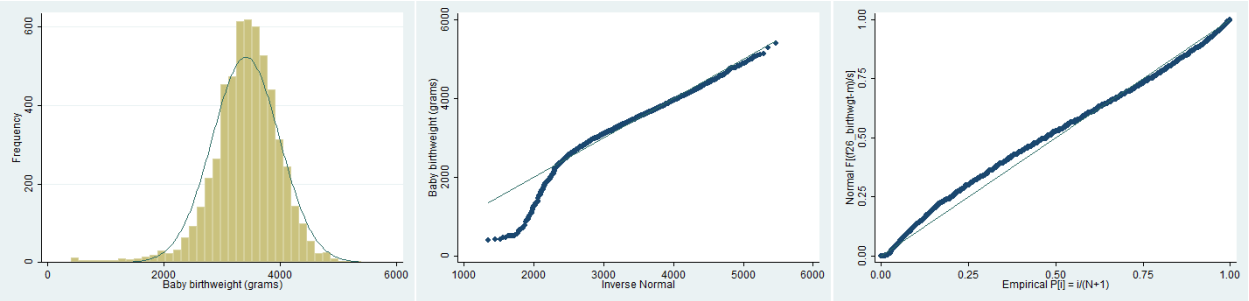
Normality tests for haemoglobin



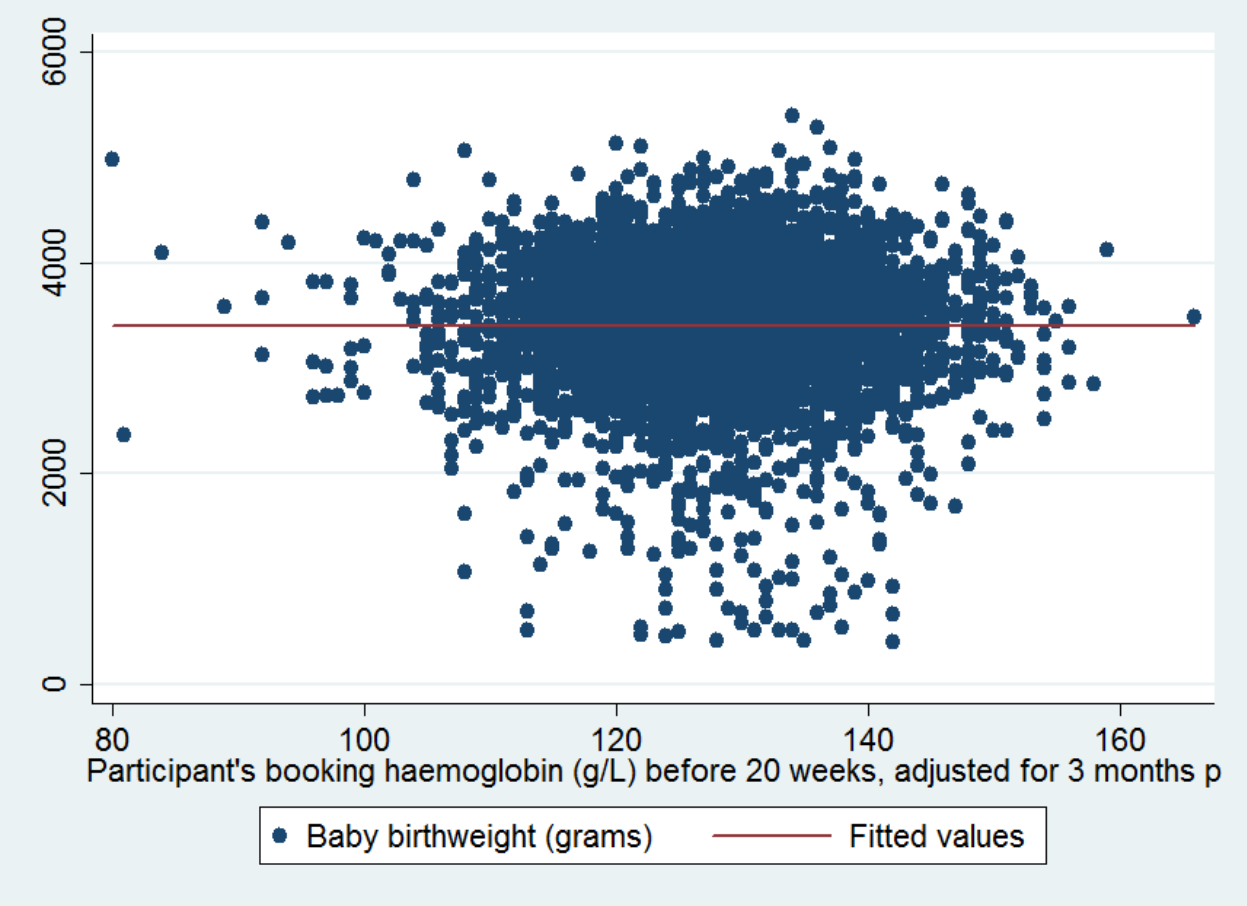
Normality tests for BMI



Normality tests for birth weight



APPENDIX 2 – Correlation between haemoglobin and birth weight



Correlation between haemoglobin and birth weight, correlation coefficient 0.0017, p-value = 0.8972.

APPENDIX 3 – Model goodness of fit

```
. estat gof // the model seems to be of good fit p > 0.05

Logistic model for adjusted_anemia_status3m, goodness-of-fit test

       number of observations =      5575
 number of covariate patterns =      1766
           Pearson chi2(1751) =      1762.82
                 Prob > chi2 =         0.4166

.
end of do-file

. do "C:\Users\Gwinyai\AppData\Local\Temp\STD0w000000.tmp"

. estat gof, group(10)

Logistic model for adjusted_anemia_status3m, goodness-of-fit test

  (Table collapsed on quantiles of estimated probabilities)

       number of observations =      5575
             number of groups =        10
      Hosmer-Lemeshow chi2(8) =         7.30
                 Prob > chi2 =         0.5049

.
end of do-file
```

APPENDIX 4 – Ethics clearance certificate

R14/49 Dr Gwinyai Masukume

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130966

NAME: (Principal Investigator)	Dr Gwinyai Masukume
DEPARTMENT:	Public Health University of Witwatersrand
PROJECT TITLE:	Birth Outcomes and Associated Risk Factors of Anaemia in Early Pregnancy in a Nulliparous Cohort
DATE CONSIDERED:	27/09/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Gill Nelson
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/09/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/~~we~~ fully understand the conditions under which I am/~~we~~ are authorized to carry out the above-mentioned research and I/~~we~~ undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/~~we~~ undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature

Date 6 OCTOBER 2013

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES