Prenatal zinc and vitamin A supplementation
A study on the impact of prenatal micronutrient supplementation in rural Indonesia

Endy P. Prawirohartono
“We are guilty of many errors and many faults, but our worst crime is abandoning the children, neglecting the foundation of life. Many of things we need can wait. The child cannot. Right now is the time his bones are being formed, his blood is being made, and his senses are being developed. To him we cannot answer “Tomorrow”. His name is “Today”.

(Gabriela Mistral, 1948)
Abstract

Objectives: To study the effects of prenatal zinc and vitamin A supplementation on birth size, neonatal morbidity, infant mortality, and growth in children up to two years of age.

Subjects and Methods: From September 1995 to December 1999 pregnant women in Purworejo District, Central Java, Indonesia with gestational age <17 weeks (n=2173) were recruited to and participated in a community-based, individually randomized, placebo controlled, double blinded study aiming to evaluate the impact of supplementation (vitamin A, zinc, vitamin A + zinc) during pregnancy on maternal morbidity and pregnancy outcomes. We analyzed secondary data from that study regarding birth size, neonatal morbidity and infant mortality of the 1956 infants born alive. A subsample of infants (n=343) was followed until 2 years of age concerning growth, feeding practices and morbidity. Outcomes were tested using the chi-square test, ANOVA, ANCOVA, and Cox’s proportional hazard function.

Results: Birth weights in the zinc [mean ± Standard deviation (SD): 3.16 ± 0.52 kg], vitamin A (3.08 ± 0.46 kg) or the combined vitamin A and zinc (3.10 ± 0.59) groups did not differ from placebo (3.09 ± 0.50 kg) after adjustment for maternal pre-pregnancy weight, weight gain during pregnancy, and parity (P=0.70). Birth lengths of infants born to mothers supplemented with zinc or vitamin A were in average 0.3 cm and 0.2 cm longer than those in the placebo group after adjustment for maternal height, pre-pregnancy weight, weight gain during pregnancy, and parity (P=0.04). The impact of prenatal supplementation on infant mortality and neonatal morbidity was not significant. There was a small effect of prenatal vitamin A supplementation on postnatal growth in height-for-age z-score (HAZ). The absolute differences between the vitamin A only and vitamin A + zinc groups at 3 and 9 months were 0.34 SD and 0.37 SD, respectively, and the absolute difference between the vitamin A only and zinc only groups at 18 months was 0.31 SD. Defining growth faltering as downward crossing of ≥2 major percentile lines, 50-75% of the children were faltering within 9 months of age, whereas 17% and 8% were <-2 SD for growth in weight-for-age z-score (WAZ) and HAZ, respectively. Prenatal supplementation did not reduce the prevalence of growth faltering.

Conclusions: Prenatal vitamin A and zinc demonstrates a small but significant impact on birth length, but it does not have any protective effect on infant mortality and neonatal morbidity. Prenatal vitamin A supplementation had a small but significant effect on postnatal length growth until 18 months of age, but no effect on weight gain, growth rate and it did not reduce the prevalence of growth faltering.

Keywords: zinc, vitamin A, prenatal supplementation, birth size, growth, morbidity, mortality
Abstract in Indonesian

Tujuan: meneliti pengaruh suplementasi zink dan vitamin A pada masa prenatal terhadap ukuran tubuh bayi baru lahir, morbiditas neonatal, kematian bayi, dan pertumbuhan anak sampai dengan umur dua tahun


Hasil: Berat badan lahir pada kelompok zink [mean ±standar deviasi (SD): 3.16 ± 0.52 kg], vitamin A (3.08 ± 0.46 kg) atau kombinasi vitamin A dan zink (3.10 ± 0.59) tidak berbeda secara bermakna dibandingkan dengan plasebo (3.09 ± 0.50 kg) setelah dikontrol oleh berat badan ibu sebelum hamil, pertambahan berat badan selama hamil, dan paritas (P=0,70). Panjang lahir dari kelompok ibu yang disuplementasi dengan vitamin A atau zink 0,2 cm dan rata-rata 0,3 cm lebih panjang dibanding kelompok plasebo setelah dikontrol oleh tinggi badan ibu, berat badan ibu sebelum hamil, pertambahan berat selama hamil, dan paritas (P=0,04). Pengaruh suplementasi selama masa prenatal terhadap kematian bayi dan morbiditas neonatal tidak bermakna. Suplementasi masa prenatal mempunyai efek yang lemah terhadap pertumbuhan bayi yang diukur dengan indikator tinggi badan menurut umur (HAZ). Perbedaan absolut panjang badan anak antara kelompok vitamin A dan kombinasi vitamin A dan zink pada umur 3 dan 9 bulan adalah 0,34 SD dan 0,37 SD; dan perbedaan absolut antara kelompok vitamin A dengan zink pada umur 18 bulan adalah 0,31 SD. Dengan menggunakan kriteria growth faltering sebagai penurunan garis pertumbuhan memotong ≥ 2 persentil major, 50-75% anak mengalami growth faltering pada umur 9 bulan, dan hanya 17% dan 8% yang terdeksi mengalami growth faltering bila digunakan kriteria WAZ dan HAZ <- 2SD. Suplementasi vitamin A dan zink pada masa prenatal tidak menurunkan prevalensi growth faltering.

Kesimpulan: Suplementasi vitamin A dan zink pada masa prenatal menunjukkan adanya pengaruh lemah tetapi bermakna terhadap panjang lahir, tetapi tidak menunjukkan efek protektif terhadap kematian bayi dan morbiditas neonatal. Suplementasi vitamin A pada masa prenatal mempunyai efek lemah tetapi bermakna terhadap pertumbuhan panjang badan sampai dengan umur 18 bulan, tetapi tidak disertai pengaruh terhadap kenaikan berat badan, laju pertumbuhan dan tidak menurunkan prevalensi growth faltering.
Original papers

This thesis is based on the following papers:


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Abbreviations and acronyms

ANC      Antenatal care
BMI      Body mass index
CHN-RL  The Community Health and Nutrition Research Laboratories
CI       Confidence interval
DNA      Deoxyribonucleic acid
GH       Growth hormone
HAZ      Height-for-age z score
HPA      Hypothalamic pituitary adrenal axis
hPL     Human placental lactogen
HR       Hazard ratio
HRS      Household Registration System
IGF      Insulin-like growth factor
IUGR     Intrauterine growth restriction
LBW      Low birth weight
LMP      Last menstrual period
OR       Odds ratio
RE       Retinol equivalent
RR       Relative risk
SD       Standard deviation
SGA      Small for gestational age
UNICEF   United Nations Children’s Fund
UNIMMAP  The United Nations International Multiple Micronutrient Preparation
WAZ      Weight-for-age z score
WHO      World Health Organization
WHZ      Weight-for-height z score
ß HCG    ß human chorionic gonadotropin
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Introduction

“Windows of opportunity” to improve maternal and childhood health

The ultimate goal of childcare is achieving overall optimal health, growth and development of the children. Unfortunately, many children in developing countries are already from birth affected by poor health. Low birth weight (LBW) is one example where suboptimal health of the mother, sometimes starting long before conception is transmitted to her progeny. In 2000, the overall prevalence of LBW was 15%, 7.0% in developed countries, 17% in less developing countries, and 19% in least developing countries (United Nation Children’s Fund and World Health Organization, 2004). If the child remains under conditions, which does not restore the child’s health or nutritional status, the growth will be affected. In 2010, it was estimated that 160 million children in developing countries had poor growth as measured by stunting, i.e. low length- or height-for-age. Although stunting in the Southeast Asian countries showed a dramatic decrease from 47% in 1990 to 27% in 2010, in other parts of developing regions such as Africa stunting has stagnated at about 40%. It is widely recognized that stunting is the result of multiple determinants and circumstances, including preconception, intrauterine, and postnatal malnutrition (de Onis et al, 2011).

Maternal nutrition before conception, during pregnancy and in the postnatal period can influence child health outcomes. The earlier efforts are made to prevent poor nutrition, the larger the opportunities to prevent negative impacts on child health and growth. Integration of actions involving program managers, policymakers, and researchers to improve maternal, infant, and child nutrition as early as possible is needed (Figure 1) (Dewey & Huffman, 2009).

Many infants in developing countries are born small for gestational age (SGA). Most of these children never catch-up growth and the deficits in length compared to children born in affluent areas become larger as the child grows older. About 50% of the deficit is attributable to low birth length or stunting at birth, and the rest to growth faltering after birth. The latter is due to several factors, including the trajectory of growth established in utero, suboptimal postnatal nutrition due to lack of exclusive breastfeeding and proper complementary feeding, and infections (Dewey & Huffman, 2009).
INTRODUCTION

Windows of opportunity

Figure 1. “Windows of opportunity” for intervention from preconception through pregnancy, the period of exclusive breastfeeding, and the target age of complementary feeding.

Maternal factors, in particular maternal malnutrition, contribute to small birth size, including low birth weight and length. In developing countries, maternal malnutrition or low body mass index (BMI) is frequently found in young mothers. To improve child nutrition according to the “windows of opportunity” it is important to improve the preconception nutritional status of the potential mother i.e. improve the nutritional status of adolescents and delaying childbearing (Dewey & Huffman, 2009).

Schrimpton et al (2009a) used medical records from four refugee camps in Bhutan to retrospectively estimate the prevalence of LBW 1994-2001. Two years after settlement into the camps there was a quick drop of the LBW rate to approximately a third of that estimated at outside the camps. The surprisingly low LBW rate in the refugee camps, in average 11%, was achieved within 5 years of settlement and indicates that the basic needs of mothers were met, including food, water and sanitation, antenatal care, and education. Another factor that may have contributed to the low LBW rate was discouraging of pre-pubertal marriage in the refugee camps, which was common in the community outside the camps. In adolescent mothers who are still growing, the maternal-foetal competition for nutrients causes decrease of birth size (Shrimpton et al, 2009a). This phenomenon supports the benefit of intervention during the preconception phase or the first “window of opportunity” (Figure 1).

The second “window of opportunity” is interventions during pregnancy. It has been acknowledged that health and nutritional status of pregnant women affect
the pregnancy outcome. Women who enter pregnancy with poor nutritional status will have infants suffering from intrauterine growth restriction (IUGR) (Belkacemi et al, 2010). SGA infants have increased risks of low Apgar score at five minutes, sepsis, intracranial haemorrhage, intrauterine and neonatal death, necrotizing enterocolitis, and respiratory complications (Damodaram et al, 2011), short stature in adult population, possible pubarche in females, and increased risk of adult diseases including stroke, type 2 diabetes mellitus, heart failure, obesity, and hypertension (Chernausek, 2012). Several studies of prenatal supplementation including both macro- and micronutrients have been published. A meta-analysis, that evaluated the benefit of balanced protein energy supplementation or protein, providing not more than 20% of the total energy intake, showed a decreased prevalence of IUGR by 31% (Imdad & Bhutta, 2011). Other meta-analyses that evaluated the effects of prenatal multi-micronutrient supplementation compared to standard iron or iron and folic acid or placebo showed an improved birth weight (Fall et al, 2009; Shah & Ohlsson, 2009), decreased prevalence of LBW (Fall et al, 2009; Kawai et al, 2011; Shah & Ohlsson, 2009), and SGA (Fall et al, 2009; Kawai et al, 2011).

The third “window of opportunity” involves promoting exclusive breastfeeding for babies up to six months of age. Following birth, mothers should be motivated to breastfeed their infants as soon as in the first hour of life. This time is a critical window not only for improving breastfeeding practices, but also for addressing the mother’s need and her nutrition during this vulnerable time period (Dewey & Huffman, 2009).

Improving complementary feeding for children 6 to 24 months of age is the fourth “window of opportunity”. Issues during this period of time include the importance of continued breastfeeding and improving the quality and accessibility of food available for complementary feeding (Dewey & Huffman, 2009).

**Prenatal factors influencing birth size**

Intrauterine growth involves complex mechanisms influenced by maternal factors including nutritional status and maternal health factors, as well as genetic factors (Figure 2) (Christian, 2010). Poor maternal nutritional status before and during pregnancy including maternal short stature and low BMI have negative effects on foetal growth and pregnancy outcomes (Black et al, 2008).
There are several mechanisms through which maternal under-nutrition affects foetal growth:

a) Plasma volume expansion occurs early during pregnancy, increasing uterine and placental blood flow, facilitating nutrient and oxygen transport to foetus (Bernstein et al, 2001). Although the red blood cells mass is increased, it lags behind leading to physiologic anaemia during pregnancy. Insufficient plasma volume expansion will result in preeclampsia and IUGR and pregnant women who are stunted have higher risk of decreased plasma volume expansion and poor foetal growth (Christian, 2010; Rosso et al, 1992).

b) Placental growth, vascularization and function in terms of transfer of nutrients to the foetus are other key factors for foetal growth and birth weight. Placental growth and volume is influenced by maternal pre-pregnancy weight, weight gain during pregnancy and food intake during pregnancy (Christian, 2010; Rao et al, 2001). Imprinted genes are a class of genes found in placenta and foetal tissues that have critical roles in foeto-placental development. Imprinted genes in the placenta control the supply of nutrients, whereas in the foetus they control the nutrient demand by regulating foetal growth. The action of imprinted genes is controlled by deoxyribonucleic acid (DNA) methylation...
that is influenced by environment factors including nutrition. The availability of methyl donors, i.e. from vitamin B12, folic acid, and amino acids may alter the DNA methylation (Christian, 2010; Reik et al, 2003).

c) Endocrine factors, such as insulin-like growth factor (IGF)-1, growth hormone (GH)-2, and human placental lactogen (hPL), have a strong relationship with foetal growth. These hormones are sensitive to maternal nutritional status and regulate foetal growth and homeostasis through trophoblast invasion, direct affecting placental development and function, and through the creation of peripheral insulin resistance allowing preferential glucose supply to the foetus (Christian, 2010).

d) The micronutrient status of pregnant women is also an important factor influencing birth size (Brown et al, 2001). Iron deficiency and anaemia in pregnancy causing hypoxia may induce stress and influence the foetal hypothalamic pituitary adrenal (HPA) axis including elevated corticotropin-releasing hormone, which is a risk factor of preterm labour in animals (Allen, 2001). Micronutrients need to be increased during pregnancy. When maternal nutrition status is adequate or she has mild nutrient deficiencies, the placenta and foetus are prioritized over most other maternal tissues. On the contrary, when maternal deficiency is severe, health and survival of the maternal organism is preserved (Redmer et al, 2004; King, 2000).

**Postnatal factors influencing growth, morbidity, and mortality**

A framework developed by UNICEF clearly describes the factors affecting child nutrition and growth (Black et al, 2008). The *immediate* factors are inadequate dietary intake and diseases, especially infectious diseases (diarrhoea, respiratory tract infection etc.), and inadequate childcare, which apart from directly influencing childhood malnutrition and poor growth also affect dietary intake and the occurrence of diseases. Breastfeeding and appropriate complementary feeding is needed to support optimal nutritional status and growth of children. The *underlying* factor, poverty causes household food insecurity, unhealthy household environment, and lack of health services facilitate the *immediate* factors influencing child nutritional status and growth. The socio-economic and political context cause lack of financial, human, physical and natural capital leading to poverty. The short-term consequences of child malnutrition and poor growth are increasing morbidity, mortality, and disability, whereas long-term the consequences are reduced adult size, intellectual ability, economic productivity, reproductive performance, and increasing the metabolic and cardiovascular diseases.
Prenatal supplementation

Researchers have for decades paid attention to the effects of iron deficiency on the pregnancy outcome, since iron deficiency is the most common nutrient deficiency found among pregnant women. Iron supplementation has been encouraged to maintain the haemoglobin levels during pregnancy (Allen, 2000). However, pregnant mothers with a haemoglobin level between 95 g/L and 105 g/L have a low risk of LBW and preterm labour, whereas those with low (<95 g/L) or high levels (≥120 g/L) has increased risk of preeclampsia and IUGR (Steer, 2000). Due to higher requirements of folic acid during pregnancy due to rapidly dividing cells in the foetus and increasing urinary losses supplementation is recommended in addition to iron. However, a meta-analysis by Peña-Rosas and Viteri (2009) showed diverging results. Although prenatal iron and folic acid supplementation improved the haemoglobin levels of the mothers and decreased iron deficiency anaemia, data failed to demonstrate that iron supplementation alone or with folic acid among pregnant women without anaemia or with mild or moderate anaemia benefitted in terms of maternal health, foetal health, or pregnancy outcome from the supplementation. There was no support from this meta-analysis that folic acid prevents neural tube defects.

A number of studies have evaluated the effects of prenatal zinc supplementation on pregnancy outcome mainly birth weight. Out of 17 studies published 1984-2009, 13 reported no association between prenatal zinc supplementation and birth weight, three studies reported positive association, and one reported negative association (Gebreselassie & Gashe, 2011). However, the overall meta-analysis showed no association either using fixed or random effect models. The failure of the studies to show effects of iron, folic acid or zinc supplementation on maternal and foetal health indicates that multiple micronutrient deficiency often coexist especially in low and middle income countries. It is difficult to evaluate the effects of all potential micronutrients, as well as their possible interaction. A greater effect may be achieved by multiple micronutrient supplementation rather than single nutrient supplementation (Haider & Bhutta, 2006).

The use of multiple micronutrients has been proposed by some authors as an alternative to the recommendation of iron and folic acid supplements during pregnancy. In 1999, WHO, UNICEF and the United Nations University agreed on a multiple micronutrient supplement that contain 15 vitamins and minerals for trials targeting pregnant women. The composition of the supplement later became known as the United Nations International Multiple Micronutrient Preparation (UNIMMAP) (Shrimpton et al, 2009b). Table 1 shows the composition of UNIMMAP in comparison to the supplements used in the thesis. Whereas several studies have documented the effects of UNIMMAP on birth weight (Shah & Ohlsson, 2009; Kawai et al, 2011; Fall et al, 2009), no study has shown effects
on other measures of birth size including length, and few studies have shown effects on infant morbidity and mortality. Also, evidence of the long-term effects of prenatal supplementation on child growth is lacking.

**Table 1.** Comparison of the composition of the World Health Organization/UNICEF/United Nations University multiple micronutrient supplement (United Nations International Multiple Micronutrient Preparation [UNIMMAP]) and micronutrients used in the Zibuvita study.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>UNIMMAP</th>
<th>Zibuvita Treatment group</th>
<th>Zibuvita Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>800 µg</td>
<td>2400 RE (2400 µg)</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>200 IU</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>10 mg</td>
<td>2 mg</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>70 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>1.4 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>1.4 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Niacin</td>
<td>18 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>1.9 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>2.6 µg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Folic acid</td>
<td>400 µg</td>
<td>250 µg</td>
<td>250 µg</td>
</tr>
<tr>
<td>Iron</td>
<td>30 mg</td>
<td>60 mg</td>
<td>60 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>15 mg</td>
<td>20 µg</td>
<td>-</td>
</tr>
<tr>
<td>Copper</td>
<td>2 mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Selenium</td>
<td>65 µg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Iodine</td>
<td>150 µg</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Zibuvita study**

The Zibuvita (Zink ibu hamil vitamin A, zinc and vitamin A for pregnant women) study was initiated in 1995. The study involved 2173 pregnant women from a rural area in Central Java, Indonesia with a presumably high prevalence of nutrient deficiencies. At that time, the government of Indonesia recommended pregnant women to consume 60 mg iron and 250 µg folic acid for 90 days. The policy of the Indonesian government was in line with the prenatal supplementation policies practiced in other countries.

Vitamin A and zinc are believed to have a critical role in maintaining the immune competence and reduce the risk of puerperal fever and sepsis in pregnant women (Cox et al, 2006; Christian et al, 2009). The first aim of the Zibuvita study was to evaluate whether adding 20 mg zinc and 2400 RE vitamin A to the standard iron folic acid supplementation would reduce the prevalence of perinatal complications. The second aim was to evaluate the effect of vitamin A and zinc on the iron status of pregnant women regularly consuming iron and folic acid sup-
The researchers hypothesized that adding vitamin A would improve the iron status of pregnant women beyond what iron and folic acid supplementation alone would do. However, they realized that adding zinc to iron might be problematic. Negative effects of zinc on iron absorption had been reported. However, few studies had evaluated the effect of zinc on iron status in pregnant women. Moreover, no study has evaluated the combination of zinc and vitamin A on iron status of pregnant women. Nurdiati (2001) showed that vitamin A substantially reduced the risk of maternal postpartum infection and that both vitamin A and zinc supplementation had a positive impact on iron status during pregnancy and the postpartum period, but the effects were related to initial iron status. The *third aim*, which is the focus of the present thesis, was to evaluate the effects of maternal supplementation with vitamin A and zinc on birth size, neonatal morbidity, infant mortality and postnatal growth.

Figure 3 summarizes the conceptual framework of the study.

**Figure 3.** The relationship between prenatal micronutrient supplementation, some explanatory variables and study outcomes (birth size, neonatal morbidity, infant mortality, and child growth).
Methods

Study site

Figure 4. Purworejo district is located in the Central Java Province, west of Yogya-karta.

The study was performed in Purworejo district, Central Java. The Indonesian archipelago consists of approximately 17,000 islands located between Asia and Australia, and between the Pacific and Indian Oceans (Figure 4). There are five major islands, namely Java, Sumatra, Kalimantan (Borneo), Sulawesi (Celebes), and Papua, and the remaining ones are groups of islands namely Maluku (Moluccas) and Nusa Tenggara. More than 80% of Indonesia’s territory is covered by water. Indonesia is a tropical country with two seasons, dry season that extends from May to October and rainy season from November to April. Indonesia has 33 administrative provinces. Each province is divided into districts and municipalities, whereas each district and municipality is divided into sub-districts. The lowest administrative unit is the village. In 2007 there were 370 districts, 96 municipalities, 6131 sub-districts, and 73,405 villages (Statistics Indonesia, 2008).
**Mother and child health in Indonesia**

Maternal and child characteristics according to the 2007 nationwide survey are presented in Table 2. One out of three (31%) mothers aged 15-49 years completed primary school, 57% were currently employed, 58% lived in rural areas, 93% received ANC from skilled providers, and 53% of deliveries took place at home. Premature delivery rate was low (2.3%). Participation in prenatal iron and folic acid supplementation as recommended by the government was low; only 29% of the pregnant women took iron and folic acid tablet for at least 90 days, and only 45% consumed vitamin A until 2 months after delivery.

**Table 2.** Mother and child characteristics for Indonesia 2007 Source: Statistics Indonesia (2008).

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Category</th>
<th>Sample size</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother (15-49 years):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td>No education</td>
<td>32,895</td>
<td>2270</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Some primary</td>
<td>32,895</td>
<td>5559</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>Complete primary</td>
<td>32,895</td>
<td>10,066</td>
<td>30.6</td>
</tr>
<tr>
<td></td>
<td>Some secondary</td>
<td>32,895</td>
<td>6776</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>Complete secondary</td>
<td>32,895</td>
<td>5954</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>Secondary and above</td>
<td>32,895</td>
<td>2270</td>
<td>6.9</td>
</tr>
<tr>
<td>Employment</td>
<td>Not currently employed</td>
<td>32,895</td>
<td>1118</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Not employed in past 12 months</td>
<td>32,895</td>
<td>12,928</td>
<td>39.3</td>
</tr>
<tr>
<td></td>
<td>Currently employed</td>
<td>32,895</td>
<td>18,849</td>
<td>57.3</td>
</tr>
<tr>
<td>Residence</td>
<td>Urban</td>
<td>32,895</td>
<td>13,745</td>
<td>41.8</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>32,895</td>
<td>19,150</td>
<td>58.2</td>
</tr>
<tr>
<td>ANC</td>
<td>From skilled providers</td>
<td>14,043</td>
<td>13,102</td>
<td>93.3</td>
</tr>
<tr>
<td></td>
<td>Meet recommended schedule</td>
<td>14,043</td>
<td>9198</td>
<td>65.5</td>
</tr>
<tr>
<td>Delivery</td>
<td>At private sectors</td>
<td>16,504</td>
<td>6007</td>
<td>36.4</td>
</tr>
<tr>
<td></td>
<td>At public sectors</td>
<td>16,504</td>
<td>1601</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>At home</td>
<td>16,504</td>
<td>8698</td>
<td>52.7</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>16,504</td>
<td>99</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Missing data</td>
<td>16,504</td>
<td>99</td>
<td>0.6</td>
</tr>
<tr>
<td>Premature birth</td>
<td>≥90 days during pregnancy</td>
<td>14,043</td>
<td>4101</td>
<td>29.2</td>
</tr>
<tr>
<td></td>
<td>60-89 days during pregnancy</td>
<td>14,043</td>
<td>1152</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>&lt;60 days during pregnancy</td>
<td>14,043</td>
<td>4761</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>14,043</td>
<td>2892</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>Do not know</td>
<td>14,043</td>
<td>1137</td>
<td>8.1</td>
</tr>
<tr>
<td>Iron and folic acid supplementation</td>
<td>&gt;90 days during pregnancy</td>
<td>14,043</td>
<td>4101</td>
<td>29.2</td>
</tr>
<tr>
<td></td>
<td>60-89 days during pregnancy</td>
<td>14,043</td>
<td>1152</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>&lt;60 days during pregnancy</td>
<td>14,043</td>
<td>4761</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>14,043</td>
<td>2892</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>Do not know</td>
<td>14,043</td>
<td>1137</td>
<td>8.1</td>
</tr>
<tr>
<td>Vitamin A 2 months after delivery</td>
<td>Not weighed</td>
<td>16,504</td>
<td>2789</td>
<td>16.9</td>
</tr>
<tr>
<td>Children (&lt;5 years):</td>
<td>&lt;2500g (LBW)</td>
<td>16,504</td>
<td>908</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>≥2500g</td>
<td>16,504</td>
<td>12,592</td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td>Do not know</td>
<td>16,504</td>
<td>215</td>
<td>1.3</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>Ever breastfed</td>
<td>16,504</td>
<td>15,710</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td>Exclusively breastfed until 6 months</td>
<td>1686</td>
<td>546</td>
<td>32.4</td>
</tr>
<tr>
<td>Complementary feeding</td>
<td>As recommended</td>
<td>4612</td>
<td>1900</td>
<td>41.2</td>
</tr>
<tr>
<td>Diseases</td>
<td>Diarrhoea</td>
<td>15,925</td>
<td>2182</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>Respiratory tract Infection</td>
<td>15,925</td>
<td>1784</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Fever</td>
<td>15,925</td>
<td>5032</td>
<td>32.0</td>
</tr>
</tbody>
</table>
METHODS

The prevalence of LBW was 5.5%. Breastfeeding initiation was high (95%), however, only 32% of infants were exclusively breastfed until 6 months of age. The prevalence of diarrhoea, respiratory tract infection and fever were 14%, 11% and 32% respectively. The neonatal, infant, child, and under-five mortality rates were 19, 34, 10, and 44 per 1000 live births respectively.

Zinc and vitamin A deficiency among pregnant women in Indonesia

Brown and coworkers (2001) estimated the prevalence of zinc deficiency in Southeast Asian countries including Indonesia at 71%, however, there are few studies of the prevalence of zinc deficiency among pregnant women. The prevalence of zinc deficiency in lactating women in West Java (1997) and Nusa Tenggara (2001) was 24% and 39% respectively (Nurdiati, 2001).

The prevalence of low serum retinol (<0.35 µmol/dL or <10 µg/dL) among pregnant women in West Java was 2.5%, whereas the prevalence of marginal serum retinol status (<0.70 µmol/dL or <20 µg/dL) was 31% (Suharno et al, 1992).

Purworejo district, Java island

Purworejo district consists of 16 sub-districts and 494 villages. The total area of the district is 1035 km², where 306 km² is rice fields and the remaining are forest (68 km²), plantation (0.12 km²), meadow (1.8 km²), and dry land. The land contour of this district varies from 2 to 325 meter above sea level, the containing lowland, highland and mountain areas. In 1997, the total population was 750,922, 368,751 males and 382,171 females. A majority (87%) were living in rural areas. Half (49%) of the population was 15-49 years old group, while the under-five, 5-14 years, and 50 years and older constituted 9%, 21% and 21% respectively. Only 22% of the population had secondary education or higher.

The district has 1 government hospital, 4 private hospitals, 24 community health centres, 62 auxiliary health centres, 4 maternity clinics, and 170 village health posts. Health services are provided by 35 general physicians, 23 dentists, 359 paramedics, and 57 midwives (Purworejo District Government, 1997).

Study population

The study population constitutes of infants born to mothers recruited in a community-based, double-blinded randomized controlled trial (the Zibuvita study) aiming to evaluate the impact of supplementation (vitamin A, zinc, vitamin A + zinc) on maternal morbidity and pregnancy outcomes. The Zibuvita mothers were recruited from a longitudinal surveillance system that was initiated in 1994 (Figure 5).
Longitudinal surveillance system: basic demographic indicators, morbidity, nutritional status, health service.

**Figure 5.** The scheme of the longitudinal studies.

**Longitudinal surveillance system**

In June 1994 a surveillance program was initiated in Purworejo district by the Community Health and Nutrition Research Laboratory (CHN-RL). The CHN-RL surveillance system was implemented as a collaborative effort between the School of Medicine Gadjah Mada University and the Ministry of Health through the District Health Office of Purworejo and the Central Java Provincial Health Office. Information about maternal and child health was collected, including use of health services, levels and trends of infant mortality, and participation in family planning programs. The main goal of the activities was to develop surveillance models which are effective and efficient and that can be used by program implementers to improve health and reduce the mortality, especially in women and children, through better planned and implemented programs. The CHN-RL received assistance from national and international organizations, including the World Bank, the John Hopkins University, WHO, UNICEF, Umeå University and other agencies (Nurdiati, 2001).

The longitudinal surveillance followed a sample of 10% of the district population (n=17,000 households). Through this surveillance system women of reproductive age (15-49 years of age) could be reached. These women were visited every 90 days by project staffs and invited to participate in the research projects, if they fulfilled each study’s enrolment criteria. The routine data collection included basic demographic indicators (birth, marriage, migration and death),
morbidity status, nutritional status (including weight), and health services indicators. This longitudinal surveillance system could identify women in early pregnancy (Nurdiati, 2001).

The Zibuvita study

A surveillance system to monitor the onset of pregnancy in women of reproductive age was established. Female interviewers made home-visits to those women out of 25,067 who consented, to detect the occurrence of pregnancy monthly. Detection of pregnancy was based on recording the date of the last menstrual period (LMP), which was confirmed with a b-human chorionic gonadotropin (b-HCG) pregnancy test in the field. Between September 1995 and December 1999, 2173 women who were pregnant for no more than 120 days and who consented to participate were randomized to a treatment group (vitamin A, zinc, vitamin A + zinc, or placebo) (Nurdiati, 2001).

The Pronak study

Infants born from women in Zibuvita study were included in the Pronak (Prospektif anak, prospective study in children) study that monitored child growth and development, anthropometry, feeding, and morbidity from birth to 4 years and some of the infants were monitored until 6 or 7 years old.

The Zinak study

The Zinak (Zinc Iron anak, zinc and iron study in children) study randomly included infants into zinc and iron supplementation groups and monitored the infants from 6 to 12 months of age (Lind et al, 2003; Lind et al, 2004).

Prenatal supplementation in the Zibuvita Study

The micronutrient capsules given to the pregnant mothers from inclusion until delivery contained either 2400 REs of vitamin A as retinyl palmitate; or 20 mg of zinc sulphate; or the same dose of both vitamin A and zinc sulphate; or placebo. All capsules also contained 2 mg dl-α-tocopherol as an antioxidant and 350 mg of soybean oil, 20 mg of beeswax and 8 mg of lecithin as capsule filler. Mothers were randomly allocated in a 1:1:1:1 ratio in blocks of twelve based on a list of treatment numbers derived from a pseudo-random number generated in the software SAS version 5 (SAS Institute, Inc., Cary, NC, USA). The treatment allocation sequence was prepared and held at the University of Newcastle, New South Wales, Australia. All investigators, field and laboratory staffs and participants were blinded to the treatment code until all field data had been collected,
and preliminary data analysis by coded groups had been completed. Field workers distributed capsules and monitored compliance in the home of the women, resulting in 70% of supplements consumed.

**Data collection**

Figure 6 shows the timing of data collection of the Zibuvita study and Pronak study. At the start of the Zibuvita study, trained field workers collected basic and socio-economic status (SES) (number of family members, mother’s age, education, occupation, parity, drinking water, sanitation facility) from the participating women through home interviews and invited mothers to participate in either non-intensive or intensive follow up.

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<th>24</th>
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</thead>
<tbody>
<tr>
<td>SES</td>
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<td>Pregnancy Delivery</td>
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<td>Anthropometry</td>
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<td>Breastfeeding</td>
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<tr>
<td>Morbidity Mortality</td>
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</tbody>
</table>

**Figure 6.** Data collection by age of child (months).

There were 2173 pregnant women were with gestational age <17 weeks included in the Zibuvita study.

Infants born to Zibuvita mothers within the study period were, after approval of caregivers, included in one of two child follow-up studies, the Zinak and Pronak studies. The anthropometric (weight and length) data were collected monthly from birth to 12 months of age, and then again at 18 months and 24 months of age. The breastfeeding practices were collected using the 24-hour recall method, weekly during the first 2 months, biweekly during the third and fourth months, monthly from 5 to 18 months of age, and finally at 24 months of age.
After the delivery field workers visited the mother and her baby collecting morbidity data daily up to 20 days in the postnatal period. The mother was asked whether the baby had any of the following symptoms: fever, the baby looked weak or ill, hypothermia (“cold temperature”), diarrhoea, vomiting, breathing difficulties, and seizure (“convulsion”). After the perinatal period (>1 month) number of days with symptoms of diarrhoea and respiratory infections were collected monthly, in which the parents were asked for occurrence of symptoms of diarrhoeal disease or respiratory infection on the day of visit by the field worker, followed by the same questions for the day before, two days before, and so forth until 14 days prior to the visit.

**Characteristics of Paper I and Paper II**

The characteristics of Paper I and Paper II are showed in Table 3.

**Table 3.** Characteristics of the study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Paper I</th>
<th>Paper II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aim</td>
<td>Evaluate impact on birth size, neonatal morbidity and infant mortality</td>
<td>Evaluate impact on growth and growth faltering of children up to two years of age</td>
</tr>
<tr>
<td>Study design</td>
<td>Randomised controlled trial</td>
<td>Randomised controlled trial</td>
</tr>
<tr>
<td>Study population</td>
<td>Infants born to mothers from the Zibuvita study</td>
<td>Subset of infants born to mothers from the Zibuvita study</td>
</tr>
<tr>
<td>Sample size</td>
<td>1956 infants</td>
<td>343 infants</td>
</tr>
<tr>
<td>Main outcome variables</td>
<td>Birth weight, birth length, neonatal morbidity, infant mortality</td>
<td>Growth and growth faltering of children up to two years of age</td>
</tr>
</tbody>
</table>

**Main outcome variables and covariates**

Method of measurement of the main outcome variables used to evaluate the effect of prenatal supplementation of birth size, neonatal morbidity and infant mortality (Paper I) and child growth and growth faltering (Paper II) are presented in Table 4.
METHODS

Table 4. Method of measurement of main outcome variables and covariates

<table>
<thead>
<tr>
<th>Outcome/covariate</th>
<th>Method of measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Evaluation of birth size, neonatal morbidity and infant mortality</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Outcomes:</strong></td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td>A portable electronic scale with the newborn completely naked within 48 hours of life with an accuracy of 10 g. The measurements were taken in triplicate, and the means recorded.</td>
</tr>
<tr>
<td>Birth length</td>
<td>A locally produced wooden measuring board within 48 hours of life with an accuracy of 0.1 cm with the child in supine position. The measurements were taken in triplicate, and the means recorded.</td>
</tr>
<tr>
<td>Neonatal morbidity</td>
<td>Daily data on symptoms of fever, if the baby looked weak or ill, hypothermia (“cold temperature”), diarrhoea, vomiting, breathing difficulties, or seizures (“convulsion”) after birth until 20 days.</td>
</tr>
<tr>
<td>Infant mortality</td>
<td>Data of infant death after birth until 1 year of life.</td>
</tr>
<tr>
<td><strong>Covariates:</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy weight</td>
<td>Weight of the mother before pregnancy with cloths as light as possible with an accuracy of 0.1 kg using an electronic scale.</td>
</tr>
<tr>
<td>Weight gain</td>
<td>Weight gain during pregnancy in kg.</td>
</tr>
<tr>
<td>Parity</td>
<td>Number of times a women has giving birth.</td>
</tr>
<tr>
<td>Maternal height</td>
<td>Height of the mother measured using a portable stadiometer with an accuracy of 0.1 cm.</td>
</tr>
<tr>
<td><strong>Evaluation of growth and growth faltering:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Outcome:</strong></td>
<td></td>
</tr>
<tr>
<td>Growth</td>
<td>Weight-for-age z-score (WAZ), height-for-age z-score (HAZ), and weight-for-height z-score (WHZ) at 0, 3, 6, 9, 12, 18, and 24 months.</td>
</tr>
<tr>
<td>Growth faltering</td>
<td>The downward crossing across two of the 5th, 15th, 25th, 50th, 75th, 85th, or 95th percentiles lines in the individual growth chart or if either WAZ or HAZ or WHZ are &lt;-2 SD in the WHO 2007 growth standard.</td>
</tr>
<tr>
<td><strong>Covariates:</strong></td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td>See above.</td>
</tr>
<tr>
<td>Birth length</td>
<td>See above.</td>
</tr>
<tr>
<td>Duration of exclusive breastfeeding</td>
<td>Number of months when a baby only received breast milk.</td>
</tr>
<tr>
<td>Duration of diarrhoea</td>
<td>Number of days a child passes three or more liquid or semi-liquid stools during 14 days prior to the visit.</td>
</tr>
<tr>
<td>Duration of respiratory illnesses</td>
<td>Number of days a child suffering from a runny nose or cough, with or without fever during 14 days prior to the visit.</td>
</tr>
</tbody>
</table>
METHODS

Data management
A system of quality control of the information collected was running in order to ensure good quality of data. Field and laboratory data were collected on pre-coded forms that were subject to a system of editing before data entry. Field supervisors monitored the quality and completeness of the data collection, and conducted a series of data checks in the field. The major steps included listing of all households, sampling of surveillance households, initial enumeration and baseline survey, data editing and printing of household record books, and finally updating of the household record books. This process was initiated every 90 days. The cycle of data collection, editing in the fields, data entry, identification of errors and logical inconsistencies, ensured that soon after the end of cycle the files were available for routine surveillance analyses for further analyses as part of research project.

A household registration system (HRS) was developed for surveillance activities including data entry, data cleaning, basic data analyses and reporting. The HRS system provided checking for errors such as mismatching data types, out of range values, incorrect identifiers and out of range dates. These allowed the data clerks to identify the possibly logical inconsistencies encountered during data entry and to return the forms to the field staff for corrections. In addition, the dSurvey software was used for data entry and data cleaning for each specific research project (Nurdiati, 2001).

Imputations for missing values of length (16%) were done by assuming a linear growth pattern; for example, we imputed missing length data at 9 months of age as: (length at 8 mo + length at 10 mo)/2.

Data analyses
Anthropometric data were transformed to z-scores using Epi Info, and then imported to SPSS for statistical analysis. Statistical significance was set at P<0.05 and two-sided hypothesis tests were used. Differences in basic characteristics of the subjects between supplementation groups for dichotomous data or proportions were tested using the chi-square test and analysis of variance (ANOVA) respectively.

The differences in birth weight and birth length by supplementation groups were analysed using analysis of covariance (ANCOVA) after adjustments with potential covariates (maternal height, pre-pregnancy weight, weight gain during pregnancy, and parity), included in the analysis if there was no collinearity. Collinearity was indicated if the values of tolerance inflation factors were <0.05 and <0.5 respectively. Factors related to infant mortality (death of infant under 1 years of age) were analysed by using bivariate logistic regression and multivariable logistic regression for factors with a P value <0.20; the odds ratio (R) and 95%
confidence interval (CI) were calculated. ANOVA was done to test the differences in the prevalence of morbidity between supplementation groups.

Two-way ANOVA was used to test the main effects and interaction of vitamin A or zinc supplementation on WAZ, HAZ and WHS at 0, 3, 6, 9, 12, 18 and 24 months, as well as the absolute and relative weight and length growth rates from birth until 24 months of age. ANCOVA was subsequently used to adjust for possible confounders and effect modifiers of prenatal supplementation on postnatal child growth. To identify determinants of time to first growth faltering Cox’s proportional hazard function was used and the hazard ratio (HR) and 95% CI were calculated.
Results

Main findings

Paper I showed that prenatal zinc or vitamin A supplementation
• Alone but not together improved birth length but had no effect on birth weight
• Had no effect on the neonatal morbidity and the infant mortality

Paper II showed that prenatal vitamin A supplementation
• Had a small but significant effect on postnatal growth of children’ length until 18 months of age
• Had no effect on other anthropometric measures
• Did not reduce the prevalence of growth faltering

Impact of prenatal vitamin A and zinc supplementation on birth weight and birth length

Prenatal zinc and vitamin A did not improve birth weight but improved the birth length (Table 5). Infants born to mothers who were prenatally supplemented with zinc and vitamin A were 0.3 cm and 0.2 cm longer than placebo (P=0.04). Overall, mean birth length of infants from zinc or vitamin A were 48.8 cm and 48.7 cm respectively, whereas the mean of birth length in the placebo group was 48.5 cm. This difference remained after adjustment for potential covariates (maternal height, pre-pregnancy weight, weight gain during pregnancy, and parity) (P=0.04).

Table 5. Impact of prenatal zinc and vitamin A supplementation on mean birth weight (BW) (kg) and birth length (BL) (cm) by sex.

<table>
<thead>
<tr>
<th>Supplementation</th>
<th>Girls BW</th>
<th>Girls BL</th>
<th>Boys BW</th>
<th>Boys BL</th>
<th>Total BW</th>
<th>Total BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>3.09</td>
<td>48.7</td>
<td>3.23</td>
<td>49.0</td>
<td>3.16</td>
<td>48.8</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>3.06</td>
<td>48.6</td>
<td>3.10</td>
<td>48.8</td>
<td>3.08</td>
<td>48.7</td>
</tr>
<tr>
<td>Zinc + vitamin A</td>
<td>3.09</td>
<td>48.5</td>
<td>3.11</td>
<td>48.4</td>
<td>3.10</td>
<td>48.5</td>
</tr>
<tr>
<td>Placebo</td>
<td>3.03</td>
<td>48.3</td>
<td>3.14</td>
<td>48.6</td>
<td>3.09</td>
<td>48.5</td>
</tr>
</tbody>
</table>

The covariates may have similar effects on the birth length, however, the statistical analysis showed no signs of collinearity among the covariates. There was no difference in birth length between mothers supplemented with vitamin A + zinc as compared with placebo. Maternal nutritional status and sex were thought to modify the effect of prenatal micronutrient supplementation on birth size. However, the stratification according to maternal nutritional status (cut-off point of BMI=18.5 kg/m²) and sex did not reveal any significant differences (P> 0.05).
The prevalence of LBW did not differ by supplementation groups (vitamin A=9.3%, zinc=9.5%, vitamin A + zinc=10.8%, and placebo=9.1%, P= 0.80).

**Impact of prenatal vitamin A and zinc supplementation on the neonatal morbidity and infant mortality**

The prevalence of neonatal illnesses within 20 days postpartum according to supplementation groups varied. We concluded that supplementation with zinc alone, in combination with vitamin A, or vitamin A alone had no effect on illness symptoms (Figure 7).

The Pronak study provides data of child mortality up to one year old or even more. Out of the 1956 infants, 18 died before 28 days of age (neonatal mortality=9.2/1000 live births), whereas 36 died before 1 year of age (infant mortality=18/1000 live births). Prenatal supplementation did not have any effect on neonatal or infant deaths (Table 3, Paper I). After adjustment for potential covariates SGA (OR=3.3; 95% CI: 1.04-11), being a boy (OR=2.4; 95% CI: 1.01-3.6), and unimproved drinking water (OR=2.4; 95% CI: 1.01-5.6) significantly increased the risk for infant deaths. On the other hand being primipara (OR=0.30; 95% CI: 0.11-0.81) and grand multipara (OR=0.07; 95% CI: 0.01-0.62) decreased the risk for infant death compared to parity of 2 to 4.
Figure 7. Prevalence of illness symptoms within 20 days of life by supplementation group.
Impact of prenatal vitamin A and zinc supplementation on child growth and growth faltering

Change in WAZ

Change in WAZ, HAZ, and WHZ are shown in Figure 8. The thesis used a factorial design that was able to test the interaction between zinc and vitamin A. Although vitamin A seemed to increase WAZ more than the other groups, two-factor ANOVA and ANCOVA testing the main effects and interaction of prenatal supplementation with vitamin A or zinc resulted in a significant main effect of vitamin A on WAZ (P=0.04) and interaction between zinc and vitamin A only at 6 months of age (P=0.048). However, this effect disappeared after adjusting for birth weight and length, duration of breastfeeding, diarrhoea and respiratory illness (P= 0.67) (Paper II, Table 2).

Change in HAZ

Similar to the change in WAZ, vitamin A looked like had a better effect on HAZ compared to other groups. However, two-factor ANOVA identified a significant main effect of zinc at 6 months (P=0.002) and significant negative interaction between vitamin A and zinc at 9, 12 and 18 months of age (P= 0.03; P= 0.04; and P= 0.02, respectively) (Paper II, Table 2), but after controlling for covariates, the difference in HAZ at 3 months became significant (P= 0.03) and the differences at 9 (P=0.04) and 18 months of age (P=0.04) remained statistically significant. The absolute differences between the vitamin A only and vitamin A + zinc groups at 3 and 9 months were 0.34 SD and 0.37 SD, respectively, and the absolute difference between the vitamin A only and zinc only groups at 18 months was 0.31 SD.

Change in WHZ

Finally, for WHZ, two-factor ANOVA did show a significant main effect of vitamin A (P= 0.04) on WHZ at 6 months, but no significant interaction effects of prenatal vitamin A and zinc supplementation. There was no effect of supplementation on the growth rate during any age period for weight, height or weight for height (data not shown). Figure 8 depicts the change in WHZ according to supplementation groups at different age.
Figure 8. The effects of prenatal zinc and vitamin A supplementation on change in WAZ, HAZ, and WHZ at 0, 3, 6, 9, 12, 18, and 24 months of age.
RESULTS

**Growth faltering**

![Figure 9](image)

**Figure 9.** Prevalence of growth faltering up to 2 years of age, defined as either crossing two or more percentile lines (solid lines) or growth <-2 SD below the reference population (broken lines) for weight for age (A) and height for age (B).

Figure 9 shows that growth faltering was more prevalent when defined as downward crossing of two or more major percentile lines compared with growth <-2 SD. Using either method, the prevalence of growth faltering increased with age (Figures 9 A and B). The majority of the children had crossed two or more major percentile lines by 9 months of age.

To assess the effects of risk factors on growth faltering up to two years of age, prenatal zinc and vitamin A and other risk factors were included in statistical analysis. Prenatal supplementation did not affect the risk of growth faltering. Birth length was the only covariate to be significantly associated with this risk in the present study. An increase in birth length of 1 cm reduced the risk of growth faltering by 9% (HR=0.910; 95% CI 0.831–0.997) (Paper II, Table 3).
Discussion

This thesis shows that prenatal zinc and vitamin A given to pregnant women with high risk of micronutrient deficiencies has an impact on birth length, but not birth weight. When supplementing these women with a daily dose of 20 mg zinc during pregnancy birth length increased by 0.3 cm compared to placebo, whereas supplementation with vitamin A increased birth length by 0.2 cm. Supplementation with a combination of zinc and vitamin A did not affect birth length. There was no effect of either supplement on birth weight and the intervention did not affect the prevalence of LBW, neonatal morbidity up to 20 days of life or infant mortality up to one year of age (Paper I). Only prenatal vitamin A supplementation affected postnatal growth. The children in the vitamin A group were taller than the children in the zinc and zinc and vitamin A groups, but not different from the placebo group. The supplementation did not have any effect on any other anthropometric measure, on the rate of growth or on the prevalence of growth faltering (Paper II). These results indicate that supplementing pregnant women with zinc and vitamin A have small and inconsistent effects on birth size and postnatal child growth.

Impact of prenatal vitamin A supplementation on birth weight

An important goal of prenatal micronutrient supplementation is to improve birth weight and decrease the prevalence of LBW. LBW is related to several poor health outcomes later in life (Onwuanaku et al, 2011; Mackay et al, 2011, Calkins & Devaskar, 2011). In this thesis prenatal supplementation with vitamin A and zinc did not increase birth weight or reduce the prevalence of LBW. This lack of effect should be compared to other studies. Meta-analyses have shown inconsistent effects of prenatal micronutrient on birth weight (Shah & Ohlsson, 2009; Kawai et al, 2011; Fall et al, 2009; Gebreselassie & Gashe, 2011). Prenatal supplementation using multiple micronutrients such as UNIMMAP increased birth weight ranging from 23 g (95% CI: 8.3-36 g) (Fall et al, 2009) to 54 g (95% CI: 36-72 g) (Shah & Ohlsson, 2009). A difference in the studies behind these meta-analyses from this thesis is the composition of supplements, where the studies finding an effect on birth weight used supplements that contained 15 vitamins and minerals, whereas the supplements used in this thesis contained only zinc and vitamin A. Other important factors, i.e. the studies being performed in developing or low-income settings on mothers with sub-optimal nutritional status and when the mothers were recruited in terms of gestational age were comparable. A meta-analysis looking at only prenatal zinc supplementation did not find any positive effects on birth weight (Gebreselassie & Gashe, 2011). Two studies that evaluated
the impact of multiple micronutrient supplementations during pregnancy have been done in Indonesia (The Supplementation with Multiple Micronutrients Intervention Trial (SUMMIT) Study Group, 2008; Sunawang et al, 2009). Neither of the studies showed significant effects on birth weight. One can speculated that pregnant women living in developing or low-income setting suffer from multiple micronutrient deficiencies, and that supplementation with only zinc and vitamin A was insufficient to have an effect on the growing foetus.

**Impact of prenatal vitamin A and zinc supplementation on birth length**

This thesis showed that prenatal vitamin A and zinc improved birth length (Paper I). Gupta et al (2007) reported that infants born to mothers prenatally supplemented with multiple micronutrients were 0.80 cm (95% CI: 0.03–1.57 cm) longer compared with placebo. The Indian study used 29 different micronutrients including vitamin A and zinc; the supplementation period started between gestational weeks 24-32, and recruited only women with a baseline BMI <18.5 kg/m². Other individual studies showed no effects of maternal zinc supplementation on birth length (Caulfield et al, 1999; Goldenberg et al, 1995; Merialdi et al, 2004). This thesis and the study by Gupta et al (2007) involved pregnant women from developing countries with similarities in nutritional status and gestational age at recruitment, but differed in the composition of supplements. A meta-analysis concluded that there was no significant effect of prenatal micronutrient supplementation on birth length in any of the individual studies (range, -0.2 cm to + 0.7 cm), and there was no significant effect in the meta-analysis (pooled estimate, +0.06 cm; p=0.20) (Fall et al, 2009).

The diverging results of prenatal zinc supplementation on birth length between this thesis and other studies are difficult to interpret. The basic characteristics of this thesis and other studies were similar with the exception of the study by Goldenberg et al (1995), which recruited pregnant women with a better nutritional status, and assumed higher zinc intakes and zinc levels at baseline. One possible explanation to the diverging results across studies is the greater variation in birth length measurements, indicating that the measurement of birth length is more complicated than measuring birth weight.

The different effects of prenatal micronutrient supplementation on birth weight and birth length should be interpreted with caution. Since zinc influences metabolic functions, the synthesis of nucleic acids and proteins, cellular growth, and morphogenesis (King, 2000), both zinc deficiency and supplementation will affect birth weight and birth length. Theoretically, several of these factors may influence intrauterine growth of weight and length. One study evaluated the impact of prenatal zinc supplementation on femur length (Merialdi et al, 2004). Although
the supplementation significantly improved femur length with mean difference of 0.1 mm to 0.7 mm compared to placebo, the birth weight and birth length were similar in both groups. The researchers argued that it is unlikely that the effects of maternal zinc on foetal femoral growth would be sufficient to affect birth length in such a way that it will be detectable with standard neonatal anthropometric assessment.

Studies on vitamin A supplementation during pregnancy reported no effect on birth weight. Panth et al (1990) reported that the means of birth weight of infants born to mother who prenatally supplemented with 1800 µg vitamin A per day >12 weeks, 8-10 weeks, and non-supplemented were 2.86 kg, 2.70 kg, and 2.68 kg respectively; however the differences in birth weight were not significant. This finding indicates a homeostatic mechanism to maintain the transfer of vitamin A to the foetus. Furthermore, vitamin A supplementation given postnatally, only minimally benefited for infant growth with severe vitamin A deficiency (Dibley & Jeacocke, 2001). The prenatal vitamin A (30 mg β-carotene + 5000 IU pre-formed vitamin A) supplementation given to HIV-1 infected pregnant women did not affect the prevalence of LBW, preterm, and SGA (Fawzi et al, 1998). The researchers argued that in such women the absorption of vitamin A was poor, therefore a higher requirements of vitamin A was needed. Coutsoudis et al (1999) reported that vitamin A (5000 IU retinyl palmitate and 30 mg β-carotene) given to pregnant women between 28 and 32 weeks gestation, and a dose of 200,000 IU of retinyl palmitate at delivery could reduce preterm delivery among HIV infected South African women by 6%. The benefit of prenatal vitamin A in this study was thought as a counteraction of vitamin A on anaemia during gestation, which increased the risk of preterm delivery. It is likely that prenatal vitamin A supplementation has different effects on gestation and birth weight in different populations (Dibley & Jeacocke, 2001). Another possible cause is due to difference in maternal plasma concentrations. Masters et al (2007) showed a correlation between maternal retinol or carotenoids and birth weight and head circumference of the infants. Infants whose mothers had plasma retinol below median weighed 125.9 g less and had 0.31 cm smaller head circumference than did infants whose mothers had high plasma retinol. There was no evidence of the relationship between maternal plasma vitamin A and birth length. The mechanism, by which prenatal supplementation influences birth outcomes are not well understood. The researchers hypothesized that vitamin A plays an important role in cell proliferation and has antioxidant activity, which might protect tissues by reacting with oxygen free radicals. Reactive oxygen species and oxidative stress are related with poor foetal growth in animal studies.
Impact of prenatal vitamin A and zinc supplementation on neonatal morbidity

In this thesis we found no effects of prenatal vitamin A or zinc supplementation on neonatal morbidity, i.e. during the first 28 days post-partum. The effect of prenatal micronutrient supplementation on early neonatal morbidity was also reported in a study from rural Nepal (Christian et al, 2008). Prenatal micronutrient supplementation had no effect on 10-day morbidity after birth. The risk of getting sepsis, birth asphyxia, acute lower respiratory infection, and hypothermia were similar among supplementation groups. The Nepalese study used similar methods to collect morbidity data as did we in the thesis. The lack effects of prenatal micronutrient supplementation on neonatal morbidity in study by Christian and coworkers (2008) may be explained by the fact that all groups received vitamin A, a potent anti-infective agent. The thesis included vitamin A only in two groups, but again we could show no effect (Paper I). Also an earlier study failed to show an impact of maternal low dose of vitamin A supplementation on foetal loss and early infant mortality (Katz et al, 2000). Osendarp et al (2001) reported that maternal zinc supplementation during pregnancy resulted in reductions in acute diarrhoea [risk ratio (RR) 0.84 95% CI 0.72-0.98], dysentery (RR 0.36 95% CI 0.25-0.84) and impetigo (RR 0.53 95% CI 0.34-0.82) among LBW infants in Bangladesh. Iannotti et al (2010) reported that prenatal zinc supplementation reduced the likelihood the infants experiencing diarrhoeal episodes of acute diarrhoea lasting longer than seven days [odds ratio (OR) 0.66 95% CI 0.43-0.99] in Peru. The different effect of prenatal zinc supplementation on diarrhoea between the studies from Bangladesh and Peru and the thesis are that study in Bangladesh analysed the effects of supplementation only in LBW infants at six months of age, and study in Peru evaluated the effect of supplementation up to one year of age, whereas this thesis evaluated the effect of supplementation on neonatal morbidity. Zinc has an important role in the immune system and theoretically prenatal zinc supplementation will improve foetal immune status as well (Shankar & Prasad, 1998). However, previous studies have not been able to show the benefit of prenatal zinc supplementation on perinatal or neonatal mortality (Kawai et al, 2011; Ronsmans et al, 2009), which is similar to the thesis.

Impact of prenatal supplementation on infant mortality

Infant mortality was a major outcome in this thesis. However, we could not show that prenatal vitamin A or zinc supplementation reduced the number of infants dying before one year of age (Paper I). Studies evaluating effects of prenatal multiple micronutrient supplementations on infant mortality have been scarce.
In a Nepalese study, Christian et al (2003) found a non-significant higher survival up to one year of age among those given folic acid with or without added iron or iron + zinc compared to a control (vitamin A) group. The researchers reasoned that the failure of the multiple micronutrient supplements to reduce infant mortality was maybe related to the disproportionately higher rate of high birth weight in the multiple-micronutrient group (RR=1.7; 95% CI: 1.1-2.7). When prenatal multiple micronutrient supplements decreased low birth and decreased infant mortality on one side, a higher birth weight was associated with an increased risk of birth asphyxia (RR=1.5; 95% CI: 1.04-2.1) an important cause of neonatal death on the other side. Although this thesis and the study by Christian et al used different types of supplements, the similar effects of prenatal micronutrient supplementation in both studies indicate may two aspects. First, prenatal micronutrient supplementations do not directly influence infant mortality. Second, although birth weight is commonly considered an indicator of infant health and survival, infant mortality may not always be mediated through increased birth weight.

**Impact of prenatal vitamin A and zinc supplementation on child growth and growth faltering**

In Paper II we show that prenatal supplementation with vitamin A had a small but significant effect on length growth during infancy. Few studies have evaluated the effects of prenatal micronutrient supplementation on postnatal child growth. In a study by Vaidya et al (2008), which evaluated the effects of prenatal supplementation with either a multiple micronutrient or an iron + folic acid supplement on growth until 2.5 years of age showed that weight, head, chest and mid-arm circumference, and triceps skin folds remained higher in the multiple micronutrients group, but with no effect on height. The minor effects of prenatal vitamin A and/or zinc supplementation on growth in the present study indicate that intrauterine growth is influenced by multiple factors and complex mechanisms. Animal studies indicate that in the progeny, maternal vitamin A deficiency has been related to LBW, indicating IUGR, and increased morbidity, poor growth or stunting until 6 months of age (Tielsch et al, 2008). From animal studies it has been hypothesised that vitamin A stimulates growth by a direct role in cell replication (Zile et al, 1979). In rodents, moderate vitamin A deficiency during pregnancy has been reported to reduce lung weight and muscle development in the offspring (Downie et al, 2005). Zinc is essential for the activity of over 300 enzymes involved in processes such as mitosis, DNA synthesis, and gene activation and expression. Some 82% of pregnant women worldwide are likely to have inadequate zinc intake (Osendarp et al, 2003). Animal studies have shown that mean birth weight, birth length, and tail length was lower in litters from iron or
zinc-deficient dams (Shahbazi et al, 2009). Also, postnatal zinc supplementation to zinc-deficient children has been shown to improve growth (Olsen, 2006).

Interactions between vitamin A and zinc may have decreased the effects of prenatal supplementation on growth in the thesis. Children of mothers supplemented with the combined vitamin A and zinc supplement were shorter than those who were supplemented with vitamin A only at certain ages. We have no measure of either vitamin A or zinc status of the mothers after supplementation, so we cannot say whether this interaction was evident in the mothers as well (Paper II). Interactions between vitamin A and zinc during pregnancy have been shown in animal studies. One study showed an increase in hepatic vitamin A concentrations in foetuses where the dams had been fed decreasing amounts of zinc, reflecting impaired mobilization of vitamin A. Another study, of pregnant rhesus monkeys, demonstrated that above a certain threshold of plasma zinc, vitamin A transport was not dependent on plasma zinc concentration, but that below the threshold, vitamin A release and transport from the liver was strongly influenced by plasma zinc concentrations. In humans, very few studies have shown interactions between vitamin A and zinc. One study indicated a weak and non-significant correlation between liver zinc and vitamin A content in foetuses and stillborn infants and infants who died in the first 4 months of life (Christian & West Jr., 1998). These studies shed little light on our findings, as the women were supplemented with both vitamin A and zinc and still their progeny grew less well. Therefore, the conflicting results of this study should be interpreted with caution, requiring biological explanations unavailable at this time; they may also indicate chance findings.

In this thesis using downward crossing of two or more major percentile lines as the marker for growth faltering, a substantial proportion of children had signs of faltering in weight and height before 6 months of age, and the majority of children were faltering by 9 months of age (Paper II). Stunting and wasting, among children under 5 years of age are associated with significant morbidity and mortality throughout life (Checkley et al, 2008; Shrimpton et al, 2001). In this thesis, the prevalence of growth faltering was lower if weight or height z-scores <-2 SD were used. Therefore, crossing major percentile lines may be an earlier sign of growth faltering than reaching z-scores <-2 SD and may offer opportunities for earlier intervention to reverse the faltering growth trend.

Prenatal micronutrient supplementation from the public health perspectives

Policy makers should prioritize the fight against preventable causes of LBW since it has severe short, medium, and long-term adverse effects on the health during childhood and in adult life. Preterm LBW infants have short-term consequences
such as higher mortality and morbidity rates compared to term LBW (Onwuanaku et al, 2011), medium term consequences including infant and childhood morbidity, poor postnatal growth and development and impaired immune function (Christian, 2010; Mackay et al, 2011), and long-term consequences such as obesity, dyslipidemia, hypertension, coronary heart disease, type 2 diabetes mellitus, stroke, kidney failure, liver failure, lung abnormalities, reduced bone mass, Alzheimer’s disease, depression, anxiety, schizophrenia, and cancer (Calkins & Devaskar, 2011; Skilton et al, 2011). These short, medium, and long-term consequences of LBW produce a large burden of health problems and substantial costs for communities and nations where the prevalence of LBW is high. Prenatal supplementation with multiple micronutrient mixes may have an impact in increasing birth weight and reducing the prevalence LBW.

Another advantage of prenatal micronutrient supplementation from public health perspective is its simplicity. Although the strategy is simple, experiences with the low adherence to prenatal iron supplementation indicate that it needs adequate program support, sufficient delivery of services, and patient factors are anticipated, including adverse effects from the supplements and misunderstanding of the supplement schedule (Gross et al, 2006). Zeng et al (2009) reported a high adherence of prenatal multiple micronutrients in China. They found that the mean adherence among pregnant women taking folic acid, iron-folic acid, and multiple micronutrients were 92%, 92% and 93%, respectively. A study in Mali showed a comparable high adherence to the prenatal multiple micronutrient supplements of 95%, whereas the adherence of the iron and folic supplement was 92% (Aguayo et al, 2005). This high adherence was related to how policy makers organized the program, such as that the supplements were distributed directly to the women, in other words their access to supplements was guaranteed, the health workers were trained and supervised, the home visitors provided consistent and easily understandable information and counselling, and the women were encouraged to participate in ANC through payment to cover the costs of such care. Ensuring the supply of micronutrients, adequate training and supervision of health workers, and attendance to antenatal care visits are needed in order to enhance supplement usage. The policy makers, health providers, and home visitors might explore the common barriers and common motivators among consistent and inconsistent users as an input for the program (Zeng et al, 2009; Aguayo et al, 2005). Tessema et al (2009) reported that among minority women in USA, common barriers were prenatal supplement qualities, adverse effects, and poor communication from health care providers about the benefits of use. Common motivators among consistent users included social network reinforcement of daily intake and fear of adverse effects to the foetus if prenatal supplements were not taken. Common barriers among inconsistent
users included scepticism towards the efficacy and necessity of prenatal supplements and the health care provider assenting to non-adherence.

Moreover, prenatal micronutrient supplementation is compatible. Overall, 78% of pregnant women reported multivitamin use, compared with 47% of women who were not pregnant (Sullivan et al, 2009). Several observations, such as in Pakistan indicated that prenatal micronutrient supplementation was acceptable to pregnant women due to low adverse effects such as vomiting and abdominal discomfort (Bhutta et al, 2009); whereas a study in China found that only 2.8% of women withdrew because of self-reported side effects of folic acid, 3.9% because of side effects of iron-folic acid, and 4.2% because of side effects of multiple nutrients (Zeng et al, 2009). Costs of the program of multiple micronutrient supple- mentations in community also were reported to be low. Zeng reported that only 22% of the cost (US$8.80 per pregnancy or US$0.05 per day based on the mean consumption of 165 supplements in pregnancy) was for supplements, including purchase of the capsules and transportation (Zeng et al, 2009); whereas a study in Peru showed the annual cost of the multiple micronutrient supple- mentation per community member was US$1.51 and the cost-effectiveness ratio was US$0.12 per 1% of prevented anaemia per community member (Lechtig et al, 2006).

A small effect of prenatal zinc and vitamin A on birth length, but no effects on birth weight, neonatal morbidity, infant mortality and child growth indicate that prenatal supplementation with these micronutrients only cannot be recom- mended. The use of multiple micronutrients given to pregnant women improved birth weight according to previous studies. Dalmiya et al (2009) underlined the concern about the risk of early neonatal mortality associated with the use of multiple micronutrients given to pregnant women although not statistically significant. They encouraged government should provide multiple micronutrient supplements instead of iron-folic acid as is normal practice among women in countries where the trials were conducted. The international agencies, non- governmental organizations, and donors have to support this action when solid evidence is available and policy exists.

**Methodological considerations**

This thesis has strength and limitation. Major strengths of this thesis include the targeting of prenatal supplementation to pregnant women with presumably a high prevalence of micronutrient deficiencies using a strong study design, and starting early in pregnancy aiming to optimize the effects of supplementation on foetal growth. This thesis showed adequate sample size, minimal loss of follow up (7%), and good compliance (70%). Finally, covariates that might modify the effects of the intervention were taken into account for in the statistical analysis.
DISCUSSION

Limitation of this thesis is lack of access to important data, such as biochemical markers, dietary intakes during pregnancy, and accuracy of exact postnatal time when birth size were measured. The lack of biochemical markers data, e.g. serum concentrations of relevant micronutrients of the participants either at baseline or at delivery limit our knowledge on the true prevalence of micronutrient deficiencies in these women, the chance to explore if certain subgroups, i.e. those with a particular micronutrient deficiency responded differently than any other subgroup, and to see whether the intervention was sufficient to have an impact on the zinc and/or vitamin A status of the participating women. Further, we lack data on the dietary intakes during pregnancy, a factor that might have affected the micronutrient status and thus possibly the outcomes in our study. Also, the exact postnatal time when measuring birth weight was not noted, and the time limit of measuring weight within 48 hours of birth might have been too long when trying to compare small between group differences in birth weight.

Although the study design and the randomization process would have taken care of large between-group differences, these limitations affect the possible explanation we may give when trying to elucidate the restricted effects of the intervention.

In the analyses of the postnatal effects of the supplementation we could only include 343 children, i.e. 17% of the total cohort of 1956 live births. The reason behind this was the civil disruption experienced in Indonesia following the Asian economic crisis. The disruption decreased staff size and data collection activities; therefore large numbers of participants could not be followed-up.

When measuring birth weight and birth length, timing is crucial. In the thesis, the goal was to measure birth size within 48 hours post partum, since some mothers gave birth at home and there may be some time between the birth and the time when the fieldworkers could reach the mothers. However, this lag time may have been too generous when measuring outcomes such as birth weight. Moreover, measuring length or height is more complicated than weight. This practical problem may have influenced the validity of length or height data.

Collecting morbidity data using recall may result in measurement bias. In the thesis, fieldworkers collect data of neonatal morbidity daily by home visits. This method minimizes recall bias since symptoms recalled from the mothers were familiar to them.

Implication for future studies

The small but positive effects of prenatal vitamin A supplementation on child growth in the thesis add to a body of conflicting results concerning maternal supplementation studies, suggesting that more research is required. Future studies on the impact of prenatal supplementation should clarify the mechanisms
and effects of actions and interactions of micronutrients, alone or in combination, on postnatal child growth, bringing deeper understanding to the public health benefits of prenatal supplementation and better tools to prevent childhood growth faltering from the earliest possible date. The long-term effects of prenatal micronutrient supplementation on growth are also interesting evidence to be elucidated. Translational research aiming to understand the mechanism of different effect of micronutrient supplementation on intrauterine growth might be important. Finally, animal studies could provide information the impact of micronutrients on the growth of different tissues and show effects of supplements during different periods of foetal growth and development.
Conclusion

This thesis concludes that 2400 RE vitamin A or 20 mg zinc given daily to pregnant women who were already receiving 60 mg iron and 250 µg folic acid during pregnancy had small effects on birth length, but had no effects on child growth up to two years old compared to placebo.

- Zinc or vitamin A supplementation alone but not together during pregnancy improved birth length compared to placebo, but had no effect on birth weight.
- Zinc or vitamin A or combination of zinc and vitamin A supplementation during pregnancy had no effect on neonatal morbidity.
- Zinc or vitamin A or combination of zinc and vitamin A supplementation during pregnancy had no effect on infant mortality.
- Zinc or vitamin A or combination of zinc and vitamin A supplementation during pregnancy had no effect on child growth up to two years old.
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