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MICRONUTRIENTS AND PREGNANCY OUTCOME: A REVIEW OF THE LITERATURE

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ABSTRACT

There is considerable evidence supporting the role of various micronutrients in determining pregnancy outcomes such as low birth weight and prematurity. While some nutrients have been studied extensively (e.g.:calcium, zinc) much less is known about others (e.g.:vitamin B-complex). Methodologically, there is considerable variation in the types of study design ranging from cross-sectional studies to well designed randomized placebo-controlled trials (RCTs) most of which were conducted in developing countries among women who were not deficient and therefore less likely to benefit from the interventions. Inadequate sample size was another problem in many studies. The key conclusions of this review of the literature are 1) there is strong evidence primarily from developed countries that zinc, calcium and magnesium supplementation could improve birth weight, prematurity and hypertension particularly in high risk groups 2) folic acid can prevent neural tube defects, but the evidence on whether iron/and or folic acid supplements reduce the prevalence of low birth weight (LBW), prematurity and maternal mortality is limited 3) Severe iodine deficiency results in increase pregnancy loss, mental retardation and cretinism, but less is known for other outcomes especially in the case of marginal iodine deficiency 4) vitamin A supplements might reduce maternal mortality and perhaps LBW 5) vitamin C may play a role in the etiology of prematurity and 6) the B-complex vitamins, copper and selenium may have a role but very few experimental studies have been conducted to date. Most importantly, although there is evidence of interactions among several micronutrients at the metabolic level, very little is known about the significance of interactions for pregnancy outcomes. There is a need for well designed RCT's that will examine the role of selected nutrient interactions and multi vitamin-mineral supplements in improving pregnancy outcomes especially in developing countries where nutrient deficiencies do not occur in isolation and multiple micronutrients deficiencies are common.

Key Words: Micronutrients, Pregnancy, Vitamin B-Complex, Folic Acid, Low birth weight.

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Pregnancy has long been recognized as a vulnerable period during which the health and well-being of both the unborn child and mother are at risk. Adverse pregnancy outcomes can be divided into those relating to the infant and to the mother. Infant related outcomes include fetal loss namely miscarriages, abortions and still births and, in the case of live births, both premature delivery (<37 weeks) and/or intra-uterine growth retardation (IUGR). Low birth weight (LBW) defined as <2.5 kg is the better known outcome and can be due to preterm delivery and to IUGR. Other adverse outcomes at birth include mental retardation and birth defects such as neural tube defects. In the case of maternal outcomes, both maternal mortality and morbidity are extremely important in many developing countries. Nearly 600,000 women die each year in developing countries from complications of pregnancy, childbirth and unsafe abortion, and many of these deaths are preventable (1). Other outcomes of public health significance which are often directly linked to maternal mortality include hemorrhage, anemia, obstructed labor and hypertension.

Of the various adverse pregnancy outcomes, LBW and maternal mortality are better known and are major public health problems in developing countries. Nearly 1 out of every 5 infants born in developing countries are LBW, compared to only 6% in developed countries. These estimates vary significantly by region: Latin America and the Caribbean, 11%; East Asia and the Pacific, 11%; Sub Saharan Africa, 16%; and South Asia, including India, Bangladesh, Pakistan and Nepal, 34% (2). While prematurity accounts for about 55% of low birth weight in most developed countries, only 25% of LBW infants are born premature in developing countries. In other words, the majority of LBW infants in developing countries are born full term (3). IUGR as well as other LBW infants are at higher risk of morbidity and death than newborns of normal weight. They are also at risk of growth retardation during the postnatal period with possible long term adverse effects on physical and mental performance (4). Clearly interventions aimed at improving nutrition and growth during pregnancy could play a key role in preventing lifelong physical and mental deficiencies.

The major causes of many adverse pregnancy outcomes especially in developing countries include the poor nutritional status of the mother, hypertension, malaria and other infections during pregnancy(5). Adequate prenatal and obstetric care with counseling especially high risk pregnancies could prevent many of these adverse outcomes. Ante-natal care often includes a package of health and nutrition interventions such as immunization, iron supplements, screening for complications etc., in many settings (6). The nutritional status of the woman (both prior to conception and during pregnancy) is increasingly being recognized as an important underlying determinant of pregnancy outcomes. Kramer (7) in his classic review and meta-analysis of studies on the determinants of LBW concluded that maternal nutritional factors both prior to pregnancy (pre-pregnant height and weight) and during pregnancy (low caloric intake/ inadequate weight gain) accounted for more than 50% of the levels of intrauterine growth retardation seen in rural areas of developing countries. Several studies have established the importance of adequate energy intake during pregnancy in relation to outcomes such as LBW (8, 9). New evidence suggests that micronutrient deficiencies may be an important cause of LBW.

Energy and protein intakes and average pregnancy weight gains are much lower in developing countries compared to industrialized countries (10). Micronutrient malnutrition is also common in pregnancy. For example, more than half the pregnant women in South Asia and Sub-Saharan Africa are anemic, most of which is due to iron deficiency (11). Other reported deficiencies include iodine, zinc, vitamin A, and vitamin B-complex including thiamine, riboflavin, folic acid and B_{12}. Conditions such as night-blindness as a result of vitamin A deficiency (VAD) and endemic goiter due to iodine deficiency during pregnancy are not uncommon in many parts of the world (12-14). Although nationally representative data on the prevalence of various micronutrient deficiencies in women of reproductive age are lacking, recent dietary surveys, especially from the Collaborative Nutrition Research Projects (CRSP) in Mexico, Kenya and Egypt, have shown that multiple micronutrient

deficiencies, rather than single deficiencies, are common and, that low dietary intakes and poor bioavailability of micronutrients account for the high prevalence of this complex of multiple deficiencies (15-18).

Micronutrients could play an important role at various stages of a woman's life. For example, micronutrient malnutrition during early childhood can adversely affect early childhood morbidity and growth in young girls which in turn determine her attained nutritional status i.e. height, prepregnancy weight and BMI(19). Severe deficiency diseases such as rickets due to a lack of vitamin D in early childhood can result in pelvic deformation which in turn increases the risk for obstructed labor and maternal mortality during childbirth. The role of micronutrients during adolescence, the other period of rapid growth and increased nutrient requirements, has received attention only recently. For example, the provision of iron supplements during adolescence may reduce the risk of low iron stores and anemia before pregnancy. Similarly, ensuring adequate intakes of calcium, phosphorus and magnesium could promote bone mineralization during the early adult years and thereby reduce the risk of pre-eclampsia during pregnancy. Last but not least, the role of folic acid in the prevention of neural tube defects serves as an excellent example of the importance of peri-conceptual nutritional status (20-22).

The main objective of this paper is to review the literature on the role of *micronutrient deficiencies during pregnancy* in causing poor pregnancy outcomes. This paper will focus primarily on the following outcomes: LBW, pre-term births, premature rupture of membranes (PROM), abortions and pre-eclampsia. Although maternal mortality and morbidity are extremely important outcomes, they have not been addressed in detail due to limited availability of research studies. The evidence linking various single micronutrients and these pregnancy outcomes is reviewed first, followed by a discussion of nutrient interactions and multiple micronutrient deficiencies. Although cross-sectional studies are taken into account, greater emphasis is given in the conclusions to evidence from well designed prospective studies and double blind randomized controlled trials (RCT's). The methodological issues that were considered in evaluating the studies are briefly described in the following section.

1. Methodological Issues:

The studies included in this review were identified using a combination of techniques, namely extensive bibliographic database searches (primarily Medline) and secondary references from published studies and review articles. Key words such as pregnancy, birth weight, and prematurity were used in combination with the several vitamins and minerals.

1.1 Study Design:

A variety of study designs, ranging from cross sectional to randomized placebo-controlled trials were used in the studies reviewed. Many of the cross-sectional studies often compared the levels of selected micronutrients in the mother at delivery and/or in cord blood and examined the association with pregnancy outcomes such as LBW. Although these studies are useful, their major limitation is that no inferences of causality can be made primarily due to lack of temporality and the non-experimental design (23). The lack of temporality can be addressed by conducting prospective follow up studies, in which micronutrient status is measured at various stages of pregnancy and linked to subsequent pregnancy outcomes. However, the absence of a control group, the role of possible confounding factors which are not always measured, and the fact that nutrient intakes are often highly correlated make it difficult to identify true causal relationships. Nevertheless, these studies provide valuable information and contribute to the accumulation of necessary knowledge which is required before the next step, i.e. an experimental trial in which a particular nutrient or combination of nutrients is provided to one group and compared to a control group. However, the design of these trials is very important. For example,

the lack of a placebo for the control group can contribute to biases due to differences in measurement as well as in the subject's response to treatment. Comparability of the experimental and control groups on factors such as access to prenatal care that might affect the outcomes is essential to avoid biases due to confounding. *The double-blind randomized placebo-controlled intervention trial* is the design of choice that addresses many of these concerns (23).

1.2. Sample Size Considerations:

A major reason for failing to detect significant effects (Type II error) even in well designed RCT's is inadequate sample size. Sample size areas should be estimated carefully prior to the study. The sample size required is a function of I) the expected difference in the outcome measures, ii) the standard deviation of the outcome measure, iii) level of significance specified (e.g. p=0.05), iv) whether one tailed or two tailed test of significance is specified, and v) power desired. A two tailed test is preferred if one could expect either benefits or adverse effects from the intervention, and at least a power of 80% is usually recommended for most hypotheses testing. Using methods described by Cohen et al (24) the smallest detectable difference between control and experimental groups in birth weight in studies with varying sample sizes is shown in Table 1 for both one and two tailed tests; these estimates assume a significance level of p=0.05, power =80% and a standard deviation of 450 g in birth weight as found in Guatemala (8). A sample of at least 200 per group would be required to detect a difference of about 100 g which represents 0.22 S.D. To detect even smaller differences, say 50 g, even larger sample sizes are needed. The choice of the expected effect depends on the setting. For example, a difference of 100 g is of biological significance and can be expected in many developing country settings (8, 9). The effect size which is presented in standard deviation units can also be used for other outcomes.

TABLE 1: Smallest Detectable Difference Between Control and Experimental Groups in Birth Weight in Studies with Varying Sample Sizes[1]

Difference in Birthweight (Δ) between groups (g)	Effect size (Δ/S.D.)[2]	Sample Size/Group	
		One Tailed	Two Tailed
45	0.1	1237	1571
90	0.2	310	393
135	0.3	138	175
180	0.4	78	99
225	0.5	50	64
270	0.6	35	45
315	0.7	26	33
360	0.8	20	26
450	1.0	13	17

[1] Assumptions are power = 0.8 and level of significance p=0.05;
[2] Standard Deviation S.D. = 450 g

1.3. Sample Selection:

The choice of the study sample is extremely important and can affect both the internal and external validity of a study (23). Often, the failure to detect any benefits of intervention may be related to the fact that the populations in which they were conducted did not really need it, i.e. they were not deficient. For example, in studies from developed countries, the micronutrient status based on biochemical indicators such as serum retinol levels, may not be low enough to see an effect. Absence of measures of response to treatment also make it difficult to interpret the efficacy of the treatment.

1.4. Single nutrients versus multiple nutrients:

Most studies tend to examine a single nutrient at a time which is useful in understanding the underlying mechanisms. However, the limitation of this approach is that it does not account for nutrient interactions and a single nutrient may not be adequate to improve pregnancy outcomes in settings where multiple nutrient deficiencies are more common (developing countries), since some other nutrient may become the limiting nutrient.

1.5. Ethical Considerations:

The use of a placebo for the control group can pose ethical problems if the nutrient in question is provided or recommended as part of standard treatment based on other outcomes. For example, the World Health Organization (WHO) recommends that most governments provide iron supplements to treat anemia; such treatment is also thought to prevent LBW. Thus, it would be unethical to conduct placebo controlled trials for iron in most settings.

2. Single micronutrients:

2.1. Vitamins

2.1.1. Vitamin A:

Vitamin A is essential for normal fetal growth and development. There is strong evidence to support that vitamin A is essential for maintaining normality during the later stages of pregnancy, when fetal organs undergo extensive cell proliferation and development (25). Animal studies have shown that VAD during gestation lead to a higher rates of fetal resorption, organ malformations and abortions. Fetal and placental growth are also compromised when maternal vitamin A status is poor (19). Although there is strong morphological and biochemical evidence of these effects in the animal model, there are few well-designed studies that evaluate the effect of vitamin A deficiency on fetal growth and other pregnancy outcomes in humans.

Observational Studies:

Several cross-sectional retrospective studies have documented an association between low vitamin A concentrations in umbilical cord blood and fetal growth retardation (low birth weight, reduced length) (27-31). The absence of similar associations in some earlier

studies may have been due to inadequate power (small sample sizes) and/or little evidence of vitamin A deficiency in the populations studied (32,33). In a case control study of 39 premature and 32 term infants, Shenai et al (30) found that mean plasma vitamin A concentration, retinol binding protein (RBP) concentration and vit A:RBP molar ratio was significantly lower in infants born prematurely than in infants of term gestation. Shah et al (34) found a significant association between fetal growth (fetal weight expressed as a % of expected weight) and fetal liver retinol concentration. Maternal serum retinol was also strongly associated with fetal liver retinol concentrations ($p<0.01$) independent of gestational age. More recently, a trend of increasing birth weight with increasing concentrations of vitamin A in umbilical cord blood of Guatemalan infants was reported (28). In particular, a strong association was observed among IUGR cases with adequate ponderal index ($r=0.14$, $p=0.004$) which is more common in many developing countries. Finally, in a study from England, vitamin A concentrations in cord blood were associated with birth weight, head circumference, length and gestational age, even after controlling for confounding factors (27). It has also been shown in some studies that serum retinol and β-carotene levels are reduced among women with pre-eclampsia (35-38). In a study from Nigeria, Ziari et al (38) found that the mean serum retinol and β-carotene levels for both the pre-eclamptic and eclamptic groups of pregnant women were significantly lower compared to those of healthy age-matched control women in the third trimester ($p=.004$). A major problem with many of these studies, however, is the inability to decipher the nature of the association, i.e. does pre-eclampsia lead to reduced levels of vitamin A or vice-versa ? Poor vitamin A status has also been associated an increased susceptibility to infection with HIV during pregnancy (39).

Although mild to moderate vitamin A deficiency and frequent infections that would affect vitamin A utilization are common during pregnancy in many parts of the world (12, 40), there are few well designed prospective studies examining its impact on pregnancy outcomes including birth weight. Two prospective observational studies conducted in the U.S. examined the association between vitamin A status during mid-gestation with later birth outcomes, while controlling for several potential confounding factors (41, 42). While the earlier study with the smaller sample size ($n=138$) found a significant association between total carotene and birth weight, the later study ($n=423$) failed to detect any association between blood levels of retinol and total carotenes and birth weight. This may have been due to the fact that the women were not vitamin A deficient.

Experimental studies:

Four experimental studies that examined the benefits of vitamin A supplementation during pregnancy in Asian women who were at risk for VAD were identified (40, 43-45). Although these studies were placebo controlled and there were significant improvements in serum retinol at delivery, the observed differences in birth weight were not statistically significant, primarily due to the small sample size ($n<50$). For example, in the study by JayaRao et al (43) from India, mean birth weight was 2.76 ± 0.09 kg for the babies born to women who received 3000 μg of vitamin A from about 18 weeks of gestation ($n=22$) compared to 2.61 ± 0.07 for those born to the women in the control group who received a placebo containing 300 mg calcium lactate ($n=18$). In the study by Howell et al (44) among Asian women of Indo-Pakistani origin ($n=29$) living in England, vitamin A supplementation (8000 IU) began only from 28 weeks of gestation which may have been too late. In another study from India (45), 450 women received routine iron supplements

(60 mg ferrous sulphate) and in addition the experimental group were allocated during various stages of gestation (12-24 weeks) to varying durations (6-10 and >12 weeks) of vitamin A supplementation (6000 IU/day). Significant improvements in both maternal and newborn vitamin A status were observed following vitamin A supplementation. Although delivery data were available for only 50 women, the mean birth weight of the infants born to the women who received at least 12 weeks of vitamin A supplementation was 2.86 kg compared to 2.68 kg for the control group. The limitations of this study are I) small sample sizes and poor statistical power, ii) it is not clear whether the allocation to treatment groups was random, iii) the lack of blinding since no placebo was used for vitamin A and iv) extremely high losses to follow up. In a larger well designed RCT conducted in Indonesia, the benefits of providing vitamin A supplements in addition to routine iron supplements during this pregnancy was examined (40) and the combined intervention was most effective in reducing the prevalence of anemia compared to iron or vitamin A only. Unfortunately no data on other pregnancy outcomes were reported. In summary, there is clearly a need for better designed intervention trials to examine whether improving vitamin A status leads to improved pregnancy outcomes especially in populations where vitamin A deficiency is common. One such trial is currently underway in Nepal and a variety of outcomes, including maternal mortality, birth weight and pre term births are being monitored. Preliminary results indicate a 44% reduction in maternal mortality in the groups receiving a weekly supplement of 7000 RE of Vitamin A (either as β-carotene or retinyl palmitate) compared to the women who received the placebo during pregnancy. Interestingly, the reduction in the β-carotene group (RR = 0.5; 95% CI: 0.4, 0.96) was greater than that seen in the retinyl palmitate group (RR = 0.52; 95% CI: 0.31, 0.8) suggesting a potential role for antioxidants. Also, most of these deaths were prevented during the early postpartum period (12 weeks) (46). These results will be useful in making recommendations to improve vitamin A status of pregnant women using either low doses of vitamin A or other food based interventions during pregnancy in populations where VAD is a significant public health problem.

The fear of the teratogenic effects of high doses of vitamin A during early pregnancy is an important concern, especially with the use of high dose supplements in populations with normal vitamin A status (47). Martha et al (48) in a retrospective case-control study of over 6000 women, found that women who took vitamin A supplements alone or in combination with multivitamins had an increased risk of having infants with cranio-facial and cardiac malformations. However, some of the limitations of this study are recall bias and inadequate information on dietary intake and dose of vitamins. Rothman et al (49) in a prospective cohort study of 22,748 pregnant women in the US found a 3-5 fold increase in the risk of defects associated with cranial neural crest tissue among women with high vitamin A intakes. Specifically, the adjusted odds ratio was 3.5 (95% CI = 1.7, 7.3) for babies born to mothers who consumed more than 15,000 IU of preformed vitamin A per day, from food and supplements compared to those born to mothers who consumed 5000 IU or less. In the case of vitamin A from supplements alone (multivitamins and retinol), the relationship was even stronger (OR=4.8 95% CI = 2.2,10.5) for mothers who consumed more than 10,000 IU of vitamin A per day compared to those who consumed 5000 IU or less per day. Retinol intake was assessed through a detailed diet, frequency of ingestion of specific food items, history and use of vitamin supplements during the first trimester. However, the teratogenic effects of vitamin A may be related to the form of vitamin A ingested. Buss et al (50) demonstrated in a cross over experimental study, where ten healthy volunteers were given different forms of retinol (5 doses of retinol as palmitate;

50 and 150 mg retinol as an oral supplement, 50 and 150 mg as fried calf liver and 3, 9 or 30 mg by IM injection) that the peak plasma concentration of all -trans retinoic acid which is the principal teratogenic metabolite of retinol was up to 20 times higher in the case of supplements compared to the same dose in liver. This finding led the authors to conclude that liver and supplements are not of equivalent teratogenic potential and that the consumption of cooked liver by pregnant women would not pose significant additional teratogenic risks. More recently, Mills et al (1997) (51) demonstrated in a geographically based case-control study in the U.S. that periconceptual consumption of vitamin A at doses > 8000 IU or > 10,000 IU per day was not associated with an increased risk of malformations in general, cranial neural crest defects or neural tube crests. These findings support the idea that moderate doses of vitamin A do not pose any additional risk.

2.1.2. Vitamin B complex

2.1.2.1. Thiamine:

Thiamine deficiency is not uncommon in many developing countries especially during pregnancy when the requirements are substantially greater than normal (52-54). It is generally recommended that daily allowances for thiamine be increased by 0.5 mg during pregnancy and lactation (55). Thiamine deficiency in pregnant rats has been shown to cause severe IUGR in the progeny as indicated by reduced body weight, placenta weight and liver weight (56, 57). More importantly, thiamine deficiency could impair brain development since thiamine dependent enzymes play an important role in cellular energy metabolism necessary for lipid and nucleotide synthesis in the developing brain (58). Heinze and Weber (59) measured the concentration of thiamine in blood cells and plasma in pregnant women between 28 and 39 weeks of pregnancy and found that women who gave birth to infants with severe IUGR had significantly lower blood cell thiamine content compared to normal. In normal pregnancies, blood cell thiamine values dropped from 230 nmol/l to 170 nmol/l in the 28th to the 39th week of gestation. In contrast, cases with IUGR, thiamine levels were only around 140 nmol/l in the 30th week of gestation and decreased slightly to 130 nmol/l in the 39th week of gestation. However, the thiamine values in plasma were not significantly different in normal pregnancies and pregnancies with IUGR in this study. Also, we do not know if the authors controlled for other confounding. No experimental studies were identified and more well designed studies, especially in deficient populations are needed to understand the effects of thiamine deficiency during pregnancy.

2.1.2.2. Vitamin B_6:

Vitamin B_6 is an important co-enzyme involved in protein metabolism and plays a major role in the development of the central nervous system (60). Animal studies have shown that maternal vitamin B_6 deficiency can have harmful effects on the fetus (61-65). However, the relationship between vitamin B_6 status and pregnancy outcomes has not been well studied in humans. In two observational studies conducted in the mid 70's, vitamin B_6 status was not associated with birth outcomes including birth weight (66,67). However later studies reported significantly lower Apgar scores for infants born to mothers with lower dietary intakes and lower serum levels of vitamin B_6 compared to those with adequate intakes (68,69). While only serum levels at delivery were used in the study by Roepke and Kirksey (68), a better design was used by Schuster et al (69) who had assessed vitamin B_6 status at two stages of pregnancy i.e. at the initial prenatal visit and at 30 weeks

of pregnancy. Mean birth weight was 131 g less among those with poor vitamin B_6 status, but this difference was not statistically significant, probably due to limited sample size. Kaminetzky et al (70) also reported that vitamin B_6 levels were low in teenage mothers who gave birth to LBW infants. In a double blind RCT, Schuster et al (71) found that infants of mothers taking 7.5 mg or more supplemental pyridoxine had a significantly higher one minute Apgar score compared to infants of mothers who received less than 5 mg. However, there was no difference in five minute Apgar scores. Although maternal and cord plasma pyridoxal phosphate (PLP) levels were significantly higher ($p<0.005$) when supplementation was 7.5 mg or more, no significant differences were seen between the groups in birth weight and length as well as placental weight. Stratification by plasma levels of PLP or controlling for other factors such as race, parity, alcohol or tobacco use and prepregnancy weight did not alter these findings. Although this is a well designed study with information on both dietary and biochemical measures and other confounding variables, the sample size (n=50) was clearly inadequate to detect important differences in outcomes such as birth weight (see Table 1).

2.1.2.3. Folic Acid:

Folic acid deficiency may be associated with a variety of pregnancy complications including spontaneous abortions, bleeding, abruptio placentae, preeclampsia, limited fetal growth and congenital malformations. The active form of folic acid is the coenzyme tetrahydrofolate which is required for the synthesis of pyrimidines and purines and the synthesis of Deoxy ribonucleic acid (DNA). In rapidly dividing cells, folate deficiency may lead to alterations in DNA synthesis and chromosomal aberrations (72, 73). These important functions of folic acid clearly indicate its role in fetal growth and development. Although poor folate status either preconceptual or during pregnancy can be causally linked with the occurrence of neural tube defects (20, 22) there is less evidence on the effects on other pregnancy outcomes such as LBW and prematurity. Several investigators have reported mixed findings on the association of poor folate status measured post-partum in mothers and infants with fetal growth retardation (23, 74, 75). Mukherjee et al (76) found that high plasma folate levels were also associated with the occurrence of complications ($p<.008$) and with fetal distress ($p< .002$). Abortion was more frequently associated ($p<.02$) with folate levels in the lowest quartile. However, both the limited small sample sizes and nature of the data do not permit any causal interpretations. The findings of the prospective follow up studies and experimental trials examining the relationship between folate status and pregnancy outcomes is described in the following sections. A summary of the study design and results of the experimental trials is presented in Table 2.

Observational Studies:

Several non-experimental studies have shown that folate status is associated with the occurrence of neural tube defects (77-79). However, studies have examined other outcomes. In a longitudinal study of 116 pregnant women, Pietrzik et al (80) found that the serum folate levels were significantly lower ($p< .05$) among the 37 women who aborted, than the controls. There were no statistically significant differences in cobalamin, zinc or iron levels in the two groups. In contrast, Neiger et al (81) in a prospective follow up study found that among pregnancies complicated by first trimester bleeding, a known

TABLE 2: Description of Experimental Trials of Folic Acid Supplementation And Pregnancy Outcomes

S. No.	Investigator (Yr)	Study Site	Nature of Intervention[1]: Experimental Group	Control Group	N	Wks Preg	Results[3]: BW	PM	Abortion
1.	Iyengar et al, 1975	India	500 µg FA+ 60 mg Fe	60 mg Fe	189	20-28	↑	nr	-
2.	Giles et al, 1971	Australia	5 mg FA+ 200 mg Fe	200 mg Fe	620	10-30	-	-	↑
3.	Rolschau, 1979	Denmark	5 mg FA + MVM	MVM without FA	36	21-25	↑	nr	nr
4.	Blot et al, 1981	France	350 mg FA+105 mg Fe	Fe	109	28	↑	↓	nr
5.	Czeizel et al, 1993	Hungary	MVM (incl 15 mg FA)	trace elements	4704	<0[2]	↓	-	-
6.	Kirke et al, 1992	Ireland	I) 0.36 mg FA ii) 0.36 mg FA + MVM	MVM without FA	354	<0[2]	-	nr	-

[1] FA = folic acid; Fe = iron; MV = multivitamins; MVM = multivitamin mineral; n = sample size
[2] Supplementation stopped at 12 weeks, whereas others were until delivery
[3] BW = birth weight; PM = prematurity; PIH = pregnancy induced hypertension; nr = not reported

risk factor of abortion, low folate status was not associated with an increased risk of pregnancy loss and adverse outcomes. Of the 151 women included in this study, 52 (34%) had low folate levels.

Five prospective follow up studies in which the relationship between folate status and fetal growth retardation was examined were identified (82-86), but three of them used the same population.(82-84) In a prospective follow up study of 295 African Americans, who are considered to be at increased risk for poor pregnancy outcomes in the US, infants born to women in the upper two quartiles of serum folate levels measured at 30 weeks of gestation had significantly higher mean birth weight ($p=.0003$) and lower prevalence of growth retardation ($p= .001$) compared to the rest even after controlling for maternal race, infant sex, smoking, previous LBW and body mass index (BMI) (82). It should be noted that all subjects were provided with 325 mg of folic acid supplementation, although compliance may have been poor. Interestingly, the psycho social profile of these women which was considered to be predictive of improved compliance did not alter the relationship between folate status and birth weight. Using the same study population, Tamura et al (83) reported that serum folate levels at 30 weeks, but not at 18 weeks of gestation was associated with improved birth weight and Apgar scores of the new born even after controlling for potential confounding factors. They also found no correlation between serum folate and serum zinc refuting the notion that folic acid has an adverse effect on maternal zinc nutriture and pregnancy outcome. However, since the supplement contained both iron and folic acid, it is difficult to attribute the results to folate status. The other major limitation is the absence of data on compliance. More recently, Neggers et al (84) analyzed the dietary intake data from the same study and found a small but significant positive association between maternal folate intake and birthweight after adjusting for the other potential confounders. These intakes were based on 24 hour recalls at 18 and 30 weeks of pregnancy. An increase of 47.6g in infant birthweight was found when maternal intake increased from the 10^{th} to the 90^{th} percentile. Frelut et al (85) also found a positive relationship between maternal erythrocyte folate in the third trimester and birth weight ($p<.02$). Maternal folate intake was also positively associated with newborn blood folate ($p<.05$). Finally, a recent prospective, longitudinal, observational study of women from Camden, NJ, found that both low dietary intakes of folate (≤ 240 µg/day) and lower concentrations of serum folate measured at 28 weeks of pregnancy were associated with an increased risk (two fold) of pre-term delivery and LBW even after controlling for maternal characteristics, energy intake and other correlated nutrients (86). These findings clearly suggest the role of folic acid in fetal growth and are consistent with data from the animal model (squirrel monkey) (87).

Experimental studies:

Several experimental trials that were conducted during the 1970's have conflicting results on the effect of folic acid supplements on prematurity and birth weight (88-95). While some of these studies suggest the potential for folic acid in improving birth weight, they need to be interpreted with caution due to problems related to study design, especially the biases introduced by the large losses to follow up (7). In contrast, the later RCT's provide better evidence for the role of folic acid in reducing the risks for neural tube defects (20-22). Unfortunately these studies were conducted primarily in developed countries and do not shed any light on the role of folic acid for other pregnancy outcomes such as LBW which is common in developing countries. Two early experimental studies,

conducted in India (88) and in South Africa (89), reported increased birth weight using a design which compared iron to iron-folate supplementation. However the large drop out rates in both these studies is a major drawback. In another RCT conducted in Denmark, 40 pregnant women between 21-25 weeks were matched with respect to parity, pre pregnant weight, tobacco, age and housing conditions and were allocated to two groups:(1) the experimental group received vitamins, 250 mg ferrofumarate and 5 mg folic acid and 2) the control group was given similar tablets without folic acid (90). The infants in the folic acid group were 12.7 % heavier than those in the control group ($p<.01$). The plasma folic acid was 105% higher ($p<.001$) and erythrocyte folic acid was 34% higher ($p<.001$) in the folic acid group as compared to the control group. There was a significant correlation between erythrocyte folic acid at birth and birth weight ($r=.53$, $p<.001$). Two other trials also found that folic acid status during pregnancy was positively related to gestational length (91,92). Blot et al (92) reported that mean gestational age was 40.7 weeks in the folic acid group compared to 39.9 weeks in the control group. In contrast, three studies conducted in developed countries (93-95) showed no benefits of folic acid supplements on fetal growth. For example, Giles et al (93) in a randomized placebo controlled intervention trial with 620 pregnant women in Australia failed to detect significant differences in birth weight or maternal hematocrit between the folic acid and the placebo group. However, these findings are difficult to interpret in the absence of information on the nature of the placebo, method of randomization, compliance, and other confounding variables such as maternal weight, BMI and dietary intakes. In summary, the data are conflicting regarding the effect of folic acid on fetal growth and the absence of good experimental studies that examine the benefits of folic acid in reducing the low birth weight, prematurity and other complications is a major drawback.

2.1.2.4. Vitamin B_{12}:

Vitamin B_{12} is an essential nutrient present only in animal products or in contaminated foods (fecal matter). It is involved in various metabolic functions and its deficiency can cause defective DNA synthesis, a reduced rate of cell multiplication and metabolism disorders which lead to megaloblastic anemia and other neurological abnormalities (96, 97). Diets in many developing countries often have little or no animal products and are therefore low in vitamin B_{12} intakes. Vitamin B_{12} deficiency has been reported in both pregnant and lactating women when the requirements are increased. However, there is limited evidence directly relating vitamin B_{12} status with adverse pregnancy outcomes such as LBW and prematurity (98). Shojania (99) reported that severe vitamin B_{12} deficiency during pregnancy may cause intra-uterine death. An earlier follow-up study by Temperly et al (100) found a significant correlation between prematurity and low initial serum vitamin B_{12} levels of pregnant women. Recently, in a cross sectional study among 188 French women, Frery et al (101) found that among smokers birth weight was significantly but negatively correlated with cord ($r=-0.42$, $p<0.05$) and maternal vitamin B_{12} ($r=-0.46$, $p<0.05$) measured at delivery. This relationship remained even after controlling for ethnicity and parity not with maternal vitamin B_{12} level. Although the reasons for this relationship is unclear, it may be related to the role of vitamin B_{12} on lipid metabolism specific to smokers (102,103).

2.1.3. Vitamin C:

Recent evidence suggest a role for vitamin C in pregnancy outcome especially premature rupture of membranes (PROM) which in turn may have an adverse effect on the duration of pregnancy. Weakening of the chorioamnion membrane has been observed in PROM, which may be attributed to low collagen content or proteolysis due to free radicals, both of which are affected by vitamin C (104-107). Originally, Wideman et al (108) had reported a correlation between low plasma ascorbate and an increase in PROM incidence. More recently, Casanueva et al (109) reported an association between low levels of vitamin C in white blood cells measured at delivery and premature rupture of membranes among Mexican women in a case-control cross-sectional study. Infections were also found more frequently in the PROM group when the women had low levels of vitamin C. Barrett et al (110) also found an association between PROM and amniotic fluid ascorbic acid level but not with serum ascorbic acid levels. Serum beta carotene levels were also lower in the PROM group. The two groups had comparable past obstetric history. Although these studies clearly suggest a role for vitamin C, a randomized intervention trial is needed.

2.1.4. Vitamin D:

Vitamin D plays an important role in calcium homeostasis and is essential for normal bone growth. In a retrospective study, Marya et al (114) compared pregnancy outcomes from random samples of 25 Indian women who had received 1,200 U vitamin D with 375 mg calcium daily in the third trimester, 20 women who had received 2 large doses of 600,000 IU Vitamin D in the 7^{th} and 8^{th} months of pregnancy, and 75 who did not take vitamin D supplements during pregnancy served as controls. There also significant improvements in serum calcium, proteins and inorganic phosphorus levels in the maternal and cord blood at delivery in both the supplemented groups. Unfortunately, it is not clear whether the groups were similar, and more importantly, this was not a prospective double blind randomized trial. A RCT was conducted in England, in which pregnant immigrant Asian women who are at increased risk for vitamin D deficiency were randomly allocated to received either 1000 IU of ergocalciferol (vitamin D) (n= 59) or placebo (n-67) during the last trimester of pregnancy (112). Maternal daily weight gain was significantly higher in the treatment group (63.3 g) compared to the controls (43.3 g), and almost twice as many infants in the control group (29%) were SGA compared to the treatment group (15%). The 10th weight percentile based on Gairdner's standards was the cutoff used to define SGA (113). Mean birth weight was about 120 g greater (3157 ± 61 g) in the treated group compared to the control group (3034 ± 64 g), but this difference was not statistically significant due to the small sample size. There were also no significant differences in the other anthropometric measurements such as crown-heel length, triceps skinfolds and head circumference of the infants at birth. Infants in the control group had larger fontanelle and lower plasma calcium levels suggesting impaired ossification of the skull. An earlier randomized controlled trial of vitamin D supplementation during the last trimester also failed to detect any effects on birth weights (114), but the sample size of only 50 women may have been limiting to detect important differences. Although maternal serum vitamin D levels improved, maternal and cord levels of calcium were not affected. Recently, Brunvand et al (115) in a cross sectional study of 30 Pakistani women with known vitamin D deficiency found a positive correlation between the level of ionized calcium in maternal serum and the crown-heel length of the infant ($r=0.65$, $p=.002$). The maternal serum parathyroid hormone was related inversely to the crown -heel length ($r=-0.47$, $p=0.01$).

These findings suggested that lack of calcium and not vitamin D deficiency had a negative effect on fetal growth. In summary, although not conclusive, studies to date suggest that vitamin D possibly affects fetal growth through an effect on calcium homeostasis.

2.1.5. Vitamin E:

Vitamin E is known to play a role in ensuring normal reproduction, and may have adverse effects. In the animal model, Martin and Hurley (116) showed that plasma vitamin E levels were significantly higher in the pups born to rats that received vitamin E supplements, but, resorption rates, litter size, weight, and survival of pups and fetal malformations were not affected by excess Vitamin E. Vitamin E deficiency can result in anemia and neurologic abnormalities (117), although little is known on the role of vitamin E during pregnancy in humans. Cross sectional studies have failed to demonstrate any significant association between birthweight and maternal plasma vitamin E concentrations at birth (27,118) except for one in which maternal serum vitamin E levels at delivery were significantly lower among women with low birthweight infants (119). In a longitudinal study in Nigeria, 100 pregnant women were followed up to study the association of vitamin E and pregnancy outcomes (120). All subjects were recruited by 12 weeks of gestation and venous blood samples were obtained monthly until delivery. They also received prophylactic iron, folic acid and antimalarials but no vitamin supplements. Although mean birth weight increased as cord plasma vitamin E level increased in both males and females, maternal vitamin E level was not associated with infant birth weight. No correlation was found between maternal and fetal plasma vitamin E concentrations at delivery. Surprisingly, the authors do not report the relationship between maternal vitamin E status during early pregnancy and birth weight. The role of other confounding factors were also not considered. In a recent cross-sectional study, serum vitamin E levels of the pre eclamptic and eclamptic women were 15 % and 30 % lower respectively than those of the corresponding controls ($p<.01$) (38). Uotila et al (1993) (121) also reported similar findings.

2. 2. Minerals

2.2.1. Calcium:

Calcium (Ca) is required for the development of the fetal skeleton but also plays an important role in neuromuscular function and blood coagulation. A deficiency of this nutrient can adversely affect pregnancy outcomes both by impairing growth and development of the fetus, as well as by altering membrane permeability and excitability and smooth muscle contractility which in turn could affect blood pressure as well as lead to premature uterine contractions and subsequent delivery.

Several randomized clinical trials using calcium supplementation have been conducted to date in which the role of calcium in reducing pregnancy induced hypertension (PIH) and other adverse pregnancy outcomes such as prematurity and LBW has been examined. In an early double blind RCT trial conducted in 52 pregnant women, a significant reduction of 4-5 mm in both systolic and diastolic blood pressure values was found compared to the placebo ($p< .05$). The treatment was either 1.5 g Ca per day ($n=25$) or a placebo ($n=27$) after the 26th week of pregnancy and all patients were given prenatal vitamins containing 200 mg Ca and 100 mg of Mg (122). Although the incidence of PIH was greater in the

placebo group (11.1%) compared to the treatment group (4%), these differences were not statistically significant which may have been due to the small sample size (n=52). Adjustments were made for race, baseline blood pressure, maternal age , maternal weight/ height ratio and weight during pregnancy. Subsequent studies with larger sample sizes have demonstrated the effects of calcium supplementation in reducing the risks of preeclampsia and PIH. In a recent meta analysis of 14 randomized controlled trials involving 2459 women, Bucher et al (123) estimated a reduction of 5.4 mm Hg (95% CI= -7.81 to -3.00, p<.001) and 3.44 mm of Hg (95% CI = -5.2 to -1.68, p< .001) in systolic and diastolic blood pressure respectively. The odds ratio for preeclampsia in women with calcium supplementation compared with placebo was 0.38 (95% CI: .22 to .65). Most of these studies used 1.5- 2.0 g of calcium supplementation daily. However, a recently published study with a large sample (n=4589) of nulliparous women who received either 2 g of elemental calcium or placebo beginning from 13-21 weeks of gestation until delivery has raised doubts on the benefits of Ca supplements in preventing pre-eclampsia (RR=0.94, 95% CI = 0.76, 1.16). No significant differences were found in the prevalence of PIH without pre-eclampsia (15.3% vs 17.3%), or all hypertensive disorders (22.2 % vs 24.6%) (124). These findings suggest that perhaps the benefits of Ca supplementation in reducing the risk of pre-eclampsia may be limited to only women at high risk.

Fewer studies have examined the effect of calcium supplementation on the incidence of pre term births and low birth weight. Villar et al (125) in a study of high risk adolescent pregnancies in the US, found that calcium supplementation reduced the incidence of pre-term delivery (7.4% vs 21.1%, P=.007); spontaneous labor and pre-term delivery (6.4% vs 17.9 %; p= .01) and low birth weight (9.6% vs 21.1%, p= .03). The infants in the calcium group were 189 gm heavier (p=.06) than in the placebo. However no effect was observed on the incidence of IUGR and PROM. Life table analysis demonstrated an overall shift to a higher gestational age in the calcium group compared with the placebo group (log-rank test, p=.02). This study was a RCT in which 190 pregnant women below 17 years, were randomly allocated to either calcium supplementation of 2 g/day or placebo. All women also received routine prenatal vitamins containing 200 mg Ca and 100 mg magnesium per day (125). The subjects were mostly primiparas who have an increased risk of preeclampsia. The two groups were similar on various baseline characteristics. Dietary intake of calcium during pregnancy (1200 mg) and compliance, during the study were also similar in both groups. The effect of potential confounders such as compliance, urinary tract infection and chlamydial infections were controlled for by stratification. In contrast, no significant differences in pre-term deliveries and birth weight were found in the more recent studies (124,126,127). Belizan et al (126) conducted a double blind RCT in Argentina with 1194 subjects who were randomly assigned to receive either 2 g of Ca or placebo daily from the 20th week of gestation. The rate of hypertensive disorders of pregnancy were significantly lower in the calcium group (9.8%) than in the placebo group (14.8%), especially after the 28th week of gestation. The lack of effects for the other outcomes may be explained by differences in the study population. In a recent meta-analyses using data from five randomized clinical trials (125-129),

Bucher et al (123) estimated that the odds ratio (95% CI) for pre-term delivery, IUGR, and cesarian section, were 0.69 (0.48-1.01), 0.77 (0.51-1.16) and 0.8 (0.6-1.07), respectively. The data for the individual and pooled estimates for the different outcomes namely PIH, prematurity and LBW are shown in Figures 1-3. These authors concluded that while calcium supplementation during pregnancy leads to an important reduction in

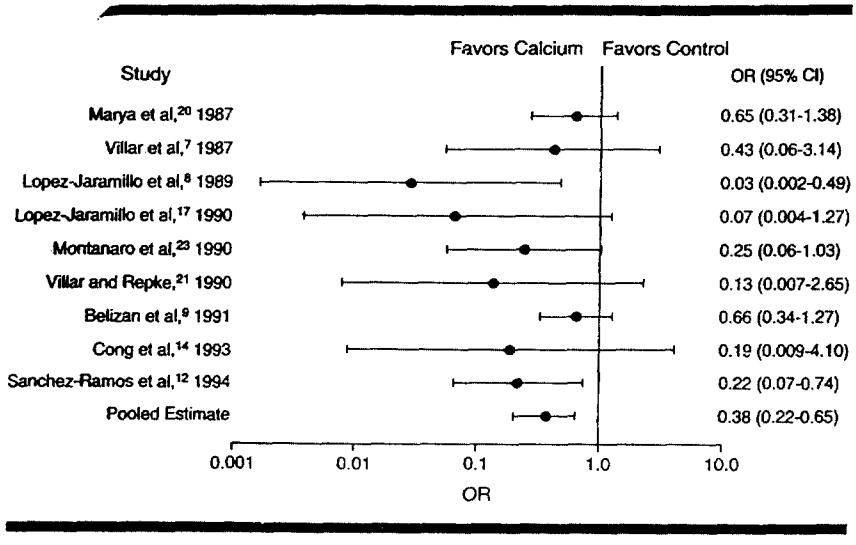

FIG 1: Calcium Supplementation in Pregnancy: Effect on Preeclampsia[1]

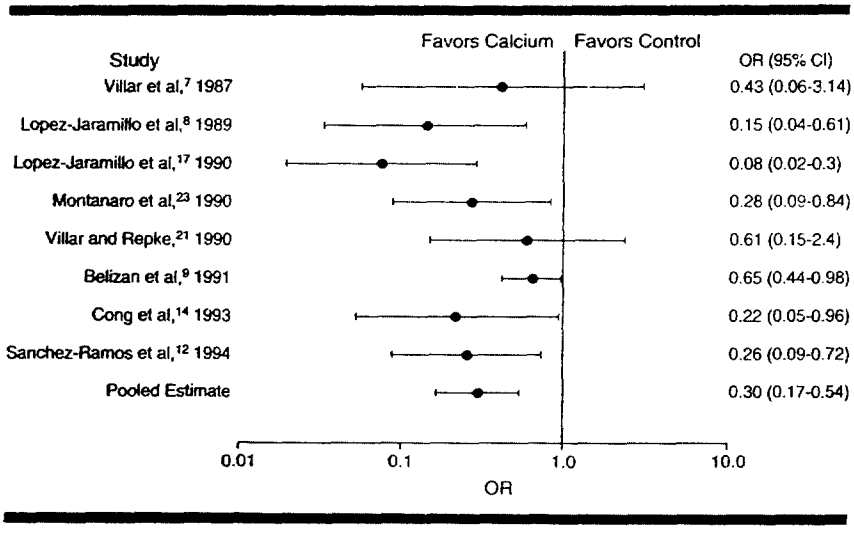

FIG 2: Calcium Supplementation in Pregnancy: Effect on Incidence of Hypertension[1]

1. From Bucher et al 1996 (112); The scale is logarithmic. OR indicates odds ratio; CI, confidence interval.

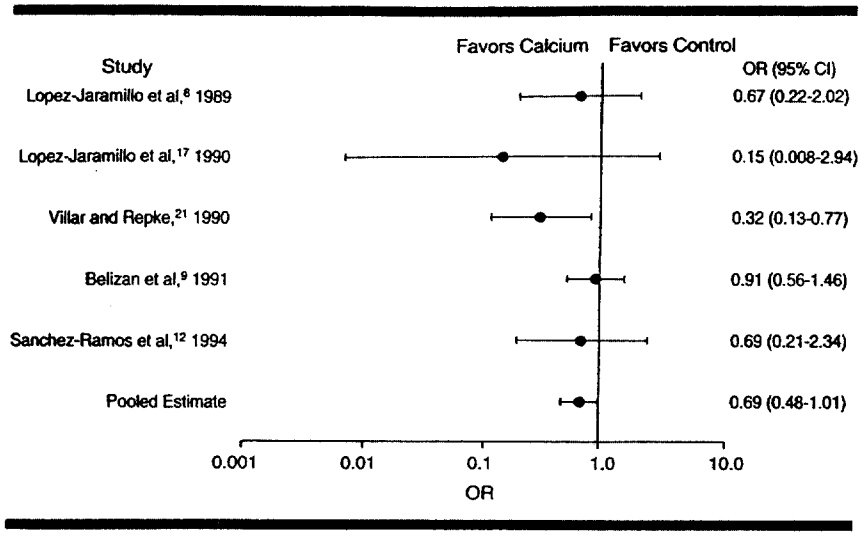

FIG 3: Calcium Supplementation in Pregnancy: Effect on Preterm Delivery[1]

1. From Bucher et al 1996 (112); The scale is logarithmic. OR indicates odds ratio; CI, confidence interval.

systolic and diastolic blood pressure and preeclampsia, the beneficial effect of calcium on major maternal and infant morbidity and mortality remains unestablished. Although, the most recently completed study suggests no benefits of calcium supplementation in reducing the number of pre-term deliveries, SGA births or fetal and neonatal deaths in normal pregnancies (124), it is important to recognize that few studies have been conducted in populations where Ca intakes are low, and therefore more likely to benefit from Ca supplements.

Belizan and Villar (130) originally proposed the relationship between Ca intakes and hypertension by pointing out that the incidence of pre-eclampsia was inversely proportional to dietary intakes of Ca worldwide. Purwar et al (131) demonstrated significant reductions in the incidence of gestational hypertension (RR = 0.28; 95% CI: 0.14; 0.59) and pre-eclampsia (RR = 0.13, 95% CI: 0.01, 0.64) in a RCT of Ca supplementation that was conducted in India where dietary intakes of Ca are very low (350 mg/day). The treatment group of nulliparous women (n=103) received 2g of calcium daily from 20 weeks of gestation till delivery, while the control group (n=93) received a placebo. Birthweight of infants in the Ca-supplemented group (2731 $\pm$ 278g) was significantly greater (p=0.016) than in the placebo group (2626 $\pm$ 309g). Although the decrease in the rates of LBW and prematurity in the supplemented group were not statistically significant, an increase of 1000 g in birthweight is of great public health significance in this population. This well-designed study in which the two groups were comparable on several baseline characteristics and compliance, provides strong evidence to support the benefits of Ca supplementation on maternal and infant outcomes in populations with low intakes.

2.2. 2. Iodine:

Iodine deficiency is now recognized as a major public health problem and as many as 800 million people live in iodine-deficient environments and are therefore at risk of the effects of iodine deficiency (14). Iodine is an essential component of the two thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) which are critical for normal growth and development starting from conception to about 2 years of age. The most important effects of iodine deficiency are goiter, hypothyroidism and cretinism. Anatomic studies show that thyroid hormone increase cell proliferation, synapse formation, dendritic proliferation and microtubular assembly, and, sustained hypothyroidism during these critical periods can lead to irreversible mental retardation (132). Fortunately, cretinism can be prevented by the correction of iodine deficiency before or during the first three months after conception. This can be done in a variety of ways including the administration of iodized oil either orally or using injections, or more commonly by the consumption of iodized salt. In a recent review of the published evidence, Delange (134) concluded that "*the administration of iodized oil before or during pregnancy prevents endemic cretinism and brain damage by correcting iodine deficiency and thyroid function in pregnant women, fetuses, neonates, infants and children*". Although biological tests examining thyroid function were not available, an early randomized placebo-controlled trial conducted in New Guinea demonstrated significant reductions in the prevalence of endemic cretinism following iodine supplementation (134, 135). The prevalence of cretins/1000 births was 1.7 among those who received iodinated oil before conception compared to 42.2 among those who received the saline placebo. These rates were 52.6 and 61.0 respectively among those who were injected after conception. Pretell et al (136) also provided similar evidence from Peru in the context of iodination program which did not follow a randomized controlled design. Although mean values were not significantly different, this study demonstrated a consistent tendency for lower birth weight, length and cephalic circumference among those children born to iodine deficient mothers compared to those who were treated with iodized oil.

The most detailed studies of iodized oil given during pregnancy have been conducted in Zaire (137-140), Algeria (141) and Malawi (139) in areas of severe iodine deficiency where endemic goiter is complicated by endemic cretinism. In a double blind RCT conducted in Zaire, pregnant women (n=983) were recruited at their first prenatal clinic visit and were allocated to receive either iodized oil (1 cc Lipiodol 1M, treated group) or placebo (control group) (138, 139). Iodine status based on biochemical (blood and urine samples) and clinical examinations was assessed at baseline for mothers and at delivery for both mothers and neonates. Both groups were similar at baseline (in terms of urinary iodine distributions), and the effect of the intervention in improving iodine status was well demonstrated. This study also showed that iodine supplementation during the last two trimesters of pregnancy was safe and prevented neonatal and infantile hypothyroidism during the first 2 years of life. The intervention had a positive impact on other important neonatal outcomes such as birth weight, infant mortality and psychomotor development. Although the increase in mean birth weight was not statistically significant for the overall group, birthweight was significantly greater ($p < 0.05$) for the children born to the subset of mothers who were iodine deficient at baseline (urinary iodine < 5 μg/dl). The mean birth weight were 2634 $\pm$ 52 g (n=112) versus 2837 $\pm$ 55 (n=98) for the neonates born to mothers who received the placebo and iodized oil respectively. Significant improvements

in infant mortality rates (167 versus 250/1000) were seen for the overall group, with greater benefits for those who were severely iodine deficient (RR=2.5) compared to those who were only mildly to moderately iodine deficient (RR=1.4). The mean development scores during the first 2 years of life was also significantly greater for the treated group (115 $\pm$ 3) compared to those in the control group (103 $\pm$ 4). A more recent study from Algeria (141) compared the benefits of oral administration of 0.5 ml of Lipiodol at various stages i.e. 1-3 months before conception, during the first month of pregnancy and during the third month of pregnancy within the context of an iodized oil prevention program. Untreated mothers served as controls. This study demonstrated significant reductions in the rates of prematurity, stillbirths and abortions in the treated groups compared to the untreated controls. Mean birth weight was similar in all the treated groups (3400 g), but was significantly greater than the untreated controls (3200 g). The major limitation of this study is that it was not a randomized placebo controlled trial. Although the two groups were similar on selected baseline characteristics, the role of other confounding factors cannot be overlooked. Finally the placebo controlled randomized study in Malawi in which 0.5 ml of iodized oil was given during the last trimester of pregnancy also demonstrated significant benefits (133). However, the results are preliminary and not completely available. The only study that failed to demonstrate any benefits of providing iodized oil, to date, was conducted in parts of Bhutan and India where severe iodine deficiency is common (142, 143). Based on the findings of low cord T_4 and elevated TSH levels in 10.4% of the neonates indicating neonatal biochemical hypothyroidism, the authors concluded that iodized oil therapy during pregnancy induced thyroid failure in the neonates and was not a recommended intervention. However, the validity of this interpretation has been questioned especially since the observed rates of neonatal biochemical hypothyroidism were not significantly different from that in study areas without an iodized oil program. The absence of urinary iodine values in mothers also make it difficult to infer whether the mothers were overloaded or not (135). Clearly ensuring adequate iodine status among women of reproductive age should receive high priority especially in iodine deficient areas of the world.

2.2.3. Iron:

Iron is an important micronutrient and is necessary for hemoglobin (Hb) synthesis and several other important functions in the body. Iron deficiency can result not only in reduced oxygen carrying capacity due to lowered hemoglobin levels, but can also affect immunity and growth and development. More than half the women of reproductive age in developing countries are anemic, primarily due to iron deficiency as a result of poor diets and increased requirements (11). The prevalence of iron deficiency anemia increases markedly during pregnancy as a result of the increased demands of maternal erythropoiesis and the unborn fetus. Thus, iron deficiency anemia during pregnancy has the potential for adverse outcomes both in the mother (for e.g: maternal mortality) and the newborn. A vast body of literature suggests maternal anemia has adverse effects on birth outcomes, particularly prematurity and perinatal mortality (144). However, the benefits of iron supplements on birth outcomes especially LBW remain unclear (145). Several studies have demonstrated the benefits of iron supplementation in reducing the prevalence of iron deficiency anemia and improving iron stores in pregnant women, but the data on the potential benefits for the fetus are far from conclusive (7, 55, 146-151). There is also surprisingly little evidence of anemia or depletion of iron stores based on serum ferritin levels in the infant of the iron depleted mother (152-154).

A serious weakness in many studies is the failure to take into account the normal hematological changes that occur in response to plasma volume expansion during pregnancy (144, 155). Even among iron sufficient women, hemoglobin levels fall during early pregnancy, reach a nadir in the second trimester of pregnancy and rise again to prepregnant levels by term (55, 156). The tendency to correlate pregnancy outcome with hemoglobin concentration measured late in pregnancy or at delivery and failure to control for energy intake and other nutritionally related confounders are some of the methodological flaws (7). For example, report conflicting findings on the relationship between anemia based on postpartum hemoglobin levels and birth weight were reported in two cross-sectional studies with small samples (n <200) from Bangladesh. Khan et al (157) found evidence of an inverse relationship but did not control for important confounding variables especially gestational age. In contrast, Hasin et al (158) found evidence of a positive relationship between hemoglobin levels at delivery and birth weight ($p < 0.05$), but this was not significant after controlling for factors such as socioeconomic status, education and sex and gestational age of the child. Similarly, no significant relationship between maternal hemoglobin and birth weight was found in a larger study from the Philippines using the same study design (159) . But, important factors such as smoking and gestational age were not accounted for. An interesting observation in this study was the increased placental weight among anaemic mothers ($p < 0.05$) which may be due to placental hypertrophy, a compensatory physiological response to ensure adequate oxygen supply to the fetus in anaemic mothers. The other major concern is that in many cases, only hemoglobin has been measured, and the absence of other measures of iron status (e.g.: serum ferritin, transferrin saturation) make it difficult to evaluate the role of iron. Anemia may result also due to other nutritional deficiencies e.g: vitamin B-12 and folate as well as non-nutritional factors. The results of the better designed prospective follow up studies and randomized controlled trials are discussed in the following sections.

Observational studies:

Several observational studies suggest that iron supplementation may reduce the prevalence of poor pregnancy outcomes such as pre-term births and low birth weight (LBW) only for severe iron deficiency anemia (152,160-163). High maternal hemoglobin or hematocrit during pregnancy have also been associated with poor birth outcomes (160, 161-164). Garn et al (160) reported that when a hemoglobin concentration below 10 g/dl was reached during any stage of pregnancy, the likelihood of low birth weight, pre-term birth and perinatal mortality increased. However, the risk of these unfavorable outcomes was greatly increased at both hematological extremes (hematocrit < 29% and > 39% or Hb <= 9 g/100 dl and >= 13 g/100 dl), even after controlling for the effects of maternal smoking, hypertension, maternal weight and other risk factors by stratified analysis. Higgins et al (165) examined the relationship between hematologic changes during pregnancy and pregnancy outcomes in a sample of 1112 Canadian pregnant women who attended a public prenatal clinic. All women received prenatal vitamin and mineral supplement containing vitamins A, B_{12}, C, D, thiamine, riboflavin, niacinamide, pyridoxine, folic acid, iron and calcium and also nutritional services. Although initial Hb was not related to infant birth weight, a significant inverse relationship was found between late antenatal Hb and infant birth weight ($p<.01$). Also, change in maternal Hb concentration during pregnancy was inversely associated with infant birth weight ($p<.025$); the greater the decrease in Hb, the higher the mean infant birth weight. No differences were seen in the low birth weight rates. The limitation of this study was that the effect of other

minerals and vitamins was not considered. Murphy et al (161) also found evidence of an U-shaped relationship between maternal hemoglobin levels during pregnancy and outcomes such as pre term births, LBW, and perinatal deaths. Mothers with intermediate values (10.4-13.2 g/dl) fared best. Although several confounding factors were not controlled for and hemoglobin levels were measured at different times of gestation, this relationship was observed across different strata of time of measurement of hemoglobin namely, less than 13, 13-19 and 20-24 weeks of pregnancy. There was also a significant relationship between PIH and booking Hb ($p<.001$) in both primiparas and multi paras. Lu et al (164) examined the relationship between maternal hematocrit at various stages of pregnancy and pre term delivery and fetal growth retardation in a large group of pregnant women (n=17,149) in Alabama who received iron and folate supplementation. Although lower hematocrits (<37%) before 20 weeks of gestation tended to be associated with an increased risk of both fetal growth retardation and pre-term delivery, these differences were not statistically significant after controlling for other risk factors. More importantly, hematocrit greater than 40% was associated with both fetal growth retardation and pre-term delivery even after adjusting for known confounders namely race, parity, age, smoking, hypertension and maternal height and weight. The strongest association (OR>2) between high hematocrit and both fetal growth retardation and pre term delivery occurred with hematocrit at or above 43% at 31-34 weeks gestation. The authors concluded that a hematocrit above 40% or more especially in late pregnancy should be considered a risk factor for poor pregnancy outcome and high hematocrit should generate more concern than low hematocrit. Although these findings are valid, it should be noted that the prevalence of anemia is low in these populations and the relationship may be different in developing countries where iron deficiency anemia is very common and access to antenatal care and compliance to iron therapy is poor. (11,166) For example, significant increases in the prevalence of LBW (65% vs 27%) have been reported among primigravida women detected with early pregnancy anemia (<8 g/dl) in a study from Papua New Guinea (163). In a large prospective study of pregnant women in Nepal where both anemia and low birth weight are common, Dreyfuss et al (167) reported a similar U-shaped between hemoglobin levels at various stages of pregnancy and outcomes such as birth weight and preterm delivery. Although iron deficiency is the major cause of anemia in this population, there were no additional measures of iron status i.e. serum ferritin.

In a prospective study of 800 pregnant women in the U.S., iron deficiency anemia was associated with a higher risk of pre-term delivery and low birth weight but not with small for gestation age births (168). The risk of pre-term delivery and LBW was 2.5 and 3 times greater in women with iron deficiency anemia respectively than in non anemic women even after controlling for several confounding factors. These relationships were seen with early pregnancy anemia and not with anemia later during pregnancy. In a follow up study of 157 Spanish women who received iron supplementation (160 mg/day elemental iron) during the second half of pregnancy, newborns whose mothers were anemic (Hb <110 g/L) in the third trimester had significantly lower red blood cell, hemoglobin and hematocrit values compared to those whose mothers were not anemic (Hb >= 110 g/L). However, there were no significant differences in serum ferritin and birth weight (169). In a recent study of 27 Turkish women who did not receive iron supplements during pregnancy, no significant associations were found between measures of iron status, i.e. hemoglobin and serum ferritin at 16 and 34 weeks of pregnancy and fetal outcomes such as infant's birth weight, gestational age and hemoglobin levels at birth and 43 months of age (170) Although serum ferritin declined during pregnancy as expected, the small sample

size of this study is a major limitation. In contrast, Achadi et al (171) demonstrated the beneficial impact of consuming iron-folate supplements at least once a week on fetal weight and length in a prospective community based study in West Java, Indonesia. Using multiple regression models that controlled for several maternal and neonatal factors, iron consumption during pregnancy was found to be a significant predictor of full term pregnancy, neonatal weight and length. Consumption of one or more iron tablets (200 mg ferrous sulphate and 0.25 mg folic acid) per week was associated with increased neonatal weight (172 g) and length (1 cm). Both adequate weight gain during pregnancy and height were significantly positively associated with neonatal weight ($p<.001$) and length ($p<.05$). Although of significant policy significance, the major limitations of this study is the absence of data on biochemical indicators which make it more difficult to interpret the findings. Also, since the supplement contained both iron and folate, we cannot isolate the effects of each nutrient.

Preliminary findings from the CDC Pregnancy Nutrition Surveillance System (PNSS) also suggest that anemia during the first trimester may be associated with an increase of LBW (172, 173). The prevalence of LBW was greater among infants born to women who were anemic during the first (9.7%) and second (11.6%) trimester compared to those born to non anemic women (7%). In contrast, women who were severely anemic in the third trimester were not at a greater risk than non anemic women. Since the time of entry varied in these data, Scanlon et al (173) defined anemia as hemoglobin values below -2 S.D. of mean values defined by weeks of gestation, and found that women who were anemic were 30% more likely to deliver infant prematurely as well as with IUGR. The risk of IUGR was also increased at the higher end of hemoglobin values, which may be related to inadequate plasma volume expansion and not iron status. The lack of data on other indicators of iron status and use of supplements however is a major limitation.

In a recent prospective follow up study of 580 African-American women, Goldenberg et al (174) found that women who were in the highest quartile of plasma ferritin levels at 26 weeks were at *increased* risk for pre-term delivery and low birth weight compared to those with lower levels. Tamura et al (175) also found that serum ferritin concentrations were negatively correlated with gestational age at birth ($p=.034$) in a similar population. These recent findings confirm earlier work (176) and suggest that increased serum ferritin levels acts as a marker of sub-clinical infections especially upper genital infections during pregnancy, since it is an acute-phase reactant, which in turn is predictive of poor pregnancy outcomes. This poses a problem when using serum ferritin as a measure of iron status, and may observe the potential benefits of improving iron status.

Experimental Studies:

Although well designed RCTs trials would provide better answers, there are few such studies. A major limitation however, is that based on ethical considerations, it would be very difficult to conduct such trials, since it would involve denying standard treatment for the control group. Many countries throughout the world including developed ones such as the U.S. provide routine iron supplementation as part of ante-natal care to compensate for the increased iron requirements during pregnancy. A brief description of experimental trials of iron supplementation that examine pregnancy outcomes is presented in Table 3. The trials published to date (147, 177-179) are affected by inadequate sample size, poor predictive power, lack of blinding and other confounding factors. More importantly there

are very few RCTs in developing countries where the problem is greater. Two RCTs with iron supplementation from Finland (179) and Denmark (147) failed to detect any significant differences in mean birth weight and the prevalence of LBW. However the lack of blinding and suitability of the control group who received selective iron supplementation is a major concern in the study by Hemminki et al (179). The same authors, in a follow up study of the same population, also reported that there was no difference between the two groups in either the number or timing of hospital visits, whereas the proportion of children admitted for convulsions was significantly higher in the routine group compared to the selective group which led them to conclude that routine iron prophylaxis during pregnancy may be harmful to infants. However biases introduced due to the high rate of loss to follow ups (56% of mothers could not be traced) and accuracy of data based on hospital records and registers cannot be ignored. Millman et al (147) had a better study design and included a placebo, but the small sample size (n=120) may have limited their ability to detect significant differences. In the iron treated group (n=63), maternal mean cell volume was negatively correlated to birth weight ($r=.29$, $p< .03$), and in the placebo treated group (n=66), maternal Hb and maternal serum erythropoietin were both inversely correlated to birth length ($r=0.26$, $p<.05$). Newborns in the iron treated group had a higher cord S-ferritin than newborns in the placebo group.

Only two RCTs of iron supplementation that were conducted in developing countries were identified (177, 178). In a trial of multi-gravid pregnant women in Gambia, Menendez et al (177) found that the mean birth weight of infants born to women who received daily supplements containing 200 mg of Fe and 5 mg of folic acid during pregnancy was 56 g greater than that of infants born to the controls who received only folic acid. Although this difference was not statistically significant, it is of biological significance and was accompanied by significant reductions in the prevalence of anemia and iron deficiency. Further, there was evidence of a positive dose-response relationship between birth weight and the degree of iron deficiency. For example, among women who took at least 80 tablets (app 3 months supply), a significant difference of nearly 100 g ($p=0.04$) was observed between the two groups. More recently, Preziosi et al (178) reported findings of a trial in which 197 women were randomly allocated to receive either 100 mg Fe/day or the placebo during the last trimester of pregnancy. This study which was conducted in a Maternal & Child Health Clinic in Niamey Nigeria was possible due to lack of routine administration of iron or folic acid supplements to pregnant women. As expected, the iron supplement contributed significantly to reductions in the prevalence of anemia and higher serum ferritin values in both mothers and their infants. More interestingly, mean length and Apgar scores were significantly higher in infants who were born to mothers in the iron group compared to those in the placebo group. Although mean birthweight was 30g higher in the iron supplemented group compared to the placebo groups, these differences were not statistically significant primarily due to the limited sample size. However, the differences diminished at 3 and 6 months postpartum. These findings suggest the strong potential of iron supplementation in reducing the prevalence of low birth weight in developing countries. Although it would not be ethically justifiable to conduct more RCT's in light of the international recommendations by WHO/UNICEF for routine iron supplementation during pregnancy, well designed prospective studies with data on pregnancy outcomes as well as biochemical indicators, supplement use and dietary intakes during pregnancy in developing country settings will be useful to examine the benefits of improving iron nutriture on pregnancy outcomes.

TABLE 3: Description of Experimental Trials of Iron Supplementation and Pregnancy Outcomes

			Nature of Intervention[1]				Results[2]		
S. No.	Investigator(Yr)	Study Site	Experimental Group	Control Group	N	Wks Preg	BW	PM	Abor-tion
1.	Higgins et al, 1982	Canada	MV	n.a.	1112	13-25	↑	nr	nr
2.	Hemminki et al, 1991	Finland	100 mg Fe	50 mg Fe[3]	2694	10	-	↓	↑[4]
3.	Milman et al, 1994	Denmark	66 mg Fe	placebo	120	14-16	-	-	nr
4.	Menendez et al, 1994	Gambia	60mg Fe + 5mg FA	5 mg FA	550	24	↑	-	nr
5.	Preizosi et al, 1997	Niger	100mg Fe	placebo	209	28	-	-	nr

[1]Fe = elemental Fe; MV = multivitamin including 2 mg folic acid + 66 mg iron; FA = folic acid; n.a. = not applicable; Hb = hemoglobin

[2]BW = birthweight; PM = prematurity; PIH = pregnancy induced hypertension; nr = not reported

[3] Supplemented only if Hb< 100g;

2.2.4. Magnesium:

A recent systematic review of intervention trials concluded that magnesium sulphate was more effective than other interventions in preventing the recurrence of seizures and the first seizure in preeclampsia (180). However, the role of magnesium in improving pregnancy outcomes such as prematurity and LBW is still controversial. Nutritional deficiency of magnesium (Mg) has been suggested as predisposing to pre-term labor (181). In a retrospective cross-sectional study, magnesium levels at delivery were positively associated with an increasing trend with birth weight (27). Kurzel (182) found that 71oout of 128 patients who had pre term labor showed significantly depressed serum Mg levels ($p<.0005$). However, the lowered Mg levels were not dependent on specific etiologies for pre-term labor: PROM, twin pregnancies, placental abruption and placenta previa, and the author concluded that it is unlikely that hypomagnesemia associated with pre term labor is due to Mg deficiency but rather reflects the initiation of uterine contractility or irritability. Since hypomagnesemia reflects the condition of uterine irritability, serum Mg could serve as a marker for patients who are at risk for progressing on to pre-term delivery and prenatal supplementation to such patients therefore has a scientific basis for preventing the recurrence of pre term delivery (182). In a case control study of 76 full term births (31 IUGR babies and 45 normal babies), the IUGR group showed significantly higher magnesium level even after controlling for other factors using multiple regression (183).

Experimental studies:

Conradt et al (181) demonstrated that magnesium supplementation was associated with reductions in the incidence of pre-term delivery, PROM and IUGR by comparing 4023 low risk pregnancies to 882 high risk pregnancies who were treated with β-sympathomimetic agents in combination with magnesium aspartame hydrochloride. The major drawbacks with this study is the retrospective design, the choice of the control group who did not receive a placebo, and that the treatment was not randomly allocated. However these findings were confirmed by Kovacs et al (184) who in a RCT of nearly 1000 women found that there were fewer premature births, smaller proportion of small for date and IUGR babies in the group who received 15 mmol magnesium sulphate supplementation compared to those who received a placebo. Similarly in a double blind study in a Swiss population, Spatling and Spatling (185) supplemented 568 women with 15 mmol of magnesium aspartame hydrochloride from 16th week of gestation and found that magnesium supplementation was associated with significantly fewer maternal hospitalizations, a reduction in the pre-term delivery and less frequent referral of the newborn of the neonatal intensive care unit. However, there were no significant differences between the two groups with respect to the placental weight, infant weight and length and head circumference. After exclusion of women who did not take medication regularly, the analysis showed a significant relation between birth weight and length, head circumference, Apgar scores with supplementation. The authors concluded that magnesium supplementation during pregnancy has a significant influence on fetal and maternal morbidity both before and after delivery. In contrast, Mg supplementation did not improve pregnancy outcomes in a double blind RCT of normotensive primigravidas (n=374) in the U.S. who were enrolled between 13 and 24 weeks of gestation. (186) The treatment was 365 mg magnesium daily, whereas all subjects received prenatal vitamins containing 200 mg elemental Ca and 100 mg elemental Mg. Although trends were observed in favor of the supplemented group, no

significant differences were found in the two groups in the incidence of preeclampsia, fetal growth retardation, pre-term labor, birth weight, gestational age at delivery or number of infants admitted to the special care unit. It should be noted that the two groups were similar at baseline and also the magnesium supplemented group had significantly higher magnesium levels at delivery. Although these studies suggest benefits of magnesium supplementation especially among women at risk for PIH, there are no such studies in developing countries where the problem may be greater.

2.2.5. Zinc:

Zinc deficiency has been associated with infertility, abortions, malformations, IUGR and other adverse pregnancy outcomes both in animals and humans (187-189). Zinc is an important nutrient during pregnancy and plays a critical role in normal growth and development, cellular integrity and several biochemical functions. It is a co-factor for several enzymes including carbonic anhydrase and DNA polymerase which are involved in protein and nucleic acid synthesis (190). While the teratogenic effects of severe zinc deficiency during pregnancy have been well documented both in animals and humans, the role of mild to moderate zinc deficiency remains controversial. Several studies of dietary intakes of zinc among pregnant women from different parts of the world show that overall mean intakes of pregnant vegetarian women tend to be markedly lower (about 8 mg/day) compared to pregnant non-vegetarians (about 10 mg/day) (189). Low intakes have also been reported among subgroups in developed countries such as the U.S. and Canada (191-194).

An extensive number of observational studies using both retrospective and prospective follow up designs have been conducted in different parts of the world to examine the relationship between zinc nutriture based on dietary intakes and biochemical indices and a variety of pregnancy outcomes. However, many of these studies have provided conflicting and inconclusive evidence and have several limitations, namely inadequate sample size, retrospective study design (e.g: use of postpartum values), lack of data on confounding factors, and poor indicators of zinc status A brief summary of the findings of these studies is presented in the following section, focusing on the better designed prospective studies. Additional details are available in a recently published extensive review of this topic (189). Details of the randomized intervention trials which provide stronger evidence on the role of zinc during pregnancy are presented in Table 4.

Observational studies:

Although several cross-sectional studies have shown that maternal and cord plasma zinc levels are significantly lower in cases with mild to moderate pre-eclampsia, the lack of prospective data and/or experimental trials do not permit inference of causality. Similarly, retrospective studies have also suggested a relationship between maternal zinc nutriture and PROM and other birth defects (189). Scholl et al (191) using a prospective design found that PROM was associated with low dietary intakes of zinc.

In a recent extensive review of zinc nutriture and pregnancy outcomes, Tamura and Goldenberg (189) reported that 22 out of 41 published studies had found significant associations between zinc status and birth weight. In an early landmark prospective study of 84 primigravida from Sweden, Jameson (1976) reported an association between serum

TABLE 4: Description of Experimental Trials of Zinc Supplementation and Pregnancy Outcomes

S. No.	Investigator (Yr)	Site	Nature of Intervention[1] Experimental Group	Control Group	N	Wks Preg	Results BW	PM	Abor-tions
1.	Hunt et al, 1984[2]	US	20 mg Zn + MVM	MVM	213	19	-	-	nr
2.	Hunt et al, 1985	US	20 mg Zn + MVM	MVM	138	14	-	-	nr
3.	Ross et al, 1985	S. Africa	4.3-12.9 mg Zn	placebo	64	<20	-	-	nr
4.	Kynast et al, 1986	Germany	20 mg Zn	no placebo	524	25	↑	-	nr
5.	Mahomed et al, 1989	UK	20 mg Zn	placebo	494	<20	-	-	nr[3]
6.	Cherry et al, 1989	US	30 mg Zn	placebo	556	<25	-	↓	nr
7.	Simmer et al, 1991	UK	22.5 mg Zn	placebo	56	14-25	↑	-	nr
8.	Garg et al, 1993	India	45 mg Zn	no placebo	168	10-30	↑	↓	nr
9.	Goldenberg et al, 1995	US	25 mg Zn + MVM	MV	580	19	↑	↓	nr
10.	Jonsson et al, 1996	Denmark	44 mg Zn	placebo	1206	<20	-	-	nr

[1]Zn = zinc; MVM = multivitamin mineral excluding zinc
[2]This study also reported significant reductions in pregnancy induced hypertension
[3]This study reported significant reduction in pregnancy complications

zinc and copper levels and adverse pregnancy outcomes (195). Zinc concentrations were significantly reduced ($p < 0.001$) during early pregnancy in women with complications such as abnormal labor or atonic bleeding. Women who delivered pre-term or post term babies also had lower serum zinc levels. However, there were no data on other factors, such as energy or protein intakes. Pioneering work by Meadows et al (196, 197) in the early 1980's also showed that mean leucocyte zinc level was significantly lower in mothers with SGA babies and their infants, when compared to mothers with normal babies. However, plasma zinc levels were similar in both groups. These findings were confirmed by other studies (198-200), although those who failed to detect an association may have been due to lack of suitability of the methods used to assess leucocyte zinc levels (189). Wells et al (200) in a prospective follow up study of 70 women in the UK found that the median maternal leukocyte zinc concentrations measured in the third trimester was significantly associated with birth weight. A maternal leukocyte zinc concentration <120 nmol/10^9 leukocytes strongly predicted a baby weighing below the 10th centile (positive predictive value = 71.9 %; negative predictive value = 91.5 %; sensitivity=64.3 %; specificity=81.8 %). The authors concluded that early third trimester maternal leucocyte zinc concentrations may be used to predict IUGR.

Mukherjee et al (76) measured plasma nutrient levels in 450 pregnant women in India, and found that maternal plasma zinc levels were inversely related correlated with fetal weight. However, a significant correlation was found between the total occurrence of feto-maternal complications specifically fetal distress, increased with decreasing levels of zinc ($p<.02$) and albumin ($p<.02$). In a cross sectional study among 437 Chinese women, found a positive correlation between birth weight and maternal serum zinc concentration ($r=.632$, $p<.001$) but a negative correlation with hair zinc concentration ($r=.-.574$, $p<.001$) was found (201). Abortion, low birth weight and congenital anomalies were not associated with low concentration in maternal plasma or hair (201). However the adequacy of these indicators, lack of adjustment for confounding factors and study design affect the validity of these conclusions.

More recently, consistent findings supporting the relationship between marginal zinc status and birth weight have been reported (27, 191, 202, 203). Two cross-sectional studies have shown a significant positive correlation between birth weight and zinc levels based on cord blood measurements (27, 203). Speich et al (202) also found a significant correlation between gestation age and erythrocyte zinc levels in the cord blood of 66 term infants. In two well controlled prospective studies serum zinc levels (< 60 μg/dl) late in pregnancy and low zinc intakes (< 6 mg/day) during pregnancy were associated with 2-5 fold increases in the risk of LBW after controlling for energy intakes during pregnancy and other confounding variables (191, 202). A low intake of zinc during pregnancy was also related to a three-fold increased risk of prematurity (191). These studies were conducted in poor urban communities in the US. Data from the Nutrition Collaborative Research Projects (CRSP) also demonstrated that maternal pregnancy intake of zinc was related positively to length among newborns in Kenya (204) and predicted neonatal habituation behavior in Egypt (205) after controlling for various other factors. Clearly, well designed intervention trials would provide stronger evidence on the role of zinc in pregnancy outcomes.

Experimental Studies:

Zinc supplementation has been associated with reductions in pregnancy complications (206-210). Although some earlier studies reported that zinc supplementation did not offer any benefits to the mother or fetus, they suffered from low compliance, inadequate power and also initial zinc status was not considered (192, 193, 210). To date, the results of ten intervention trials, most of which were randomized double-blind controlled trials have been published (see Table 4).

In one of the earliest studies, conducted among 312 pregnant women in Sweden, Jameson (211) found that daily administration of 45 mg/day of zinc from 14 weeks of pregnancy to women with low serum zinc levels (< 65 ug/dl at 14 weeks) resulted in a greater number of normal deliveries (63%) and increased gestational age compared to the controls (55%). However the lack of dietary data is a major limitation. Birth weight was also not reported. In contrast, two consecutive double-blind studies conducted among women (adults and teenagers) of Mexican descent in the U.S. (192,193) failed to detect any significant benefits of zinc supplementation on a variety of pregnancy outcomes, despite the fact that these women had low dietary intakes (50% RDA) and the intervention reduced the prevalence of women with low serum zinc levels (below - 1 S.D.). The only significant finding was the reduced prevalence of PIH among the older women. Although both these studies were well designed RCTs with measures of dietary intakes and biochemical measures, the lack of any effects as described by the authors may have been due to the fact that the dosage was inadequate and may have been started too late. They used less than half the dose used by Jameson (211) and started supplementation 6 weeks later.

In a randomized study of Zulu women, Ross et al (212) found no significant difference in birth weight of the zinc supplemented group (3088 g) compared to those who received the placebo (3171g). However, the zinc supplemented women were significantly lighter (5 kg) compared to the placebo, and this could have influenced the outcome. Small sample size and inadequate amount of zinc supplementation are other concerns. Zinc supplementation (20 mg of zinc aspartame) was associated with a significant reduction in the incidence of large for date and small for date infants, pre-term labor, premature separation of the placenta and vaginal bleeding in another RCT from Germany (209). There were no significant differences in birth weight, which may have been due to the fact that zinc supplementation was started only around 25 weeks of pregnancy. In a larger RCT (n=700) in the U.K., Mahomed et al (210) failed to detect any benefits of Zn supplementation for various pregnancy outcomes, namely birth weight, pregnancy complications, maternal bleeding, hypertension, Apgar scores and neonatal abnormalities. However, this may have been due to the fact that the subjects were not likely Zn deficient. Although dietary intakes of Zn were low (below 50% RDA), the lack of improvement in mean leucocyte Zn levels following supplementation suggest that they may have had adequate zinc stores. Although sample size may have been limiting in detecting significant decreases in the prevalence of IUGR, it was adequate to detect differences of 100g in birth weight.

The more recently conducted zinc supplementation trials have shown more promising results. Simmer et al (208) demonstrated that zinc supplementation was beneficial to pregnant women who were at high risk of delivering small babies. The inclusion criteria were low prepregnant weight, birth of a previous SGA infant and smoking. This study

showed a 3-4 fold reduction in the prevalence of IUGR (27% controls; 7% treatment). Although mean birth weight was 107 g greater in the Zn supplemented group compared to those who received the placebo, these differences were not statistically significant. There were also significant reductions in the percentage of women who had induced labor and C-section deliveries. Cherry et al (207) also demonstrated evidence of an interaction between maternal weight and response to Zn supplements in a double blind RCT among low income pregnant adolescents. The prematurity rates were reduced in the group of women who received Zn supplements and birth length was also greater compared to the control group. In a study from India (213), significant increases in birth weight ($p< 0.001$), and gestational age ($p< 0.01$), a reduced incidence of prematurity ($p < 0.05$), reduced incidence of IUGR ($p <0.05$), and a higher Apgar score ($p <0.001$) were found in the Zn supplemented group as compared to the untreated group. The dosage used (200 mg of zinc sulphate), was higher in this study, but the lack of blinding may have introduced biases. Finally, two recently conducted randomized, double-blind trials have provided interesting evidence on the benefits of providing zinc supplements during pregnancy (206, 214). In the first study that was carried out in a poor, urban area of the US, 589 pregnant women with low serum levels of zinc were allocated randomly to one of two supplements options: a multivitamin compound with 25 mg of zinc or a multivitamin compound without zinc. The infants born to the zinc-supplemented women were heavier (126 g, $p=0.03$) and had larger head circumference values (0.4 cm; $p=0.02$). The rate of prematurity was also lower. Of note, is that women with low BMI's (<26.0) showed the greatest benefits. In the double blind RCT in Denmark, Jonsson et al (214) randomly assigned 1206 women less than 20 weeks of gestation to receive either 44 mg of zinc or a placebo. The outcome measures studied were large for gestation age (LGA), small for gestation age (SGA), PROM, pre-term labor, preeclampsia and bleeding in second or third trimester. No differences concerning any of the outcome were observed in the two groups. However, these women were healthy (middle class population) and may have been receiving adequate zinc through diet. These findings suggest that the benefits of zinc supplementation may be limited to certain subgroups.

1.2.6. Other Minerals:

1.2.6.1. Copper and Selenium:

Copper deficiency (Cu) and excess has been associated with adverse pregnancy outcomes. A study from Finland suggested a possible role for copper in pre-term PROM (215). In a cross sectional study, of 166 Zairian pregnant women, the infants with low birth weight had a mean umbilical serum selenium (Se) concentration that was lower than that of their counterparts with birth weights higher than 2500 gm ($p<.02$) (216). Umbilical serum copper concentration was also related to birth weight ($r=.197$, $p<.05$). New born selenium and copper were concentrations were also significantly positively related to head circumference. However, maternal serum indices were not associated with newborn serum trace element concentrations. In another observational study in Poland, Wasowicz et al (217) found that both Zn and Cu concentrations in plasma of pre-term infants were significantly higher than in full term infants. Mothers of pre-term infants did not differ in plasma Zn and Se levels but Cu concentrations were significantly higher as compared to mothers of full term neonates. Se and Zn levels in maternal and cord blood plasma according to birth weight group did not show any significant difference, contrary to maternal Cu concentration. Mothers giving birth to low birth weight babies had

significantly higher Cu levels as compared to those giving birth to the heaviest babies. Similarly, serum Cu measured at delivery was negatively associated with birth weight in another cross-sectional study (27). These conflicting findings clearly warrant better designed prospective studies.

3. Inter-relationship between two micro nutrients

Based on our knowledge of the interrelationships between iron, zinc and vitamin A metabolism and their role in growth and development, the combined benefits of these three micro nutrients on pregnancy outcomes warrant immediate attention. In addition, interrelationships between some of the above nutrients and folic acid have also been examined, in light of the recent attention on the importance of folic acid in determining pregnancy outcomes (20-22) and its inclusion with iron in many antenatal care programs. The specific interrelationships that are briefly reviewed in this section are those between iron and zinc, vitamin A, folic acid and vitamin C, between zinc and folic acid; and between zinc and vitamin A. It should be noted that other inter-relationships (for e.g. Cu and Zinc) have not been included.

3.1. Iron and Zinc:

The interrelationship between iron and zinc may be bi-directional. There are also reports that zinc supplements interfere with the absorption and availability of iron and vice versa. Cherry et al (218) in a randomized trial using a daily supplement of either zinc gluconate (30 mg zinc) or placebo that was combined with routine iron supplementation reported significant differences for fetal distress, fetal demise (still births and neonatal deaths), pre-term deliveries as well as infection rates. There was evidence of interactions between the iron status of the women and timing of the supplement. For example, women with low ferritin levels and who had started zinc supplementation in pregnancy had longest infant lengths, whereas among those who began zinc supplementation later, infants born to women with adequate ferritin levels were longer. Head and chest circumference were also larger in the zinc treated anemic women.

3.2. Iron and Vitamin A:

Vitamin A deficiency inhibits iron utilization and accelerates the development of anemia (219). Some studies have shown that improving vitamin A status improves hematological indices in both children and pregnant women, but the impact of this combined intervention in improving other pregnancy outcomes including birth weight is not known (40, 45, 220). In a randomized double blind trial, 251 second trimester pregnant women were supplemented with either vitamin A, iron, both or neither (40). Vitamin A and iron supplementation significantly increased Hb to 12.78 (95% CI- 10.86-14.7), one third of which was attributed to vitamin A and two thirds to iron supplementation. After supplementation the proportion of women who became non anaemic (Hb< 110 g/L) was 35% in the vitamin A supplemented group, 68 % in the iron group, 97% in the vit A + iron group and 16 % in the placebo group.

3.3. Iron and Folate:

The impact of iron and folate supplementation, which is a part of routine antenatal care to treat nutritional anemia in some parts of the world, in reducing the prevalence of low birth weight has not been studied. Achadi et al (171) in a study in Indonesia reported that the consumption of one or more iron-folate pill per week by pregnant women was associated with an increase in birth weight (172 g) and length (1 cm), compared to those who did not consume the supplement regularly.

3.4. Iron and Vitamin C:

Vitamin C is a known enhancer of iron absorption (221). However few studies have examined the benefits of this combined intervention. It is plausible to expect a much larger benefit, especially since recent studies indicate an independent role for vitamin C in PROM. A well designed RCT especially in deficient populations that would compare iron only to iron and vitamin C would be of great value, and ethically acceptable.

3.5. Vitamin A and Zinc:

Although the vitamin A-zinc connection has been extensively studied in animal models as well as to a certain extent in young children (222-225), the relevance of this interaction in pregnancy and lactation has not been described. Low levels of serum zinc and retinol levels have been reported among pregnant women with inadequate dietary intakes (33).

3.6. Folic Acid and Zinc:

In an earlier study, Mukherjee et al (76) reported that fetomaternal complications were greater among women who were in the lowest quartile of plasma zinc levels and highest quartile of plasma folate levels measured at delivery. These findings were explained by the possibility of the adverse effects of folic acid supplements on intestinal zinc absorption (226). However, Tamura et al (83) using prospectively collected data did not find any significant associations when birth weight was examined across combined quartiles of serum zinc and folate that were measured at 18 and 30 weeks of gestation. In light of the potential benefits of zinc and folic acid, clearly this interaction needs to be further studied.

4. Multivitamin-mineral supplements

There are several studies, in the literature reporting the benefit of multivitamin supplements, especially with folic acid, on the rate of neural tube defects as well as occurrence of birth defects such as cleft palate and cleft lip (227). But, few studies have examined outcomes such as birth weight, which are of great public health significance. Although the relationship between the levels of biochemical indicators of several micronutrients in maternal and cord blood at delivery with birth outcomes such as IUGR and prematurity has been examined in several cross-sectional retrospective studies (27, 32,74, 76, 203, 216, 217, 228), the nutrients were usually viewed in isolation, and very few investigators examined the significance of combinations of nutrients. The evidence from prospective longitudinal studies which included measures of dietary intakes of multiple nutrients, micronutrient status and/or use of multi-vitamin mineral supplements are presented in the following section.

Observational Studies:

Pfeffer et al (229) in a prospective follow up study of 82 pregnant Mexican women failed to detect significant differences in weight gain based on a combination of biochemical indicators of iron, zinc and vitamin C in spite of the relatively high prevalence of deficiency (15-35% for Zn, 64-88% for vitamin C and 60-90% for hemoglobin and ferritin). Prepregnant weight was the most important determinant. Besides biochemical indicators, studies examining the association of dietary intakes of several micro nutrients including supplements are also of interest. Kullander and Kallen (230) in a prospective study of drugs and pregnancy that was conducted in the 1960's in Sweden, found that the prevalence of LBW was significantly lower among women who reported the use of iron and/or multivitamin mineral supplement during pregnancy. However, data on other potential confounding factors, such as the use of prenatal care, smoking and other nutritional factors that may have been associated with supplement use are lacking. Although there is some information on the patterns of use of multi-vitamin mineral supplements among women of reproductive age especially in the U.S. (231-234), little is known on the impact of combinations of micro nutrients and/or the use of multi-vitamin-mineral supplements during pregnancy on outcomes such as including weight gain, birth weight and length at birth. Nevertheless, studies (mostly from developed countries) in which the relationship between dietary intakes of several micro nutrients and pregnancy outcomes has been examined, provide valuable insights, despite the concerns with the validity and reliability of dietary data.

In a follow up study in London, the association between dietary intakes of 513 pregnant women (before 13 weeks) and pregnancy outcome was studied (235). Mothers were asked to keep a diary of all food and drink consumed during one week. There was a significant correlation between dietary intake of several minerals (Mg, iron, phosphorus, zinc, sodium, potassium, calcium) and vitamins (thiamin, niacin, pantothenic acid, riboflavin, folic acid and pyridoxine) with birth weights below the median (3720 g). However, maternal intakes of vitamins A, B_{12}, C, D and E were not significantly correlated with birth weight, birth head circumference or birth length. A major concern with this study is that total energy intake and other confounding factors were not controlled for. Also, dietary intake during the first trimester, may be an underestimate of actual intakes since some women may have had lower intakes due to nausea. In another study of randomly selected groups of non smokers (n=97) and heavy smokers (n=72), 7 day weighed dietary intake at 28 weeks and at 36 weeks of gestation were used to assess the nutrient intake (236). After controlling for smoking, social class and alcohol consumption, nutrient intakes at 28 weeks did not have any effect on birth weight. However, intakes of protein, zinc, thiamin and riboflavin at 36 weeks had a positive effect on adjusted birth weight. Also, the change in intakes (between 28 and 36 weeks) of protein, zinc, iron, riboflavin, thiamine and pyridoxine had independent positive effects on birth weight. Birth weight was lower among smokers as compared to non smokers after adjusting for social class, and maternal height. In a study carried out in Ecuador (237) riboflavin and niacin intakes were positively associated, and thiamin intakes negatively associated with birth weight. However, these associations are difficult to interpret since the multiple regression model included many dietary variables as independent variables, without concern for multicollinearity. Finally, Johnson et al found no association between dietary intakes of over a dozen micronutrients and birth weight among low-income, African-American women (238). However, energy and protein intakes which have been shown to be related to pregnancy outcomes in deficient

populations were also not significantly associated in this study, indicating that the extent of deficiency may not be limiting in this U.S. population. For example, although low dietary intakes of iron, folate, magnesium, vitamin B_6 and calcium were reported, these intakes were greater than typically found in many developing countries. It should be noted that although the relationship between energy and protein intakes with birth outcomes such as LBW and pre-term births have been examined, there are few well designed prospective studies that have examined the association between the dietary intakes of several micronutrients during pregnancy with adverse pregnancy outcomes in developing countries where intakes are more deficient.

Experimental Studies:

Two experimental trials (20, 21) in which the primary outcome was neural tube defects have compared the efficacy of different combinations of multi-vitamin mineral supplements. While both studies demonstrated the reduction in the occurrence of neural tube defects, only one of them permits us to draw probabilistic causal associations between the use of multi vitamin-mineral supplements and birth weight; it was the prospective double blind randomized trial conducted in Hungary on a sample of 4753 women who received different combinations of micronutrient supplements from pre-conception throughout pregnancy (20). One group (Group I) was given all hydro soluble vitamins, vitamins A, D and E and calcium, phosphorus, magnesium, iron, copper, manganese and zinc. Group II was given only copper, manganese, zinc and vitamin C. Group I had a significantly lower proportion of congenital abnormalities but no differences were found in birth weight between the two groups. However, group II was given zinc, a micronutrient known to have a positive effect on prenatal growth (189, 205), which renders it unsuitable as a proper control. Also, no evidence of micronutrient deficiency was found in the women studied. However, the rates of ectopic pregnancies, miscarriages and stillbirths as well as pre-term births and LBW were significantly lower for both the supplemented groups compared to the population figures (20). Both groups also had greater mean birth weights than the general population which suggests potential benefits. Although the study design of the trial that was conducted in England (21) would have permitted comparisons in birth weight between women who received multi-vitamin supplements with and without folic acid to those who received only placebo, no data on outcomes such as prematurity and low birth weight were reported. Significant reductions in the recurrence of neural tube defects (RR: 0.28) was found among the groups who received folic acid, but there were no differences in the rates of miscarriages or stillbirths. The study population in this study however were women who were at risk of giving birth to infants with neural tube defects. Also these studies have been conducted in developed countries. To date, no studies have examined the benefits of multi-vitamin mineral supplements during pregnancy in developing countries where poor dietary intakes and multiple micronutrient malnutrition is common (15, 16). A randomized double-blind controlled trial is currently underway in Mexico in which the effects of multiple micronutrient supplementation during pregnancy will be compared to a control group of women who receive standard iron supplements during pregnancy. This trial is expected to provide answers to an important policy question whether multi-vitamin mineral supplements rather than only iron should be provided routinely to all pregnant women, particularly in developing countries.

5. Conclusions

Over 100 studies have examined the role of micronutrients during pregnancy. While some nutrients have been studied extensively (e.g.: calcium, zinc) much less is known about others (e.g.: vitamin B- complex). Also, there is considerable variation in the types of study design ranging from cross-sectional studies to well designed placebo-controlled randomized trials. Some of the common problems observed with many studies are methodological. A major limitation of many experimental trials was small sample size, resulting in inadequate power to detect significant differences between the experimental and control groups, especially for outcomes such as birth weight. The majority of the RCT's were conducted in developed countries among populations which were not deficient and thus were less likely to benefit from the intervention. More studies are needed in developing country settings where micronutrient deficiencies as well as poor pregnancy outcomes such as low birth weight are common. Finally, almost all the studies used the single nutrient approach , i.e. examining the relationship between one specific nutrient and some pregnancy outcomes. Although this approach is very useful to understand the mechanisms, nutrient deficiencies do not occur in isolation and the study of nutrient interactions and multiple micronutrient deficiencies merits more attention from a public health point of view.

The specific conclusions of this review are:

- There is strong evidence based on well designed RCT's, primarily from developed countries that *zinc, calcium and magnesium* improve pregnancy outcomes, such as birth weight, prematurity and PIH, especially in high risk groups.

- Current evidence on whether *iron* supplements reduce the prevalence of LBW and prematurity is weak. However, RCT's to answer this question is ethically unacceptable since iron supplements are the proven treatment for anemia.

- The importance of *iodine* in preventing mental retardation and cretinism is well established, but the evidence linking it to other outcomes such as LBW and prematurity is weaker especially in the case of marginal iodine deficiency.

- The role of *folic acid* in preventing neural tube defects is well established. However, the evidence that it also prevents outcomes such as LBW and preterm births is limited. Although conducting RCT's may be difficult to justify ethically, better designed studies with prospective data, especially in deficient populations in developing countries, are needed.

- Current data, based on retrospective and few poorly designed experimental trials suggest a plausible role for *vitamin A* in improving outcomes such as birth weight; these findings need to be confirmed by well designed RCT's in deficient populations.

- Some studies suggest a role for the *B-complex vitamins (thiamine, B_6, and B_{12}), copper and selenium,* but very few experimental studies have been conducted to date.

- Emerging clinical evidence on the role of *vitamin C* in the etiology of PROM and susceptibility to infections needs further validation by RCT's

- Although there is evidence of interactions among several micronutrients at the metabolic level, very little is known about the significance of these interactions for pregnancy outcomes. There is a need for well designed RCT's that will examine the role of selected nutrient interactions and *multi vitamin -mineral* supplements in improving pregnancy outcomes especially in developing countries where these deficiencies are common.

This review clearly shows that considerable advances have been made in our knowledge and understanding of the role of several micronutrients in determining pregnancy outcomes. Many of these advances have been made in the past 10-15 years and some of the questions/ doubts that were raised in Kramer's landmark review in 1987(7) have been clarified. However, it is important to recognize that some of the conclusions in this review differ from those presented in a more recent review by Gulmezoglu et al, who concluded that *malaria prophylaxis, stopping smoking and 'balanced' energy-protein supplementation* are the *only interventions* known to reduce low birth weight (6). While one does not disagree with the importance of these interventions, this review presents a more comprehensive picture of the role of micronutrients during pregnancy on a range of pregnancy outcomes bearing in mind the likelihood of underlying deficiencies of one of more of the various micronutrients in many developing countries. It should also be noted that the studies that examined the role of "balanced" energy-protein supplementation did not provide these two macronutrients in isolation(7, 8, 9, 239). The design of those studies do not permit us to isolate the role of micronutrients in improving pregnancy outcomes because in reality they were studies of food supplementation which may have also increased the intakes of micronutrients . Although an increasing number of studies are being conducted in these settings where the likelihood of detecting significant effects is greater, there remains a need for better designed studies that will provide evidence on the benefits of improving micronutrient intakes for pregnancy outcomes.

6. Recommendations for future research

As described in the section on methodological considerations, prospective follow up studies and experimental trials using a double-blind placebo controlled randomized design are highly recommended for future research. More retrospective cross-sectional studies are not needed at this juncture. Although RCT's are more expensive and time consuming, they are justifiable if they are well designed and implemented and address questions of public health significance. Well-designed prospective follow up studies with detailed information on confounding variables can also provide valuable information, and may be appropriate in case of nutrients about which less is known (e.g.: vitamin B-complex, nutrient interactions). More importantly, they may be the only alternative in cases where it is ethically unacceptable to conduct RCTs (e.g.: iron, iodine). The next step would be to move from conducting efficacy trials to those which examine effectiveness. This can be done by looking for effects in program settings. Two types of study designs are commonly used 1) Pre-post design, in which the prevalence of the outcome measures are measured before and after the intervention and 2) Comparison of the outcomes in the program area to a control area which the program does not serve. The limitations of program based research is that random allocation is often not possible and choosing an

appropriate control group is difficult. Nevertheless, if well designed with the inclusion of adequate process and outcome measures programs can provide important answers that are very relevant to the real situation in many settings. Last but not the least, this review clearly demonstrated the importance of sample size. All future studies should pay special attention to this issue and account for potential losses to follow up especially in the case of birth outcomes. For example, the minimum sample size to detect a 100 g difference in birth weight, which represents an effect size of approximately 0.22 S.D. and is of biological significance is at least 200 women per group assuming 80% power at a significance level of p=0.05 using a one tailed test. These estimates will increase if the difference to be detected is smaller, higher level of power and/or two tailed tests are considered. Bearing in mind these issues, the following recommendations are made for future research.

6.1. Basic research:

We need to understand better the relevance of micronutrient interactions in two broad areas i) bioavailability ii) metabolic effects. In light of the evidence of multiple micronutrient deficiencies, the obvious practical solution would be to improve the intakes of more than one nutrient. Although this can be achieved in different ways i.e. use of multi vitamin-mineral supplements, fortified products and dietary modification, little is known on the true availability of these nutrients when they are delivered together especially in the case of supplements and fortified products. Basic research is necessary to determine the optimal amounts of various micronutrients that should be included in these products. For example, we know that minerals such as iron and zinc compete for similar sites during absorption. These studies can be done in the laboratory using animal models as well as in humans using techniques such as isotope labeling. The effect of factors such as the baseline level of these nutrients, infections etc, also need to be investigated.

6.2. Experimental Trials:

These findings in this review call for well designed RCT's with adequate sample size that should preferably be conducted in developing countries where micronutrient deficiencies and poor pregnancy outcomes such as low birth weight are major public health concerns. Specific nutrients of interest include vitamins A, C and folic acid. Although several RCT's have been conducted in the case of calcium and magnesium, the findings need to be validated in developing country settings. Most importantly, future research should focus on the significance of specific nutrient interactions and benefits of multi-vitamin mineral supplements.

Another area of emerging concern, especially in developed countries with adequate intakes of various nutrients is the potential dangers of excess on pregnancy outcomes. Future studies can examine the significance of iron excess during pregnancy in developed countries (145) where iron deficiency is less common, yet provide routine iron supplements (e.g. U.S.).

TABLE 5: Programmatic Guidelines for the Role of Micronutrients During Pregnancy in Developing Countries

MICRONUTRIENT	Likelihood of deficiency in developing country situations	Biological plausibility of the importance of moderate deficiency in pregnancy	Strength of evidence that supplementation improves pregnancy outcome	Relative cost of micronutrient	Risk associated in giving additional micronutrient to pregnant women who do not need it
Vitamin A	+++	++	+	+	++
Thiamin	+	++	+	+	+
Vitamin B-6	+	++	+	+	+
Folic Acid	++	+++	++	+	+
Vitamin B-12	++	+++	+	++	+
Vitamin C	+	++	++	+	+
Vitamin D	+	++	+	++	++
Vitamin E	+	+	+	++	+++
Vitamin K	+	+	+	++	+
Calcium	+++	++	+++	++	++
Iodine	++	+++	++	+	++
Iron	+++	+++	++	+	++
Magnesium	++	++	++	++	+
Zinc	++	+++	+++	++	++

+ Low; ++ Medium; +++ High;

6.3. Programmatic Research:

In the case of iron and iodine where RCT's would be ethically unacceptable, well designed program based research is a feasible alternative. For example, it is well known that compliance with iron supplementation is poor in many developing countries (166, 240). Pregnant women could be allocated randomly either to supervised group to ensure high compliance or to an unsupervised group. Therefore, although iron supplements would be available to all women, the amount of supplementation in the two groups would be different. In the case of interactions, the control group could receive standard iron treatment, while the treatment group would receive combinations of nutrients ranging from two (e.g.:iron and vitamin C) to several (e.g.: multi-vitamin mineral supplements). Similarly, in the case of iodine, areas in which there are no effective programs, could be selected and iodized salt could be introduced in a phased manner. With respect to multi-vitamin mineral supplements, there is a lack of consensus or agreement on the composition of the "ideal" prenatal supplements, although they are widely available(241). In general, they tend to be based loosely on the RDA and for some nutrients such as iron and folic acid which have received more attention, higher amounts may be included. Even less is known in the potential interactions between the nutrients and their relative bioavailability. Information on the nature of interactions between various nutrients such as iron, calcium, zinc and folate in the pharmaceutical preparations, in the gastro-intestinal tract prior to absorption and during absorption would also be important for the appropriate formulation of the supplements. The cost of the supplements needs to be considered before making recommendations for programs. In order to guide programs, the likelihood of the micronutrient deficiencies, the biological plausibility of a role in determining pregnancy outcomes and evaluation of the available evidence have been ranked and presented in Table 5. The relative costs of the various nutrients and the potential risks of providing them to women who do not require them i.e. has also been included in this table. Although additional data are clearly needed, this table could serve as a template to guide further progress in this important area.

In summary, considerable strides have been made in the area of micronutrients and their importance to pregnancy outcomes. Clearly, as we move ahead with better designed studies, several public health recommendations that will reduce the burden of poor pregnancy outcomes in many settings will ensue.

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