Effects and safety of periconceptional folate supplementation for preventing birth defects (Review)

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[Intervention Review]

Effects and safety of periconceptional folate supplementation for preventing birth defects

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ABSTRACT

Background

It has been reported that neural tube defects can be prevented with periconceptional folic acid supplementation. The effects of different doses, forms and schemes of folate supplementation for the prevention of other birth defects and maternal and infant outcomes are unclear.

Objectives

This review updates and expands a previous Cochrane Review assessing the effects of periconceptional supplementation with folic acid to reduce neural tube defects (NTDs). We examined whether folate supplementation before and during early pregnancy can reduce neural tube and other birth defects (including cleft palate) without causing adverse outcomes for mothers or babies.

Search strategy

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (July 2010). Additionally, we searched the international clinical trials registry platform and contacted relevant organisations to identify ongoing and unpublished studies.

Selection criteria

We included all randomised or quasi-randomised trials evaluating the effect of periconceptional folate supplementation alone, or in combination with other vitamins and minerals, in women independent of age and parity.

Data collection and analysis

We assessed trials for methodological quality using the standard Cochrane criteria. Two authors independently assessed the trials for inclusion, one author extracted data and a second checked for accuracy.

Main results

Five trials involving 6105 women (1949 with a history of a pregnancy affected by a NTD and 4156 with no history of NTDs) were included. Overall, the results are consistent in showing a protective effect of daily folic acid supplementation (alone or in combination with other vitamins and minerals) in preventing NTDs compared with no interventions/placebo or vitamins and minerals without folic acid (risk ratio (RR) 0.28, 95% confidence interval (CI) 0.15 to 0.52). Only one study assessed the incidence of NTDs and the effect was not statistically significant (RR 0.08, 95% CI 0.00 to 1.33) although no events were found in the group that received folic acid. Folic acid had a significant protective effect for reoccurrence (RR 0.32, 95% CI 0.17 to 0.60). There is no statistically significant evidence of any effects on prevention of cleft palate, cleft lip, congenital cardiovascular defects, miscarriages or any other birth defects. There were no included trials assessing the effects of this intervention on maternal blood folate or anaemia at term.

We found no evidence of short-term side effects.

Authors' conclusions

Folic acid, alone or in combination with vitamins and minerals, prevents NTDs but does not have a clear effect on other birth defects.

PLAIN LANGUAGE SUMMARY

Folic acid supplements before conception and in early pregnancy (up to 12 weeks) for the prevention of birth defects

Folic acid is a synthetic form of folate used in supplements and fortified foods (like wheat and maize flour) to reduce the occurrence of neural tube defects (NTDs). These include spina bifida (or cleft spine), where there is an opening in one or more of the bones (vertebrae) of the spinal column, and anencephaly where the head (cephalic) end of the neural tube fails to close. Supplementation with folic acid is internationally recommended to women from the moment they are trying to conceive until 12 weeks of pregnancy. Another option recommended by the World Health Organization (WHO) is that women of reproductive age take weekly iron and folic acid supplements, especially in populations where the prevalence of anaemia is above 20%. Supplementation may also reduce other birth defects such as cleft lip with or without cleft palate and congenital cardiovascular defects. Recently, 5-methyl-tetrahydrofolate (5-MTHF) has been proposed as an alternative to folic acid supplementation. This is because most dietary folate and folic acid are metabolised to 5-MTHF. Some women have gene characteristics which reduce folate concentration in blood.

This review confirms that folic acid supplementation prevents the first and second time occurrence of NTDs and shows there is not enough evidence to determine if folic acid prevents other birth defects. Information about the safety of other current and alternative supplementation schemes and any possible effects on other outcomes for mothers and babies is also lacking. This review of five trials, involving 6105 women (1949 with a history of a pregnancy affected by a NTD and 4156 with no history of NTDs), shows the protective effect of daily folic acid supplementation in doses ranging from 0.36 mg (360 µg) to 4 mg (4000 µg) a day, with and without other vitamins and minerals, before conception and up to 12 weeks of pregnancy, for preventing the recurrence of these diseases. There were insufficient data to evaluate the effects on other outcomes such as cleft lip and palate. More research is needed on different types of supplementation programmes and the use of different types of supplements (such as 5-methyl-tetrahydrofolate -5-MTHF), particularly in countries where folic acid fortification of staple foods like wheat or maize flour is not mandatory and where the prevalence of NTDs is still high.

BACKGROUND

The main focus of this review is on the provision of folate as folic acid or 5-MTHF (with or without other vitamins and minerals) in the periconceptional period (prior to conception and in early pregnancy, before 12 weeks' gestation) to reduce the first and second time occurrence of neural tube defects (NTDs) and other birth defects. Other Cochrane Reviews and protocols focus on related topics such as oral iron with or without folic acid during pregnancy (Pena-Rosas 2009); treatment for iron deficiency and anaemia (Reveiz 2007); the use of various vitamin and multivitamin/micronutrient supplements for women during pregnancy (Haider 2006) and the effectiveness of oral folate supplementation

alone during pregnancy on haematological and biochemical parameters during pregnancy and on pregnancy outcomes (Haider 2008). This review updates and expands the scope of a previously published Cochrane Review on periconceptional supplementation with folate with or without multivitamins for preventing NTDs (Lumley 2001).

Introduction

Folate is a water-soluble B vitamin present in legumes, leafy green vegetables (such as spinach and turnip greens) and some fruits (such as citrus fruits and juices). Folic acid is the synthetic and most stable form of folate and the form often used in supplements and in fortified foods. The bioavailability of folic acid is approximately 70% higher than that of folate naturally contained in foods, although there are wide variations depending on the methodology used in the measurement (McNulty 2004).

Folate status in populations is generally assessed using static biochemical tests that directly measure folate in serum or in red blood cells (WHO 2008). Cut-off points to assess folate status have been proposed with an approach that relies on the combination of blood concentrations of the vitamin and the functional indicators in populations (Selhub 2008). The cut-off suggested to define deficiency is below 10 nmol/L (below 4 ng/mL) for serum folate, an indicator sensitive to recent usual intake; and below 340 nmol/L (below 151 ng/L) for red blood cell folate, an indicator of folate storage (WHO 2008). There are no universally accepted cut-off points to define deficiency during pregnancy, as concentrations decline over gestation and recover at delivery (WHO 2008), probably due to the physiologic haemodilution. Folate concentration measurements differ depending on the method used for assessment, particularly at the lower range of concentrations (CDC 2008a; Fazili 2007; Life Sciences Research Office 1994).

Description of the condition

Insufficient periconceptional folate and folic acid intake is associated with a number of birth defects that may also relate to genetic and environmental factors (IOM 2003) operating before conception or during early pregnancy. Birth defects can cause lifelong problems affecting health, growth and learning and may be immediately apparent after birth, or manifest later in life (Murray 1997; WHO 1999; WHO 2000). Environmental factors, including nutrition, are thought to contribute to about 5% to 10% of total birth defects (IOM 2003).

NTDs, which include anencephaly, spina bifida and encephalocoele, are congenital malformations that arise during the structural development of the neural tube, a process that is completed within 28 days after conception. In 1991, one randomised controlled trial (RCT) demonstrated that periconceptional folic acid supplementation prevented the recurrence of NTDs (MRC 1991) and in 1992 another RCT showed that a multiple micronutrient supplement containing folic acid prevented the occurrence of NTDs (Czeizel 1992). The latter results were confirmed in a public health campaign among women preparing for marriage conducted between 1993 and 1995 in China after which the risk of neural tube defects among the fetuses or infants of the women who took a folic acid supplement more than 80% of the time decreased by between 40% and 85% (Berry 1999).

While maternal intake of folate and folic acid is specifically associated with a decreased risk for NTDs they may also provide protection for other selected birth defects. There is suggestive evidence of protection from cardiovascular defects, Down syndrome, limb defects, cleft lip with or without cleft palate, urinary tract anomalies and congenital hydrocephalus (Coppede 2009; Eskes 2006; Goh 2006; Wilcox 2007). In the case of orofacial clefts, there are several similarities with NTDs: their occurrence at a similar time during embryogenesis, their involvement with the midline of the embryo, their near identical population characteristics and similar gene contributions. There is also some evidence of a suggested protective effect of folic acid use, especially for cleft lip with or without cleft palate (Wehby 2010), although this remains controversial, possibly because of the differences in dosage and type of supplementation (e.g. folic acid alone or with other micronutrients) used among studies (Botto 2004; Botto 2006). No effects have been shown in preventing pyloric stenosis, undescended testis or hypospadias. Approximately half of birth defects are limited to a single organ and the other half frequently present additional birth defects, such as heart malformations (Shibuya 1998).

Suspicion of a NTD may be raised by a maternal serum screening test during the second trimester of pregnancy which detects an elevated concentration of alpha-feto-protein. The diagnosis is confirmed by ultrasound examination during the second trimester of pregnancy. Cleft lip and palate can be identified on detailed ultrasound examination, but if there is only involvement of the palate, diagnosis by ultrasound can be difficult, and often not established until after birth. Unfortunately, these tests are not yet routinely done in most developing countries. Today, as infant mortality rates fall, birth defects are responsible for an increasing proportion of infant mortality and morbidity (Modell 1989; WHO 1997; WHO 2004). Affected infants have difficulty with feeding and later with speech development, hearing and tooth formation. Stigmatisation and discrimination may pose lifelong problems. Malnutrition and infection resulting from cleft lip or cleft palate, or both, can lead to severe illness and, in some cases, death (Shibuya 1998).

The impact of folate insufficiency on birth defects in different populations varies with each healthcare system. It partly relates to the use and coverage of preventive strategies including education and awareness of the importance of folic acid intake among women of reproductive age, access to and/or distribution of pre-pregnancy folic acid supplements, and/or fortification of staple foods with folic acid, in some cases with mandatory regulations for fortification of foods such as wheat and maize flour (CDC

2008b). Recent evidence demonstrates that public health policies which include folic acid fortification of staple foods are likely to result in a large-scale prevention of NTDs (Botto 2005; De Wals 2007; Berry 2010). It would seem reasonable to implement both interventions fully, especially in countries with a high prevalence of birth defects.

Several gene polymorphisms affect folate metabolism and are associated with reduced folate absorption and therefore increased folate needs. Some of the most studied mutations are the methylene-tetrahydrofolate reductase (MTHFR) gene and the reduced folate carrier (RFC1) gene (Chango 2000). The former affects 8% to 35% of the population, depending on ethnicity (Botto 2000; Guéant-Rodriguez 2006). In the absence of a folate-sufficient diet, these mutations are associated with increased risk of NTDs and conotruncal defects in the offspring (Van der Put 1998; Van Beynum 2006).

Folic acid intake may also affect fetal and child growth. Observational and controlled trials have showed a positive effect of periconceptional folic acid supplementation on fetal growth (Iyengar 1975; Relton 2005; Rolschau 1999), although the evidence for this association remains controversial.

Description of the intervention

In 1992, after evidence establishing the protective effect of folic acid supplementation against the first occurrence of NTDs emerged, the United States Public Health Service recommended daily supplementation with 400 µg of folic acid for all women of reproductive age (CDC 1992). The US Preventive Services Task Force (USPSTF) recommends that all women planning or capable of pregnancy take a daily supplement containing 0.4 to 0.8 mg (400 to 800 µg) of folic acid (US Preventive Services Task Force 2009). The World Health Organization recommends that women take 400 µg of folic acid from the moment they are trying to conceive until 12 weeks of pregnancy (WHO 2006). For women with a history of delivery of a baby with a NTD, have diabetes, or are receiving an anticonvulsant treatment, the recommended daily dose is 5 mg of folic acid in addition to dietary advice to increase food folate intake (IOM 2003; WHO 2006). Daily supplementation with 400 µg of folic acid in addition to iron is routinely recommended for all pregnant women (universal supplementation) to prevent anaemia (WHO/UNICEF/UNU 2001). The World Health Organization (WHO) also recommends weekly iron and folic acid supplementation (containing 2.8 mg of folic acid per week) in population groups where the prevalence of anaemia is above 20% among women of reproductive age, and where mass fortification programs of staple food-stuffs with iron and folic acid are unlikely to be implemented within the next one to two years (WHO 2009). The evidence of the effectiveness of the folic acid content of the supplements in a weekly regimen is limited, and little attention was given to the change in folic acid intake when supplements were given on a weekly as opposed to daily basis.

Recently, the use of 5-methyl-tetrahydrofolate (5-MTHF) has been proposed as an alternative to folic acid supplementation. The rationale for this is that most dietary folate and folic acid are metabolised to 5-MTHF during its passage across the intestinal mucosa. The 5-MTHF may be an adequate alternative for supplementation in the presence of MTHFR gene mutation. Four controlled trials using different doses have shown that supplementation with 5-MTHF is at least as effective as folic acid in improving folate status in women of childbearing age (Houghton 2006; Lamers 2004; Venn 2002; Venn 2003). This form of folate may also be less likely to mask haematological symptoms of severe vitamin B_{12} deficiency and it exhibits a lower interaction potential with antifolate antimalarial drugs (Nduati 2008; Pietrzik 2010), specifically sulphadoxine-pyrimethamine (SP), which might be affected by folic acid supplementation (Carter 2005; English 2006; Van Eijk 2008).

How the intervention might work

The main function of folate is as coenzyme in one-carbon transfer during the methylation cycle, a process essential for the syntheses of nucleic acids, which form part of DNA and the neurotransmitters. From these reactions it is immediately apparent why folate is so important to gene expression. Folate also plays an important role in protein synthesis and metabolism and other processes related to cell multiplication and tissue growth (WHO 2008). The main consequence of folate deficiency in adults is megaloblastic anaemia, characterised by abnormally large red-cell precursors in the bone marrow and larger than normal red cells in the peripheral blood.

The methylation of homocysteine to produce methionine (both essential amino acids) uses 5-MTHF as the methyl donor in the reaction. In folate deficiency, homocysteine accumulates in the serum resulting in negative effects for health. Elevated circulating homocysteine concentrations have been associated with an increased risk in cardiovascular disease (Refsum 2008) and late pregnancy complications such as pre-eclampsia (Makedos 2007; Patrick 2004; Tamura 2006), and possibly NTDs. Therefore, elevated plasma homocysteine may be a risk factor or, alternatively, merely a marker of risk (WHO 2008).

From the safety perspective, an observational study on the fetal origins of disease has proposed that normal to high maternal folate status coupled with low vitamin B_{12} status was associated with higher adiposity and insulin resistance in Indian babies (Yajnik 2008) which could probably have a long-term effect in the fetus later in life. Additional potentially undesired effects of folic acid supplementation come from the possible association of the use of multivitamins containing folic acid and an increase in twin pregnancies (Vollset 2005) as well as the ambiguous findings on the effects of folic acid supplementation on colonic lesions (Fife 2009; Jaszewski 2008; Wu 2009).

Why it is important to do this review

There seems to be sufficient evidence of known benefits of folic acid supplementation on NTDs (Lumley 2001) but not on other birth defects or on benefits to the mother. Furthermore, the best scheme (daily versus weekly), dose and/or form (5-MTHF versus folic acid) for providing folate supplements to women of childbearing age or during the periconceptional period are not yet established. It is also not known whether supplementation is of any benefit in places where wheat or maize flour fortification is mandatory and has proven to be effective for reducing NTDs rates. Additionally, concerns raised by some authors about the potential harm associated with high intake of folic acid merit exploration (Cole 2007; Mason 2007). This review aims to assess the evidence to respond these questions.

OBJECTIVES

This review updates the previous systematic review assessing the effects of periconceptional supplementation of folic acid to reduce neural tube defects (Lumley 2001) and aims to examine whether periconceptional folate supplementation can reduce the risk of neural tube and other birth defects (including cleft palate) without causing adverse outcomes for mothers or babies.

METHODS

Criteria for considering studies for this review

Types of studies

We have included both randomised and quasi-randomised trials. We had planned to include cluster-randomised trials if they were otherwise eligible. Other levels of evidence (e.g. cohort or case-control studies) have not been included in meta-analyses nor have they contributed to the results or conclusions, but we have considered such evidence in the discussion where relevant.

Types of participants

We included studies with women who become pregnant or were 12 or less weeks' pregnant at the time of the intervention, independent of age and parity. We have included studies focusing on women who have had a previous pregnancy affected by a neural tube defect. We excluded women who continued supplementation throughout pregnancy as the effectiveness of oral folate supplementation alone during pregnancy on haematological and biochemical parameters during pregnancy and on pregnancy outcomes are evaluated in another Cochrane Review (Haider 2008).

Types of interventions

We have included a range of interventions including supplementation with folic acid alone (FA) and with other vitamins and minerals. We have included studies where supplementation is offered in the periconceptional period and during early pregnancy. Where data were available we planned to compare:

- supplementation with FA versus no treatment/placebo/other micronutrients without folic acid;
- supplementation with FA alone versus no treatment/placebo;
- supplementation with FA + other micronutrients versus no treatment/placebo;
- supplementation with FA + other micronutrients versus other micronutrients (without folic acid);
- supplementation with 5-methyl-tetrahydrofolate (5MTHF) alone versus no treatment/placebo;
- supplementation with 5MTHF + micronutrients versus no treatment/placebo;
- supplementation with 5MTHF + micronutrients versus other micronutrients (without 5MTHF);
- supplementation with 5MTHF versus supplementation with FA.

Types of outcome measures

Primary outcomes

Infant

- Neural tube defects
- Cleft lip
- Cleft palate
- Congenital cardiovascular defects
- Other birth defects (excluding neural tube defects, cleft lip, cleft palate and cardiovascular defects)

Maternal

- Anaemia at term (defined as Hb less than 110 g/L)
- Red blood cell folate at term (nmol/L)
- Serum folate at term (nmol/L)
- Miscarriage (as defined by trialists)

Secondary outcomes

Infant

- Stillbirths (as defined by trialists)
- Neonatal deaths (death occurring in days 0 to 28 of life)

- Pregnancy termination for fetal abnormality (as defined by trialists)
- Low birthweight (less than 2500 g)
- Very low birthweight (less than 1500 g)
- Infant optimal health status at birth (as defined by trialists)
- Admission to special care for any cause (as defined by trialists)
- Macrosomia (greater than 4000 g)
- Infant insulin resistance (as defined by trialists)
- Apgar at one minute after birth (8 or greater)
- Apgar score at five minutes after birth (8 or greater)
- Preterm birth (less than 37 weeks' gestation)

Maternal

- Multiple pregnancy (2 or more fetuses at birth)
- Homocysteine at term (µmol/L)
- Serum vitamin B_6 concentration at term (nmol/L)
- Plasma or serum vitamin B_{12} concentration at term (pmol/L)
- Pre-eclampsia (defined as gestational hypertension (blood pressure higher than 140/90 mmHg and proteinuria (more than 300 mg of protein in a 24-hour urine sample)
- Any side effects (as defined by trialists)

Search methods for identification of studies

Electronic searches

We contacted the Trials Search Co-ordinator to search the Cochrane Pregnancy and Childbirth Group's Trials Register (July 2010).

The Cochrane Pregnancy and Childbirth Group's Trials Register is maintained by the Trials Search Co-ordinator and contains trials identified from:

1. quarterly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
2. weekly searches of MEDLINE;
3. handsearches of 30 journals and the proceedings of major conferences;
4. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Details of the search strategies for CENTRAL and MEDLINE, the list of handsearched journals and conference proceedings, and the list of journals reviewed via the current awareness service can be found in the 'Specialized Register' section within the editorial information about the Cochrane Pregnancy and Childbirth Group.

Trials identified through the searching activities described above are each assigned to a review topic (or topics). The Trials Search Co-ordinator searches the register for each review using the topic list rather than keywords.

Searching other resources

We contacted the World Health Organization, Centers for Disease Control and Prevention (CDC), and the International Clearinghouse for Birth Defects Monitoring Systems (ICBDMS) to identify ongoing studies and unpublished reports (correspondence is available upon request).

The international clinical trials registry platform (ICTRP) was also searched for any ongoing or planned trials. We did not apply any language restrictions.

Data collection and analysis

Selection of studies

One review author (LMR) screened all the titles and abstracts of all references identified as a result of the search strategy while JPR, AFG, and TD each assessed each a third. We resolved any disagreement through discussion.

In this version of the review all studies were reported in journal articles. In updates of the review if we identify trials published only as abstracts, or study reports containing insufficient information on methods, we will attempt to contact the trial authors to obtain further details of study design and results; if there is insufficient information for us to be able to assess risk of bias, studies will await assessment until further information is published, or made available to us.

Data extraction and management

We designed a form to extract data. For eligible studies, two review authors (TD, AFG) extracted the data using the agreed form. We resolved discrepancies through discussion. We entered data into Review Manager software (RevMan 2008) and TD and LMR carried out checks for accuracy.

When information regarding any of the above was unclear, we attempted to contact authors of the original reports to provide further details.

Assessment of risk of bias in included studies

Two review authors independently assessed risk of bias for each study using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2008). We resolved any disagreement by discussion or by involving a third assessor.

(1) Sequence generation (checking for possible selection bias)

We have described for each included study the method used to generate the allocation sequence.
We assessed the method as:

- adequate (any truly random process, e.g. random number table; computer random number generator);
- inadequate (any non-random process, e.g. odd or even date of birth; hospital or clinic record number);
- unclear.

(2) Allocation concealment (checking for possible selection bias)

We have described for each included study the method used to conceal the allocation sequence and assessed whether intervention allocation could have been foreseen in advance of, or during recruitment, or changed after assignment.
We assessed the methods as:

- adequate (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
- inadequate (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth);
- unclear.

(3) Blinding (checking for possible performance bias)

We have described for each included study the methods used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. For interventions involving the provision of nutritional supplements it may be possible to blind women, clinical staff and outcome assessors to group allocation by providing placebo preparations. Blinding has been assessed separately for different outcomes or classes of outcomes, and we have indicated where there was partial blinding (e.g. of outcome assessors).
We assessed the methods as:

- adequate, inadequate or unclear for participants;
- adequate, inadequate or unclear for personnel;
- adequate, inadequate or unclear for outcome assessors.

(4) Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations)

We have described for each included study, and for each outcome or class of outcomes, the completeness of data including attrition and exclusions from the analysis. We have noted whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusions where reported, and whether missing data were balanced across groups. Where sufficient information was reported, or supplied by the trial authors, we have re-included missing data in the analyses which we have undertaken.

Assessing bias associated with incomplete data may be particularly difficult in this topic area as the intervention may take place many months (or indeed years) before outcomes are assessed, and not all of those randomised may be eligible for all outcomes. For example, women randomised to receive supplements before conception may not become pregnant and therefore cannot experience outcomes that occur during pregnancy (such as miscarriage), or at birth (low birthweight infant). We decided that we would take a pragmatic approach and include in the denominators for pregnancy outcomes only for those women who became pregnant, rather than all women randomised. We are aware that this may introduce a serious source of bias, and we will note those outcomes likely to be affected by this, and advise caution in the interpretation of results.
We assessed methods as:

- adequate;
- inadequate;
- unclear.

(5) Selective reporting bias

We described for each included study how we investigated the possibility of selective outcome reporting bias and what we found.
We assessed the methods as:

- adequate (where it is clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review have been reported);
- inadequate (where not all the study's pre-specified outcomes have been reported; one or more reported primary outcomes were not pre-specified; outcomes of interest are reported incompletely and so cannot be used; study failed to include results of a key outcome that would have been expected to have been reported);
- unclear.

(6) Other sources of bias

We described for each included study any important concerns we have about other possible sources of bias.
We assessed whether each study was free of other problems that could put it at risk of bias:

- yes;
- no;
- unclear.

(7) Overall risk of bias

We made explicit judgements about whether studies are at high risk of bias, according to the criteria given in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2008). With reference to (1) to (6) above, we assessed the likely magnitude and direction of the bias and whether we considered it was likely to impact on the findings. We planned to explore the impact of the level of bias through undertaking sensitivity analyses - *see* Sensitivity analysis.

Measures of treatment effect

Dichotomous data

For dichotomous data, we presented results as summary risk ratio with 95% confidence intervals.

Continuous data

For continuous data, we used the mean difference if outcomes were measured in the same way between trials. We used the standardised mean difference to combine trials that measured the same outcome (e.g. serum folate concentration) but using different methods.

Unit of analysis issues

Cluster-randomised trials

We planned to include cluster-randomised trials in the analyses along with individually-randomised trials. If in the future we identify any such trials, in updates of this review we will adjust the standard error of the effect estimate from cluster trials using the methods described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2008). Meta-analyses will be carried out using the generic inverse-variance method available in RevMan (RevMan 2008). We will use an estimate of the intra cluster correlation co-efficient (ICC) derived from the trial (if possible), or from another source. If ICCs from other sources are used, we will report this and conduct sensitivity analyses to investigate the effect of variation in the ICC. If we identify both cluster-randomised trials and individually-randomised trials, we plan to synthesise the relevant information. We will consider it reasonable to combine the results from both if there is little heterogeneity between the study designs, and the interaction between the effect of intervention and the choice of randomisation unit is considered to be unlikely.

We will also acknowledge heterogeneity in the randomisation unit and perform a separate meta-analysis.

Cross-over trials

Cross-over trials are not an appropriate study design for the interventions considered in this review and have been excluded.

Studies with more than two treatment groups

For studies with more than two intervention groups (multi-arm studies) in the main analysis (comparison one) we combined groups to create a single pair-wise comparison (Higgins 2008).

Dealing with missing data

For included studies, we have noted levels of attrition. We explored the impact of including studies with high levels of missing data in the overall assessment of treatment effect by using sensitivity analysis.

We planned to carry out analyses, as far as possible, on an intention-to-treat basis, i.e. by attempting to include all participants randomised to each group in the analyses, so the denominator for each outcome in each trial would be the number randomised minus any participants whose outcomes are known to be missing. In those studies where women were recruited before conception for outcomes relating to pregnancy we have taken a pragmatic approach and included in the denominators only those women known to become pregnant.

Assessment of heterogeneity

We examined the forest plots from meta-analysis to look for heterogeneity among studies, and used the I^2 and T^2 statistics to quantify the level of heterogeneity among the trials in each analysis. If we identified substantial heterogeneity (I^2 greater than approximately 50%) we noted this in the text and explored it by pre-specified subgroup analysis. We would advise caution in the interpretation of those results where there are high levels of unexplained heterogeneity.

Assessment of reporting biases

Where we suspected reporting bias (*see* 'Selective reporting bias' above) we attempted to contact study authors, asking them to provide missing outcome data. We have not explored possible publication bias by producing funnel plots as too few studies contributed data to the review.

Data synthesis

We carried out statistical analysis using the Review Manager software (RevMan 2008). We have used fixed-effect meta-analysis for combining data where trials were examining the same intervention, and the trials' populations and methods were judged sufficiently similar. Where we suspected clinical or methodological heterogeneity between studies sufficient to suggest that treatment effects might differ between trials we repeated the analysis using a random-effects model.

Subgroup analysis and investigation of heterogeneity

Where data were available we had planned to carry out subgroup analysis for primary outcomes:

1. by scheme: daily supplementation versus weekly supplementation;
2. by dose: 400 µg/d or less of folic acid versus more than 400 µg folic acid per day;

3. by start of supplementation: before pregnancy versus during first trimester versus mixed;

4. by assisted reproduction: assisted versus non-assisted reproduction;

5. by mandatory folic acid fortification: places with mandatory flour fortification versus non-flour fortification or not mandatory;

6. by history of a pregnancy affected by a neural tube defect (recurrence): yes versus no.

We planned that for both fixed- and random-effects meta-analyses we would examine differences between subgroups by inspection of the subgroup confidence intervals; non-overlapping confidence intervals indicating a statistically significant difference in treatment effect between the subgroups. We carried out few random-effects analyses as levels of heterogeneity were generally low.

Sensitivity analysis

We planned to carry out sensitivity analysis to examine the effects of removing studies at high risk of bias (studies with poor allocation concealment) from the analysis. In this version of the review none of the trials were assessed as having poor allocation concealment. If, in updates of the review, cluster trials are included we will carry out sensitivity analysis using a range of intra cluster correlation values.

RESULTS

Description of studies

See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of ongoing studies.

Results of the search

The search strategy retrieved 117 references corresponding to 59 trials. Five trials (31 references) were included and one trial is still ongoing and its results are expected in 2011 (see Characteristics of ongoing studies).

Included studies

We included five trials involving 6105 women in the review, all of which met the pre-stated inclusion criteria. Most of the studies focused on infant outcomes and few of the results reported related to the maternal outcomes we had pre-specified in the protocol. All of the studies that we included were published before 2001. In all of them women were supplemented daily; in one trial women received less than 400 µg (0.4 mg) of folic acid per day (Kirke 1992), while in the remaining studies women consumed 800 µg (0.8 mg) (Czeizel 1994); 2000 µg (2.0 mg) per day (Laurence 1981) and 4000 µg (4.0 mg) per day (ICMR 2000; MRC 1991). In all trials women started supplementation before pregnancy and discontinued it after 12 weeks of pregnancy. Sample sizes varied among studies. All trials focused on the prevention of neural tube defects; four trials evaluated recurrence (ICMR 2000; Kirke 1992; Laurence 1981; MRC 1991) and only one occurrence (Czeizel 1994). Of the 6105 women included in the review, 1949 had a history of NTDs and 4156 had no history.

One trial compared folic acid supplementation with a placebo group (Laurence 1981), two trials compared folic acid plus multiple micronutrients versus multiple micronutrients (Czeizel 1994; ICMR 2000) although the control groups received different formulations. One trial included four comparison groups: one with folic acid with iron and calcium, one with folic acid plus iron, calcium and multiple micronutrients, another with iron, calcium and multiple micronutrients without folic acid and a control group that only received iron and calcium but did not receive multiple micronutrients nor folic acid (MRC 1991). In this study the iron and calcium in the capsules was provided as ferrous sulphate and dicalcium phosphate. In the another included study (Kirke 1992) there were three comparison groups: one with folic acid; another with multiple micronutrients and another with multiple micronutrients and folic acid. The allocation of women to the control group in this study (Kirke 1992) was not randomised but the comparison was still included in the analyses.

We did not identify any randomised controlled trials which examined either weekly supplementation or the use of 5-MTHF.

Excluded studies

We excluded 53 trials: 41 trials because their study design or scope did not match the objectives of this review; three trials reported studies performed in non-pregnant women; one trial was a non-randomised clinical trial, and eight trials were on folic acid supplementation that started in the first trimester of gestation and continued throughout pregnancy.

See Characteristics of excluded studies tables for a detailed description of the studies and the reasons for exclusion.

Risk of bias in included studies

Allocation

Sequence generation: Three trials adequately randomised the participants to the treatment groups (Czeizel 1994; Kirke 1992; Laurence 1981); one of them used block randomisation (Kirke 1992). Two multicentre trials did not report or did not state clearly the method used to generate the randomisation sequence (ICMR 2000; MRC 1991).

Allocation concealment: Two trials reported using sealed envelopes, opaque bottles or similar pills when doing the allocation of the women to treatment groups (ICMR 2000; Kirke 1992). In this last study pills were changed after one year resulting in a partial loss of blinding. The three remaining studies were reported as blinded, but were unclear in their method of concealment of treatment allocation.

Blinding

All of these trials were described as "double-blind" although it was not clear whether outcome assessors were blind to group allocation. Women in all treatment groups received capsules or tablets containing active treatment ingredients or placebo although it was not always clear whether the preparations were of identical appearance.

Incomplete outcome data

For those women becoming pregnant, loss to follow up ranged from 1% (Czeizel 1994; MRC 1991) to 20% (Kirke 1992). The remaining two trials lost less than 10% of the randomised participants.

We noted in the methods section above that for pregnancy outcomes we would include in the denominators only those women with confirmed pregnancies. In all studies women were randomised before conception and not all women became pregnant. For example, in the study by Czeizel 1994 of 7905 randomised, 5502 had confirmed pregnancy (69.6%); so 30% of those randomised did not become eligible to experience pregnancy and birth outcomes. We are aware that this makes results more difficult to interpret. Where data were available, we have provided information on the number of women randomised and the numbers with confirmed pregnancies in the Characteristics of included studies tables.

In some cases we did not find it simple to determine the denominators as detailed information on attrition at different stages was not reported. For some competing/overlapping outcomes (e.g. perinatal deaths, miscarriages and pregnancy termination for fetal abnormality) we have reported figures provided in the trial reports but we advise caution in interpreting such data.

Selective reporting

Assessing selective reporting bias was difficult as we did not have access to study protocols. We were not able to explore possible reporting bias as too few studies have been included to make allow us to carry out meaningful analyses.

Other potential sources of bias

In one study (Czeizel 1994) the randomisation code was broken twice: once to analyse the teratogenic effect of vitamin A and at the end of the trial to analyse the effect of the supplementation. One study (ICMR 2000) was terminated before expected as a consequence of the publication of the results from another trial (MRC 1991); however the calculated sample size was almost achieved.

See Characteristics of included studies tables for assessments of the methodological quality of each included trial and Figure 1 and Figure 2 for summaries of the quality of included studies.

Figure 1. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.

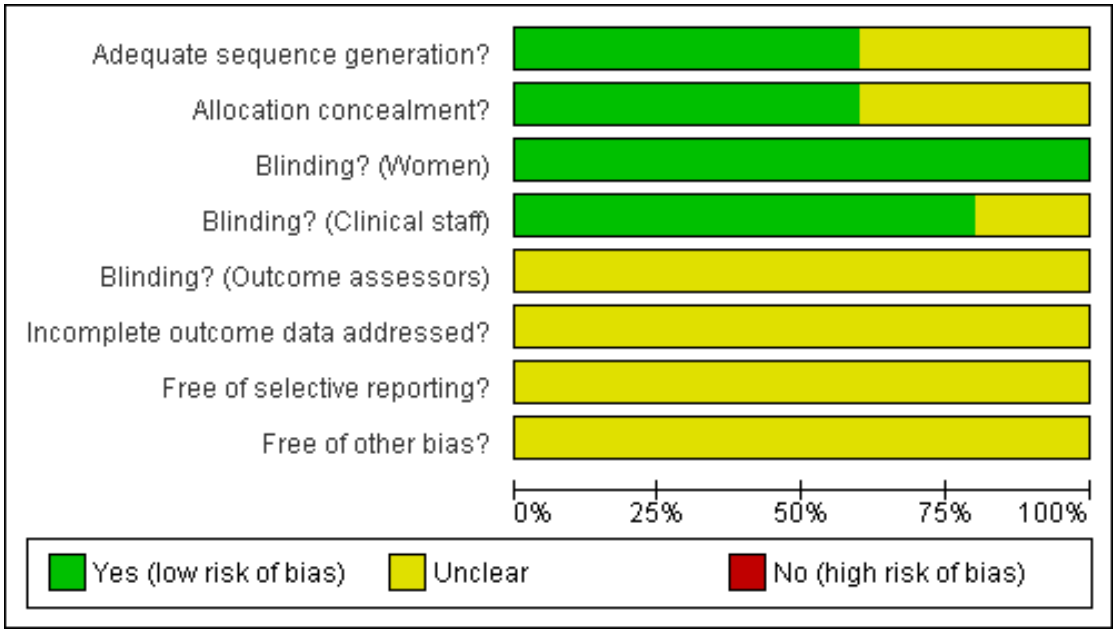
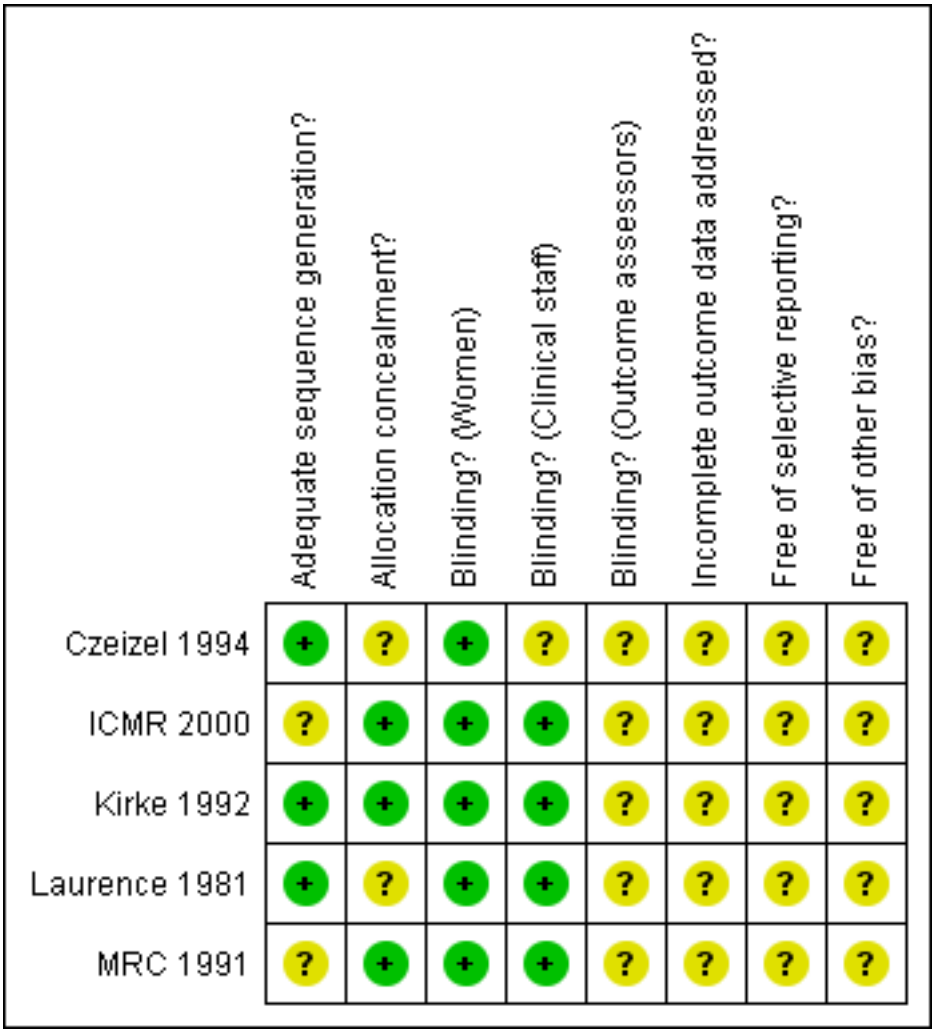


Figure 2. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.



Effects of interventions

The summary of results is organised by comparisons and by infant and maternal outcomes. *See* the Data and analyses section for detailed results on primary and secondary outcomes.

Four of the five trials included in the review recruited women with a history of NTDs and subgroup analyses suggested no clear differences in trials examining first occurrence and recurrence of NTDs. However to aid interpretation of results, along with reporting overall findings from trials, we have described the results from the trials examining first occurrence and recurrence of NTDs separately.

(1) Supplementation with folic acid versus any other interventions/placebo (five trials)

Primary outcomes

Infant outcomes

Neural tube defects (NTDs) (all)

Five trials (Czeizel 1994; ICMR 2000; Kirke 1992; Laurence 1981; MRC 1991) with 6105 women examined the prevalence of NTDs in women receiving folic acid supplementation (alone or in combination with other vitamins and minerals) compared with women receiving placebo or other vitamins and minerals (not including folic acid) (Analysis 1.1). In all of these trials, supplementation started before pregnancy, continued throughout the first trimester, and involved daily supplementation. Periconceptional folate supplementation (alone, or in combination with other vitamins and minerals) reduces the prevalence of NTDs in comparison with no intervention, placebo or multiple micronutrients without folic acid (risk ratio (RR) 0.28, 95% confidence interval (CI) 0.15 to 0.52).

Four trials recruited women with a history of NTDs (ICMR 2000; Kirke 1992; Laurence 1981; MRC 1991) and supplementation was associated with a reduction in the recurrence of a pregnancy affected by a NTD (RR 0.32, 95% CI 0.17 to 0.60). In the single trial (4156 women) (Czeizel 1994) recruiting women with no history of NTDs, and examining the first occurrence of NTDs, results favoured the group receiving supplementation, but evidence of a difference between groups did not reach statistical significance (RR 0.08, 95% CI 0.00 to 1.33); however, no cases of NTDs were observed in the supplemented group.

In one of the trials involving 364 women (Kirke 1992) the daily supplementation dose was 360 µg (0.36 mg); while results from this study favoured the intervention group they were not statistically significant (RR 0.12, 95% CI 0.01 to 2.29). In the remaining four trials (Czeizel 1994; ICMR 2000; Laurence 1981; MRC 1991) the daily dose of folic acid was more than 400 µg (0.4 mg) and NTDs were reduced in the intervention group; the difference between treatment groups was statistically significant (RR 0.30, 95% CI 0.16 to 0.56).

Cleft palate

Overall, only three babies were affected with cleft palate in the studies examining this outcome (Czeizel 1994; Kirke 1992; MRC 1991); there was no significant difference between the group receiving folic acid supplementation and controls (RR 0.66, 95% CI 0.11 to 3.92).

Cleft lip

In the three trials (Czeizel 1994; Kirke 1992; MRC 1991) reporting the number of babies affected by cleft lip, there was no evidence of a difference between groups; overall eight babies were affected by cleft lip, four in each treatment group (Analysis 1.5; Analysis 1.6).

Congenital cardiovascular defects

This outcome was reported in three trials (Czeizel 1994; Kirke 1992; MRC 1991). There was no significant evidence of a difference between experimental and control groups in the number of babies with congenital cardiovascular defects irrespective of dose of drug, or history of NTDs (Analysis 1.7; Analysis 1.8).

Other birth defects (excluding NTDs, cleft lip, cleft palate and cardiovascular defects)

Three trials reported the number of babies with other birth defects (Czeizel 1994; Kirke 1992; MRC 1991). Overall, when results from these three trials were pooled there was no significant evidence of a difference between groups (RR 0.72, 95% CI 0.48 to 1.07). However, for this outcome there was high heterogeneity (I^2 = 63%) and we repeated the analysis using a random-effects model (average RR 0.81, 95% CI 0.38 to 1.77) and the lack of effect was confirmed. In the Czeizel 1994 trial which recruited women with no history of NTDs, the difference between groups, favouring women in the supplementation group, was statistically significant (RR 0.50, 95% CI 0.30 to 0.84).

Maternal outcomes

Maternal anaemia at term (defined as Hb less than 110 g/L); red blood cell folate at term; serum folate at term

No trials reported on these outcomes.

Miscarriage

All five trials examined the rate of miscarriage in women with confirmed pregnancy receiving daily folic acid supplements compared with controls (Czeizel 1994; ICMR 2000; Kirke 1992; Laurence 1981; MRC 1991); while the number of miscarriages was increased in the group receiving supplements containing folic acid the difference between groups did not reach statistical significance (RR 1.10, 95% CI 0.97 to 1.26). There were no significant differences between experimental and control groups for any of the subgroups we examined (Analysis 1.12).

Secondary outcomes

Infant outcomes

Stillbirths

Stillbirths were reported in four trials (Czeizel 1994; ICMR 2000; Kirke 1992; MRC 1991). There was no significant evidence of any difference between treatment groups in the pooled analysis (RR 0.96, 95% CI 0.51 to 1.83), or in any subgroup analyses (Analysis 1.14).

Pregnancy termination for fetal abnormality

Overall, in the four studies examining this outcome (Czeizel 1994; ICMR 2000; Laurence 1981; MRC 1991) there were 58 pregnancies terminated for fetal abnormality; 13/2978 in the experimental group and 45/2930 in the control group. The difference between groups was statistically significant in the pooled analysis (RR 0.30, 95% CI 0.16 to 0.54). In all of these studies daily supplementation was greater than 400 µg (0.4 mg) and women started supplements before pregnancy.

Low birthweight (less than 2500 g)

The number of babies with low birthweight was examined in one trial (186 babies) (ICMR 2000). There was no significant evidence of a difference between treatment groups (Analysis 1.19).

Other infant outcomes

No trials reported results for our other pre-specified secondary outcomes:

- Very low birthweight (less than 1500 g)
- Infant optimal health status at birth (as defined by trialists)
- Neonatal deaths
- Admission to special care for any cause (as defined by trialists)
- Macrosomia (less than 4000 g)
- Infant insulin resistance (as defined by trialists)
- Apgar at one minute after birth (8 or greater)
- Apgar score at five minutes after birth (8 or greater)
- Preterm birth (less than 37 weeks' gestation)
- Long-term outcomes (as defined by trialists)

Maternal

Multiple pregnancy

The number of women affected by multiple pregnancy was examined in three trials (Czeizel 1994; ICMR 2000; MRC 1991). Overall, there was no evidence of any significant difference between women receiving supplements with folic acid and those in the controls (RR 1.32, 95% CI 0.88 to 1.98), nor was there evidence of differences between treatment groups in the subgroups we examined (Analysis 1.16).

Side effects

Only one study reported findings on side effects like nausea, vomiting, constipation or diarrhoea (Czeizel 1994) and the number of reported cases was very low in both the group of women receiving folic acid with multiple micronutrients and the control group during the pre-pregnancy and pregnancy period.

Other maternal outcomes

No trials reported on our other pre-specified secondary outcomes:

- Homocysteine at term (µmol/L)
- Serum vitamin B_6 concentration at term (nmol/L)
- Plasma or serum vitamin B_{12} concentration at term (pmol/L)
- Pre-eclampsia (defined as gestational hypertension (blood pressure higher than 140/90 mmHg) and proteinuria (more than 300 mg of protein in a 24-hour urine sample)

(2) Supplementation with folic acid alone versus no treatment/placebo (two trials)

Primary outcomes

Infant outcomes

NTDs (all)

In two trials (Kirke 1992; Laurence 1981) involving 299 women, supplementation in the experimental group was with folic acid alone (rather than with folic acid plus other vitamins and minerals). Overall, the prevalence of NTDs was lower in the group receiving folic acid supplementation compared with controls; the difference between groups was statistically significant (RR 0.32, 95% CI 0.08 to 1.34). In all of these trials women had a history of a pregnancy affected by a NTD, supplementation started before pregnancy, continued throughout the first trimester, and involved daily supplementation.

One of the trials (Kirke 1992) involved a lower dose of folic acid (360 µg). In this study the difference between groups was not significant (Kirke 1992) for this outcome.

Cleft palate

In the only study reporting this outcome, with 188 women (Kirke 1992), there were no cases affected by a cleft palate (Analysis 2.3).

Cleft lip

No babies were affected with cleft lip in the only study reporting this outcome (Kirke 1992).

Congenital cardiovascular defects

This outcome was reported in one trial (Kirke 1992). Only one baby in the no treatment/placebo group was affected with a congenital cardiovascular defect (Analysis 2.7).

Maternal outcomes

Maternal anaemia at term (defined as Hb less than 110 g/L); red blood cell folate at term; serum folate at term

No trials reported on these outcomes.

Miscarriage

Two trials (299 women) examined the rate of miscarriage in women with confirmed pregnancies receiving folic acid supplements compared with controls (Kirke 1992; Laurence 1981); there was no significant evidence of a difference between treatment groups in the number of women having miscarriage (Analysis 2.11).

Secondary outcomes

Infant outcomes

Stillbirths

Stillbirths were reported in one trial (Kirke 1992). Overall there were four stillbirths in the no treatment/placebo group; the difference between groups was not statistically significant (RR 0.13, 95% CI 0.01 to 2.46).

Pregnancy termination for fetal abnormality

One study contributed data to this analysis (Laurence 1981); the number of pregnancy terminations for fetal abnormality was reduced in the folic acid group. The difference was not statistically significant (RR 0.28, 95% CI 0.06 to 6.83).

Other infant outcomes

No trials reported on our other pre-specified secondary outcomes.

Maternal

No trials reported on our pre-specified secondary outcomes:

(3) Supplementation with folic acid + other micronutrients versus no treatment/placebo (one trial)

Primary outcomes

Infant outcomes

NTDs (all)

One trial (Kirke 1992) involving 190 women contributed data to this comparison. The dose of folic acid was 360 µg (Kirke 1992). The risk of NTDs was lower in the group receiving supplementation with folic acid and other micronutrients compared with controls (RR 0.17, 95% CI 0.01 to 3.22). In this trial, women had a history of a pregnancy affected by a NTD, supplementation started before pregnancy, continued throughout the first trimester, and involved daily supplementation.

Cleft palate and cleft lip

No babies included in the comparison were affected by cleft lip or palate (Analysis 3.3; Analysis 3.5).

Congenital cardiovascular defects

This outcome was reported in one trial (Kirke 1992). There was only one baby (in the no treatment/placebo group) affected by a congenital cardiovascular defect (Analysis 3.7).

Other birth defects (excluding neural tube defects, cleft lip, cleft palate and cardiovascular defects)

Other birth defects were reported in one trial (Kirke 1992). There were two defects in the folic acid group (2/87) and two the control group (2/103) and the difference between groups was not statistically significant (RR 1.18, 95% CI 0.17 to 8.23).

Maternal outcomes

Maternal anaemia at term (defined as Hb less than 110 g/L); red blood cell folate at term; serum folate at term

No trials reported on these outcomes.

Miscarriage

There were two cases of confirmed pregnancy suffering miscarriage among the women who received the folic acid and none in the women who received no treatment/placebo (2/87 in the folic acid group compared with 0/103 in the no treatment/placebo group) although the difference between groups did not achieve statistical significance (RR 5.91, 95% CI 0.29 to 121.46). (Analysis 3.11).

Secondary outcomes

Infant outcomes

Stillbirths

Stillbirths were reported in one trial (Kirke 1992). Overall there were four stillbirths, all in the no treatment/placebo group. However, there was no significant evidence of any difference between groups (RR 0.13, 95% CI 0.01 to 2.41).

Other infant outcomes

No trials reported on our other pre-specified secondary outcomes.

Maternal

No trials reported on our pre-specified maternal secondary outcomes.

(4) Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid) (four trials)

Primary outcomes

Infant outcomes

NTDs (all)

Four of the trials (Czeizel 1994; ICMR 2000; Kirke 1992; MRC 1991) (5806 women) included comparisons of women receiving folic acid with other micronutrients compared with women receiving other micronutrients without folic acid. There was a statistically significant difference between groups favouring those who received the folic acid supplementation (RR 0.29, 95% CI 0.15 to 0.56). In all of these trials supplementation started before pregnancy, continued throughout the first trimester, and involved daily supplementation.

Results in subgroups are identical or similar to those in comparison one.

Cleft palate

Two babies (both in the control group) were affected with cleft palate in the three studies examining this outcome (Czeizel 1994; Kirke 1992; MRC 1991); the difference between groups was not statistically significant (RR 0.66, 95% CI 0.11 to 3.92).

Cleft lip

In the three trials (Czeizel 1994; Kirke 1992; MRC 1991) reporting the number of babies affected by cleft lip, there was no evidence of a difference between groups; overall eight babies were affected by cleft lip, four in each treatment group (Analysis 4.5).

Congenital cardiovascular defects

This outcome was reported in three trials (Czeizel 1994; Kirke 1992; MRC 1991). There was no statistically significant evidence of a difference between experimental and control groups in the number of babies with congenital cardiovascular defects irrespective of dose of drug, or history of NTDs (Analysis 4.7).

Other birth defects (excluding neural tube defects, cleft lip, cleft palate and cardiovascular defects)

Three trials reported the number of babies with other birth defects (Czeizel 1994; Kirke 1992; MRC 1991). There was no significant evidence of a difference between groups (RR 0.75, 95% CI 0.50 to 1.12). In the Czeizel 1994 trial which recruited women with no history of NTDs, the difference between groups, favouring women in the folate supplementation group, was significant (RR 0.53, 95% CI 0.31 to 0.89).

Maternal outcomes

Maternal anaemia at term (defined as Hb less than 110 g/L); red blood cell folate at term (nmol/L); serum folate at term (nmol/L)

No trials reported on these outcomes.

Miscarriage

Four trials reported the number of women with confirmed pregnancy suffering miscarriage (Czeizel 1994; ICMR 2000; Kirke 1992; MRC 1991); while the number of miscarriages was increased in the group receiving folic acid the difference between groups did not reach statistical significance (RR 1.10, 95% CI 0.96 to 1.26). There were no significant differences between experimental and control groups for any of the subgroups we examined (Analysis 4.12).

Secondary outcomes

Infant outcomes

Stillbirths

Stillbirths were reported in four trials included in this comparison (Czeizel 1994; ICMR 2000; Kirke 1992; MRC 1991). There was no significant evidence of any difference between treatment groups in the pooled analysis (RR 1.36, 95% CI 0.68 to 2.75), or in any subgroup analyses (Analysis 4.14).

Pregnancy termination for fetal abnormality

Overall, in the three studies examining this outcome (Czeizel 1994; ICMR 2000; MRC 1991) there were 57 pregnancies terminated for fetal abnormality; 13/2918 in the women who received folic acid with other micronutrients and 44/2879 in the control group who received other micronutrients supplements without folic acid. The difference between groups was statistically significant in the pooled analysis (RR 0.30, 95% CI 0.16 to 0.55).

Low birthweight (less than 2500 g)

The number of babies with low birthweight was examined in one trial (186 babies) (ICMR 2000). There was no significant evidence of a difference between treatment groups (Analysis 4.19).

Other infant outcomes

No trials reported results for our other pre-specified secondary outcomes.

Maternal

Multiple pregnancy

The number of women affected by multiple pregnancy was examined in three trials (Czeizel 1994; ICMR 2000; MRC 1991). There was no significant evidence of a difference between women in the two treatment groups (RR 1.32, 95% CI 0.88 to 1.98).

Other maternal outcomes

No trials reported on our other pre-specified secondary outcomes.

(5) Supplementation with 5-methyl-tetrahydrofolate (5MTHF) alone versus no treatment/placebo

We did not identify any trials for inclusion in the review which examined this comparison.

(6) Supplementation with 5MTHF + micronutrients versus no treatment/placebo

We did not identify any trials for inclusion in the review which examined this comparison.

(7) Supplementation with 5MTHF + micronutrients versus other micronutrients (without 5MTHF)

We did not identify any trials for inclusion in the review which examined this comparison.

(8) Supplementation with 5MTHF versus supplementation with folic acid

We did not identify any trials for inclusion in the review which examined this comparison.

DISCUSSION

Summary of main results

This review addresses the effects and safety of folic acid supplementation or folic acid with other micronutrients versus no treatment/

placebo or other micronutrients (without folic acid) during the periconceptional period and the first trimester of pregnancy. This review updates and extends an earlier Cochrane Review (Lumley 2001).

To reduce the risk of neural tube defects (NTDs) for women capable of becoming pregnant, the recommendation is to take 400 μg of folic acid daily from fortified foods, supplements, or both, in addition to consuming food folate from a varied diet beginning at least one month before conception (IOM 1998; WHO/UNICEF/UNU 2001). Expert panels suggest that supplemental intake in this population should range between 400 μg and 800 μg (US Preventive Services Task Force 2009). The first recommendations were based on the amount needed to maintain an adequate haematological status, and the protective role of folic acid against NTDs was later confirmed with a public health supplementation trial carried out in China from 1993 to 1995 which showed a reduction in the occurrence of neural tube defects (NTDs) of 40% to 85% (from the south and north of the country, respectively) (Berry 1999). The results of this review clearly show that periconceptional folic acid supplementation reduces the recurrence of NTDs and although there is only one trial assessing first time occurrence of NTDs (with wide confidence intervals that cross the null value) no events were found among women supplemented with folic acid (Czeizel 1994). The evidence from early clinical trials designed to study the effect of folic acid supplementation around the time of conception on NTDs is so strong that this probably accounts for why we only found one additional RCT looking at this matter (ICMR 2000) and few observational studies published from 1995 to date that reinforce these findings (US Preventive Services Task Force 2009). There are no trials exploring the effect of 5MTHF during the periconceptional period, but it is very likely that the benefits of this intervention observed during the reproductive age and lactation (Houghton 2006; Lamers 2004) might extend to pregnancy.

Questions remain about the best dose and periodicity of folic supplementation as there is still a high prevalence of birth defects worldwide. Doses ranging from 360 μg (0.36 mg) (Kirke 1992) to 6000 μg (6 mg) (Chen 2008) a day have proven to be effective in preventing both occurrence and recurrence of NTDs; this wide response to supplementation may be determined by the baseline blood folate concentrations in each population. Improving nutrition surveillance in order to find the appropriate dose and supplementation scheme is crucial to promote a cost-effective public health policy that can reach a larger number of people.

It is very likely that the lack of results we observed on maternal and perinatal outcomes was related to the short exposure to the intervention. Currently, the World Health Organization recommends that women receive daily supplements of iron and folic acid throughout pregnancy. Some of the trials excluded in this review, where supplementation started before and continued beyond 12 weeks of pregnancy, showed positive effects on both maternal and infant outcomes and one of them also showed improved survival at seven years of age (Christian 2003). Therefore, it seems adequate to encourage periconceptional plus gestational supplementation to improve perinatal results.

All the trials included in this review were performed before many countries introduced mandatory flour fortification with folic acid. In the US, for example, the flour fortification programme was designed so that typical folic acid intake would be increased by approximately 100 µg/day and that the risk of intakes greater than 1000 µg/day (the FDA's tolerable upper level of daily intake) would be minimal (Daly 1997). Results showed that the typical intake of folic acid from fortified foods is more than twice as high as originally predicted (Quinlivan 2003). Even though the effectiveness of mandatory folic acid fortification programmes has been documented by a decline in the prevalence of NTDs in the United States, Canada, Costa Rica, Chile, and South Africa (Berry 2010), fortified foods (mandatory and not) might be providing relatively large amounts of folic acid to the entire population and some authors have suggested that the recommendation of supplementing 400 µg/day of folic acid in addition to dietary folate to women planning to become pregnant deserves to be reviewed. However, they acknowledge the lack of certainty on the minimum dose of supplemental folic acid that is effective for reducing NTDs occurrence (Dary 2009).

There are no clinical trials that review the effect of weekly periconceptional folate supplementation for the prevention of birth defects. The weekly iron and folic acid supplementation programme (WIFS) studies (WHO 2009) have proven that weekly supplementation is an effective preventive intervention to control iron deficiency among childbearing age women, with good compliance. There is no information on the impact of such schemes on birth defects. Two published studies have evaluated weekly folic acid supplementation before pregnancy (not randomised controlled trials). In the first one, carried out in Mexico, women received 5000 µg (5 mg) folic acid for three months, and their red blood cell and plasma folate concentrations significantly increased (Martinez-de Villareal 2001). This strategy was also associated with a 50% decrease in the incidence of anencephaly and spina bifida cases, and a significant reduction in infant mortality and disability after two years (Martinez-de Villareal 2002). In the second study, conducted in New Zealand, a weekly single supplement of 2800 µg (2.8 mg) of folic acid taken for 12 weeks increased women's red blood cell folate to concentrations associated with a reduced risk of bearing a child with a NTD (Norsworthy 2004).

New studies have documented that MTHFR polymorphism may be considered a susceptible gene for cardiovascular birth defects (Brandalize 2009; Marinho 2009). If MTHFR is associated with congenital heart defects (i.e. Tetralogy of Fallot), this may in part explain the lack of effect of periconceptional folic acid supplementation on cardiovascular malformations, and may warrant further study for opportunities for preventing other birth defects.

This review found a protective effect against birth defects with the use of folic acid combined with other micronutrients. From

a metabolic perspective, it is clear that niacin, thiamin, vitamin B_6, and vitamin B_{12} influence reactions that include folate. The possibility that other micronutrients apart from folic acid may have an additional protective effect has been a matter considered in several more recent studies (Czeizel 2004; Goh 2006; Thompson 2009).

Overall completeness and applicability of evidence

Periconceptional folic acid supplementation has a strong protective effect on the incidence and recurrence of NTDs, but there is not sufficient evidence to assess its effect either on the occurrence or recurrence of other defects at birth, nor on most maternal primary and secondary outcomes.

The force of the results makes this an intervention that can be applied in most settings for reducing NTDs rates. However, the women in the trials included in this review had Caucasian, Anglo-Saxon, Anglo-American and Indian backgrounds and the evidence suggests that the presence of gene polymorphisms (i.e. MTHFR gene mutation) is more prevalent among people of Latin origins (Guéant-Rodriguez 2006), thus the findings may slightly differ across all population groups. Also, there is still a need to understand the applicability of these results in endemic malaria regions where antifolate antimalarial drugs are being used, and in women living with HIV/AIDS, receiving or not receiving antiretroviral therapy. Women in the trials included in this review were actively planning pregnancy and attending for preconception care. It is estimated that 80 million women each year have unwanted or unintended pregnancies (Glasier 2006), and women seeking preconception care may be more affluent and more educated, or be seeking care as they are at high risk after previous pregnancy complications (Czeizel 1999b; Elsinga 2006). These women may be more likely to comply with prescribed supplementation schemes, and the same level of compliance and effect may not be achieved by other groups.

Quality of the evidence

The included trials were rated of medium to high quality in terms of allocation concealment and blinding, and pooling results in meta-analysis resulted in fairly low levels of between-study heterogeneity. Having said this, in trials where only a subset of the original randomised sample became eligible to experience many of the outcomes measured, results may be at risk of bias and not simple to interpret. In all the trials included in this review, studies recruited non-pregnant women (albeit women that may have been planning pregnancy) and inevitably only a proportion of those randomised had confirmed pregnancy during the study periods. As the focus of the review was on pregnancy outcomes, we decided that denominators would include only those women who became pregnant.

Restricting the analyses to pregnant women may have led to the size of the treatment effect for some outcomes being either over or under-estimated. It is possible that women who became pregnant may have been different in a number of respects from those that did not, and that the intervention may have had a different effect on those women that did or did not become pregnant. It is possible that the intervention may have an impact on the ability to become pregnant or to sustain a pregnancy during the very early stages (before pregnancy confirmation) although there is no strong evidence from the studies included in this review or in the literature to suggest that this was likely to have been the case. One study (Czeizel 1994) suggested small but statistically significant differences in confirmed pregnancy rates in women in the intervention and control groups (71.3% versus 67.9% respectively); however, this finding may have occurred by chance or been confounded by the use of in vitro fertilisation by some women (Berry 2004). The possible difference between groups in one trial (Czeizel 1994) was not borne out in other studies examining conception rates in the two treatment groups as the numbers of women with confirmed pregnancy were very similar in intervention and control groups (ICMR 2000; Kirke 1992; MRC 1991).

Interpreting the results from the review for potentially overlapping outcomes may not be straightforward and there was insufficient information in trial reports for us to be clear about whether outcomes were mutually exclusive. It was likely, but not certain, that the same fetus may have been counted in more than one outcome. For example, where NTDs were detected, these same pregnancies may have been included in the figures for pregnancy termination, but we cannot assume that all detected abnormalities led to pregnancy termination. Results for outcomes such as pregnancy termination for fetal abnormality therefore need to be interpreted with caution.

Potential biases in the review process

There were a number of potential biases in the review process. We attempted to minimise bias in several ways: two review authors independently assessed eligibility for inclusion, and data extraction, assessments of risk of bias and data entry were checked by two authors. However, carrying out reviews is not an exact science and may require a number of subjective judgements; it is possible that a different review team may have reached different decisions regarding assessments of eligibility and risk of bias. We would encourage readers to examine the Characteristics of included studies tables to assist in the interpretation of results.

Agreements and disagreements with other studies or reviews

The findings of the review are consistent with those of the previous Cochrane Review (Lumley 2001). As we have noted, the evidence

from randomised trials provides only part of the available evidence in this matter. While the evidence from trials indicates the value of folate in the prevention and recurrence of neural tube defects, evidence from case-control and other types of studies provides additional evidence on population-wide supplementation schemes, and recent research is throwing some light on the most appropriate dosing regimens to achieve the greatest health gain for mothers and babies.

AUTHORS' CONCLUSIONS

Implications for practice

The beneficial effects of folic acid supplementation for preventing birth defects depend on the timing of the intervention (before pregnancy and up to 12 weeks of pregnancy). Adequate targeting is needed to ensure the effectiveness of periconceptional folate supplementation for the prevention on birth defects.

Implications for research

The included trials were published before international recommendations for periconceptional folic acid supplementation emerged. There is a need for clinical trials using the current internationally recommended doses to determine the effect of folic acid supplementation on birth defects. Considering the high compliance of the weekly supplementation regimen, it is worth considering. Recent studies suggest periconceptional micronutrient supplementation may have a potential protective effect against paediatric cancers and folic acid may be conferring the protective effect (Goh 2007); these areas also merit further research attention.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Czeizel 1994

Methods	RCT 2-arm parallel-group design.
Participants	7905 women attending prenatal care at the Hungarian Family Planning Program (HFPP) in Hungary. Inclusion criteria were: (I) no delayed conception or infertility (i.e. no conception after more than 12 months of sexual activity without contraception), (II) not currently pregnant, (III) voluntary participation and a promise of compliance. In the first 4 years of the HFPP, there were 2 other criteria: age under 35 (women over 35 were referred to a genetic counselling clinic) and no previous wanted pregnancy.
Interventions	Women were randomly assigned to 1 of 2 groups: Group 1: women received a supplement (Elevit Pronatal®) containing 800 µg (0.8 mg) folic acid, in addition to 6000 IU (until the end of 1989) and 4000 IU (in 1990 to 1991) vitamin A; 1.6 mg vitamin B_1; 1.8 mg vitamin B_2; 19 mg nicotinamide; 2.6 mg vitamin B_6; 10 mg pantothenic acid (as calcium pantothenate); 0.2 mg biotin; 4.0 pg vitamin B_{12}; 100 mg vitamin C; 500 IU vitamin D; 15 mg vitamin E (as alpha-tocopherol-tocopherol acetate); 125 mg calcium; 125 mg phosphorus; 100 mg magnesium;60 mg elemental iron; 1 mg copper; 1 mg manganese; and 7.5 mg zinc. Group 2: women received a supplement containing 1 mg copper; 1 mg manganese; 7.5 mg zinc; 7.5 mg (calcium ascorbate) vitamin C and lactose 736.27 mg. A single tablet in either group was to be taken daily for 1 month before planned conception until 12 weeks of pregnancy (confirmed by sensitive pregnancy test after the first missed menstrual period and by ultrasonography within 2 weeks). Women were asked to ingest the tablet each day before recording the basal body temperature and to leave unused tablets in the box.
Outcomes	Maternal: weight gain, increased appetite, lack of appetite, nausea and vomiting, vertigo, heartburn, constipation, diarrhoea, ectopic pregnancy, miscarriage, spontaneous abortion, multiple birth, twin birth. Infant: NTDs and other congenital anomalies (congenital limb deficiency, cardiovascular congenital abnormalities, congenital pyloric stenosis, cleft lip, cleft palate) stillbirths, infant mortality, prenatally terminated fetuses, birthweight, low birthweight, gestational age, weight, length, and head circumference at 8 to 16 months of age, functional development tests at 8 to 16 months.
Notes	Exclusion criteria not clear. The final database included 5502 women with confirmed pregnancy.

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Yes	Women were asked about whether they agreed to their allocation on the basis of a randomisation table.

Czeizel 1994 (*Continued*)

Allocation concealment?	Unclear	Not described. It was not clear whether staff carrying out recruitment were aware of randomisation group.
Blinding? Women	Yes	Women were informed about the "blind' use of 1 of 2 kinds of tablets.
Blinding? Clinical staff	Unclear	Not clear if staff were blind.
Blinding? Outcome assessors	Unclear	Certificates were filled in by mothers and signed by physicians. If certificates were not sent back one of the co-workers visited the participants at home.
Incomplete outcome data addressed? All outcomes	Unclear	Of 7905 women randomised 69.6% had confirmed pregnancies. Loss to follow up for women with confirmed pregnancies 1%.
Free of selective reporting?	Unclear	Not apparent.
Free of other bias?	Unclear	In a comment in Czeizel 1992 it is stated that randomisation was broken twice; 1 to evaluate teratogenic effect of vitamin A and the second at the end of the trial in 1991.

ICMR 2000

Methods	RCT 2-arm parallel-group design.
Participants	466 women with a history of giving birth to a child with open NTD and planning to have another child from 5 centres: Bangalore, Mumbai, Lucknow, New Delhi and Pune, in India. Previous NTDs included anencephaly, encephalocoele, meningocoele/meningomyelocoele, cranio rachischisis, and their combinations, including complicating hydrocephalus; but isolated cases of hydrocephalus were not included in the trial. Women with a history of giving birth to a child with closed spina bifida, women with a history of diabetes mellitus or abnormal fasting and post-prandial blood sugar, epilepsy, congenital anomalies indicative of a genetic syndrome in previous NTD, or vitamin intake during 3 months prior to the enrolment and pregnancy were excluded.
Interventions	Women were randomly assigned to 1 of 2 groups: Group 1: received a supplement containing 4000 µg (4 mg) folic acid, iron (120 mg ferrous sulphate), 240 mg calcium phosphate; 10 mg zinc; 4000 IU vitamin A; 2.5 mg vitamin B_1; 2.5 mg vitamin B_2; 2 mg vitamin B_6; 40 mg vitamin C; 400 IU vitamin D and 15 mg niacin (nicotinamide). Group 2: received a supplement containing iron (120 mg ferrous sulphate and 240 mg calcium phosphate. Both tablets were to be taken by mouth daily.

ICMR 2000 (*Continued*)

	Supplementation started at least 1 month before conception up to 12 weeks of pregnancy. Compliance with supplement intake was checked with the help of a dietary card maintained by the women and number of capsules returned.
Outcomes	Maternal: parity, spontaneous abortion, induced abortion. Infant: livebirth, stillbirth, NTD (anencephaly, complicated spina bifida, other combination).
Notes	-

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	Study described as randomised.
Allocation concealment?	Yes	Containers were given a random number and sent to each centre.
Blinding? Women	Yes	Both capsules were identical and packed in similar containers.
Blinding? Clinical staff	Yes	Participants and care providers reported as blinded.
Blinding? Outcome assessors	Unclear	All women were instructed to inform the outcome of pregnancy personally or by post. In absence of the report a social worker contacted the participant to record the outcome.
Incomplete outcome data addressed? All outcomes	Unclear	466 women were randomised and 305 had a confirmed pregnancy during the study period (65.5%) . Loss to follow up after pregnancy confirmation less than 10%.
Free of selective reporting?	Unclear	Not apparent.
Free of other bias?	Unclear	After publication of MRC (1991) trial the study was stopped. Comment: calculated sample size (250 per arm, including 20% of losses to follow up) was almost completed.

Kirke 1992

Methods	RCT 3-arm parallel-group design plus a second non-randomised control group.
Participants	354 women with a previously affected pregnancy, who were not pregnant but planning to have another pregnancy attending 12 hospitals in Ireland. Women with conditions likely to result in impaired absorption from the gastrointestinal tract were excluded.
Interventions	Women were randomly assigned to one of three groups: Group 1: received supplement of 360 µg (0.36 mg) folic acid only (0.12 mg/tablet) daily. Group 2: received supplements (Pregnavite Forte®) containing 4000 IU vitamin A; 400 IU vitamin D (calciferol); 1.5 mg vitamin B_1 (thiamine hydrochloride); 1.5 mg vitamin B_2 (riboflavin); 1 mg vitamin B_6 (pyridoxine hydrochloride); 15 mg niacin (nicotinamide); 40 mg vitamin C; 480 mg calcium phosphate, and iron (252 mg ferrous sulphate) daily. Group 3: received supplements (Pregnavite Forte F®) containing 360 µg (0.36 mg) folic acid in addition to 4000 IU vitamin A; 400 IU vitamin D (as calciferol); 1.5 mg vitamin B_1 (thiamine hydrochloride); 1.5 mg vitamin B_2 (riboflavin); 1 mg vitamin B_6 (pyridoxine hydrochloride); 15 mg niacin (nicotinamide); 40 mg vitamin C; 480 mg calcium phosphate, and iron (252 mg ferrous sulphate) daily. Participants were instructed to take 1 tablet 3 times daily for at least 2 months before conception and until the date of the third missed period. Compliance was based on tablet counts and blood tests.
Outcomes	Maternal: spontaneous abortion, ectopic pregnancy. Fetal/infant: livebirth, stillbirth, occurrence and recurrence of NTD (major malformations: anencephalus, cleft lip, bilateral corneal ectasia with agenesis of the corpus callosum, polycystic kidneys with cleft lip, congenital mitral insufficiency, polydactyly, pyloric stenosis, urethral obstruction, cystic fibrosis, hydrocephalus without spina bifida, oesophageal atresia, and transposition of the great vessels; minor malformations: congenital dislocation of the hip, talipes, and scaphocephaly).
Notes	-

Risk of bias

Item	Authors' judgement	Description
Adequate sequence generation?	Yes	Block randomisation was employed, 12 subjects per block, stratified by hospital.
Allocation concealment?	Yes	Randomisation: achieved by using consecutively numbered, opaque, sealed envelopes.
Blinding? Women	Yes	Women initially received similarly presented white pills. After 1 year supplements with multiple micronutrients with folic acid were changed to commercially available tablet (colour purple) and the tablets containing multiple micronutrients without folic acid, produced by a different sup-

Kirke 1992 (*Continued*)

		plier were white. Authors stated that "It is felt that this partial loss of blinding did not materially affect the study outcome".
Blinding? Clinical staff	Yes	Study reported as double-blind.
Blinding? Outcome assessors	Unclear	Not clear if outcome assessors were aware of group allocation.
Incomplete outcome data addressed? All outcomes	Unclear	Of the 354 women randomised 281 had confirmed pregnancies (79.3%).
Free of selective reporting?	Unclear	Not apparent.
Free of other bias?	Unclear	Not apparent.

Laurence 1981

Methods	RCT 2-arm parallel-group design.
Participants	905 women resident in Glamorgan and Gwent, Wales who had a pregnancy complicated by a fetal NTD (anencephaly, encephalocoele, and spina bifida cystica) between 1954 and 1969 were traced through malformation registers, maternal and paediatric records, local authority records, and other sources. Those under 35 years of age at the time of the study were visited in their homes by medically qualified field workers and invited to participate. 218 women agreed to participate in the intervention trial.
Interventions	Women were randomly assigned to 1 of 2 groups: Group 1: received supplements containing 4000 µg (4 mg) folic acid daily (each tablet contained 2000 µg folic acid). Group 2: received placebo. Women were asked to take a tablet twice a day starting from the time contraceptive precautions were stopped. Compliance among women in group 1 was monitored at the sixth to ninth week of estimated gestation; if the serum folate concentration at this stage was higher than 10 µg/L the woman's account of taking the tablets during the earlier part of the pregnancy could be accepted as valid. If the serum folate concentration was below 10 µg/L the woman was classified as non-compliant. Compliance was not tested among women in group 2.
Outcomes	Maternal: diet, serum and red blood cell folate concentrations at 6 to 9 weeks of pregnancy, miscarriage, termination. Infant: livebirth, NTD (anencephaly, spina bifida cystica).
Notes	Exclusion criteria not clear.
Risk of bias	

Laurence 1981 (*Continued*)

Item	Authors' judgement	Description
Adequate sequence generation?	Yes	Random number tables.
Allocation concealment?	Unclear	Women were allocated to receive treatment or placebo by random numbers.
Blinding? Women	Yes	Women did not know the content of the tablets.
Blinding? Clinical staff	Yes	Study reported as double-blind.
Blinding? Outcome assessors	Unclear	It was not clear whether outcome assessors were blind to group allocation.
Incomplete outcome data addressed? All outcomes	Unclear	(Not clear, some discrepancies in figures in different papers) 218 women were randomised and there were 123 pregnancies reported (56.4%). The main study report describes outcomes for 111 women who had confirmed pregnancies.
Free of selective reporting?	Unclear	Not apparent.
Free of other bias?	Unclear	Not apparent.

MRC 1991

Methods	2 by 2 factorial RCT.
Participants	1817 participating women from 7 countries (UK, Hungary, Israel, Australia, Canada, Russia and France) at high risk of having a pregnancy with a NTD, because of a previous affected pregnancy (not associated with the autosomal recessive disorder Meckel's syndrome), that were planning another pregnancy and were not already taking micronutrient supplements. Women were excluded if they had epilepsy in case the folic acid supplementation adversely affected their treatment.
Interventions	Women were randomly assigned to one of 4 groups: Group 1: received 4000 µg (4 mg) folic acid; iron (120 mg ferrous sulphate) and 240 dicalcium phosphate daily. Group 2: received 4000 µg (4 mg) folic acid; iron (120 mg ferrous sulphate); 240 di-calcium phosphate; 4000 IU vitamin A; 400 IU vitamin D; 1.5 mg vitamin B_1;1.5 mg vitamin B_2; 10 mg vitamin B_6; 40 mg vitamin C and 15 mg niacin (nicotinamide). Group 3: received iron (120 mg ferrous sulphate);240 di-calcium phosphate; 4000 IU vitamin A; 400 IU vitamin D; 1.5 mg vitamin B_1;1.5 mg vitamin B_2; 10 mg vitamin B_6; 40 mg vitamin C and 15 mg niacin (nicotinamide) daily with no folic acid. Group 4 (control): received iron (120 mg ferrous sulphate) and 240 di-calcium phosphate

MRC 1991 (*Continued*)

	daily. Women were asked to take a single capsule each day from the date of randomisation until 12 weeks of pregnancy (estimated from the first day of the last menstrual period). The capsules used in the study were packaged in 2-week calendar "blister" packs.
Outcomes	Maternal: serum folic acid at last visit before becoming pregnant, miscarriage, ectopic pregnancy, termination of pregnancy. Infant: any fetal malformation (i.e. anencephaly, spina bifida cystica, or encephalocoele) , sex, birthweight, head circumference.
Notes	-

Risk of bias

Item	**Authors' judgement**	**Description**
Adequate sequence generation?	Unclear	Randomisation: method not clear.
Allocation concealment?	Yes	Women were allocated at random to each of the 4 groups. Separate set of random allocations were used for each centre.
Blinding? Women	Yes	Neither the doctor nor the patient knew which regimen had been allocated.
Blinding? Clinical staff	Yes	Double-blind trial.
Blinding? Outcome assessors	Unclear	Not specified.
Incomplete outcome data addressed? All outcomes	Unclear	Of 1817 women randomised 1195 had confirmed pregnancies (65.7%). Subsequent loss to follow up 7%.
Free of selective reporting?	Unclear	Not apparent.
Free of other bias?	Unclear	Not apparent.

NTD: neural tube defect
RCT: randomised controlled trial

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Atukorala 1994	195 pregnant women aged 17 to 45 years and 14 to 24 weeks' gestation from tea plantations in 5 regions of Sri Lanka attending antenatal clinics were randomly assigned to receive either a iron-fortified food (thriposha) and advised to consume 50 g/day or supplements containing 60 mg elemental iron (as ferrous sulphate) + 250 µg (0.25 mg) folic acid . The types of participants and types of interventions are out of the scope of this review.
Bailey 2005	This is a review paper and not an intervention trial. This meta-analysis included studies from 1995 to 2000.
Binns 2006	This is a cross-sectional study that documented the prevalence of mothers taking folic acid as supplements or added in fortified foods, and explored determinants of folic acid intake. This is not a randomised trial. The study design, types of participants and types of interventions are out of the scope of this review.
Botto 2006	This is a retrospective cohort study of births monitored through 13 birth defects registries monitoring rates of NTDs from 1988 to 1998 in Norway, Finland, Northern Netherlands, England and Wales, Ireland, France (Paris, Strasbourg, and Central East), Hungary, Italy (Emilia Romagna and Campania), Portugal, and Israel. The aims was to evaluate the effectiveness of policies and recommendations on folic acid aimed at reducing the occurrence of NTDs. The study design is out of the scope of this review.
Canfield 2005	This is a secondary data analysis using data reported from states to the National Birth Defects Prevention Network and examined the effect of enriched cereal-grains products fortification with folic acid on birth defects in the United States. Periods were 1995 to 1996 ("pre-fortification") and 1999 to 2000 (post-fortification) . The results suggest some modest benefit from the folic acid fortification on the prevalence of a number of non-NTD birth defects. The study design is out of the scope of this review.
Chen 2008	A randomised, population-based community intervention study was carried out in Henan, Guizhou, Hunan, and Jilin provinces of China. Ten intervention trial counties and 8 control counties were selected from these provinces. Current resident women planning a pregnancy who volunteered to participate in the follow up were included in the trial. Women from intervention counties received a supplement (Forceval®) containing 400 µg (0.4 mg) folic acid; 563 IU vitamin A; 200 IU vitamin D_2; 1.4 mg vitamin B_1; 1.4 mg vitamin B_2; 3 µg vitamin B_{12}; 60 mg vitamin C; 8 mg vitamin E, 100 µg biotin (bioepiderm); 14 mg niacin (niacinamide); 4 mg pantothenic acid, 100 mg calcium; 10 mg iron; 2 mg copper (cuprum); 10 mg zinc, 77 mg phosphorus, 30 mg magnesium, 3 mg manganese, 30 µg selenium, 100 µg molybdenum, and 4 mg potassium. Women in the control counties did not receive supplementation. Participants were followed up according to periconceptional supplementation for 2 years. Women who had a pregnancy were followed up from 28 weeks' gestation at least to pregnancy termination, and the outcome was recorded. During 2000 and 2002, all of the women having pregnancies with birth defects and women whose pregnancies were without any birth defects were interviewed. 9 NTDs were recorded from 25,444 pregnancies (NTD birth prevalence of 0.35/1000 pregnancies) in the intervention group and 48 NTDs among 26,599 pregnancies (NTD birth prevalence of 1.80/1000 pregnancies) in the control group. The protective rate was 80.4%. This is not a randomised trial.
Christian 2003	4926 pregnant women in rural Nepal participated in a cluster-randomised, double-masked, controlled trial with 5 arms. The following groups were evaluated: group 1 received 400 µg (0.4 mg) folic acid and 1000 µg retinol equivalents (RE) vitamin A; group 2 received 400 µg (0.4 mg) folic acid; 60 mg elemental iron and 1000 µg retinol equivalents (RE) vitamin A; group 3 received 400 µg (0.4 mg) folic acid; 60 mg elemental iron; 30 mg zinc and 1000 µg retinol equivalents (RE) vitamin A; group 4 received 400 µg (0.4 mg) folic

(*Continued*)

	acid; 60 mg elemental iron; 30 mg zinc; 10 mg vitamin D; 10 mg vitamin E; 1.6 mg vitamin B_1; 1.8 mg vitamin B_2; 20 mg niacin; 2.2 mg vitamin B_6; 2.6 mg vitamin B_{12}; 100 mg vitamin C; 65 mg vitamin K; 2 mg copper; 100 mg magnesium and 1000 µg retinol equivalents (RE) vitamin A; and group 5, 1000 µg retinol equivalents (RE) vitamin A alone as the control. All participating women were offered deworming treatment (albendazole 400 mg single dose) in the second and third trimester. Short- and long-term effects of antenatal supplementation were evaluated, showing a protective effect of iron-folic acid supplementation on infant mortality at age 7 years. Supplementation started at recruitment and continued until 3 month post-partum in the case of live births of 5 weeks or more after a miscarriage or stillbirth.
Daly 1995	80 non-pregnant women attending Coombe Women's Hospital, Ireland were randomised to one of four groups: control, dietary advice, fortified milk (70 µg folic acid/100 mL), and fortified milk plus dietary advice. Milk fortification was effective to raise serum and red cell folate. The types of interventions are out of the scope of this review.
Daly 1997	121 women of childbearing age employees in the Coombe Women's Hospital, Ireland were randomly allocated to 0, 100, 200, or 400 µg/day of folic acid. Red cell folate and plasma homocysteine were measured at baseline and after 10 weeks supplementation. Compliance was monitored by having the women sign a dated sheet when taking the tablet. 95 women completed the 6-month study. Double-blind randomised controlled trial to find the lowest folic acid dose that effectively reduces plasma homocysteine levels in premenopausal women. The types of participants are out of the scope of this review.
Doyle 2001	Fifty-five women who had given birth to a low birthweight baby (less than 2500 g), and who planned to have a further pregnancy, were recruited to a prospective randomised study in East London, UK. Multiple micronutrient supplementation started at 3 months postpartum and follow up lasted 6 months. The type of participants are out of the scope of this review.
Drazkowski 2002	Case reports from 4 women with epilepsy identified with low B_{12} levels using data from electronic medical records of the Barrow Neurologic Institute, Epilepsy Specialty Clinic, United States. They received supplements of either 4 or 5 mg synthetic folic acid and parenteral B_{12}. The type of study, participants and interventions are out of the scope of the review.
Eichholzer 2006	This is a review that addresses supplementation and fortification as public health policies. This is not an intervention trial.
Ejidokun 2000	Qualitative study using focus group discussions, observational data and in-depth interview to identify community perspectives and attitudes to pregnancy, anaemia, iron and folate supplements during pregnancy amongst women (n = 23), and 2 healthcare providers in Lagos, Nigeria. Maternal anaemia was not perceived as a priority health problem by pregnant women. The type of study, participants and interventions are out of the scope of this review.
Elbourne 2002	This is not an intervention trial. This paper addresses methodological issues relating to the meta-analysis of trials with cross-over designs.
Ellison 2004	31 women with singleton pregnancy were randomised to receive 400 µg (0.4 mg) folic acid once daily until 16 weeks' gestation or until the end of pregnancy. Authors found that folic acid supplementation throughout pregnancy maintains plasma homocysteine concentration. The type of participants and type of interventions are out of the scope of this review.

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Eskes 2000	This paper reviews the relationship between low vitamin status (folic acid, vitamin B_6 and B_{12}), hyperhomocysteinemia, the MTHFR gene mutation C677T, and thrombotic factors like Protein C, Protein S, antithrombin III, factor V Leiden and Activated Protein C, either alone or in combination as high risk factors for obstetrical vascular disease. This is not an intervention trial.
Field 1991	This paper reviews the effect of folic acid in NTD in humans and animals. This is not an intervention trial.
Geisel 2003	This paper reviews the effect of folic acid in NTD in humans, and ways in which knowledge of folic acid can be increased. This is not an intervention trial.
Hague 2003	This paper discusses the effects of changing levels of homocysteine in pregnancy. This is not an intervention trial.
Hayes 1996	This is a case-control study examining factors associated with orofacial clefts. Case were mothers with babies with cleft lip with or without cleft palate, controls were mothers of babies with other congenital anomalies (except NTDs). The type of study is out of the scope of this review.
Itikala 2001	Case-control study to evaluate the relation between regular multivitamin use and the birth prevalence of orofacial clefts. There was a 48% risk reduction for cleft lip with or without cleft palate among mothers who used multivitamins during the periconceptional period or who started multivitamin use during the first postconceptional month. No reductions for cleft lip with or without cleft palate or cleft palate alone were found for women who began multivitamin use in the second or third month after conception. The type of study is out of the scope of the review.
Johnston 2008	This paper reviews the effectiveness of cereal grain fortification with folic acid. This is not an intervention trial.
Khambalia 2009	In this study of 88 women in rural Bangladesh, women were randomised before pregnancy to receive daily iron and folic acid or folic acid only. Both treatment groups received folic acid. Once pregnancy was confirmed women were withdrawn from the study and received routine care which included folic acid supplements.
Lee 2005	A total of 131 apparently healthy pregnant women were assigned to 1 of 5 groups: group 1 (control) received no supplement; group 2 received 30 mg elemental iron (as ferrous sulphate) plus 175 µg (0.17 mg) folic acid daily from the first trimester until delivery; group 3 received 60 mg elemental iron (as ferrous sulphate) plus 350 µg (0.35 mg) folic acid from the first trimester until delivery; group 4 received 30 mg elemental iron (as ferrous sulphate) plus 175 µg (0.17 mg) folic acid daily from the 20th week gestation until delivery and group 5 received 60 mg elemental iron (as ferrous sulphate) plus 350 µg (0.35 mg) folic acid from the 20th week gestation until delivery. Authors found that improvements in iron and folate nutriture were highly dependent on when the supplement program was initiated, but both supplement doses were equally effective. The influence of folic acid supplementation on maternal folate status was not as pronounced as was the influence of iron supplementation on iron status. The type of study and type of participants are out of the scope of this review.
Mandishona 1999	112 women aged between 12 and 50 years from a population of 425 rural people participating in ongoing family genetic studies in the Murehwa and Zaka districts of Zimbabwe and in Mpumalanga Province, South Africa to assess the effect of consumption of a traditional beer, rich in iron, in the regular diet for preventing iron deficiency. The type of study, participants and interventions are out of the scope of this review.

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Manizheh 2009	In this trial in Iran 246 nulliparous women were randomised to receive daily folic acid from early pregnancy; either 0.5 mg per day or 5 mg per day. Both groups received folic acid, and supplements continued throughout pregnancy.
Mathews 1999	Authors investigated the possibility of merging 2 studies: the MRC Vitamin study, a randomised controlled trial of folic acid supplementation (4 mg/day) among women in whom a previous pregnancy had been affected by NTD; and the Prospective Study of Nutrition, Smoking and Pregnancy Outcome, an observational study that gathered detailed information on periconceptional nutrition from nulliparous women in the UK (women who had undergone infertility treatment were excluded). This is analysis of secondary data. The type of study is out of the scope of this review.
Melli 2008	203 nulliparous pregnant women with a singleton pregnancy in their first trimester, attending the antenatal outpatient clinic Tabriz, Iran with no history of hypertension and folic acid supplementation. Women were divided into 2 groups: group 1 was given 5000 µg (5 mg) per day and group 2 received 500 µg (0.5 mg/day) folic acid. In addition to the plasmatic homocysteine concentrations during the first trimesters and at delivery, the incidence of pregnancy-induced hypertension, pre-eclampsia and eclampsia and were compared. The incidence of any type of hypertension was 2% with regimen 1 compared to 11% with routine regimen. The type of intervention is out of the scope of this review.
Molster 2007	The paper reported the results of a survey of knowledge, attitudes and behaviour with regard to food fortification with folic acid amongst a randomly selected sample aged 18 years or older in Australia. This is not an intervention trial.
Nelen 2000	A case-control study. Homocysteine (fasting and afterload), folate (serum and red cells), pyridoxal 5*-phosphate, and cobalamin concentrations were measured in 123 white women who had at least 2 consecutive spontaneous early pregnancy losses each and compared with 104 healthy controls from the University Hospital Nijmegen St. Radboud, The Netherlands. Elevated homocysteine and reduced serum folate concentrations were risk factors for recurrent spontaneous early pregnancy losses. The type of study is out of the scope of this review.
Nguyen 2009	Forty non-pregnant women aged between 18 and 45 years, who had not taken folic acid supplements, from the Motherisk Program, The Hospital for Sick Children, Canada were randomly assigned to 1 of 2 groups: group 1 received a daily supplement (PregVit®) containing 1100 µg (1.1 mg) folic acid; 2700 IU b-carotene; 30 IU vitamin E; 12 µg vitamin B_{12}; 120 mg vitamin C; 250 IU vitamin D; 3 mg thiamine; 300 mg calcium; 3.4 mg riboflavin; 20 mg niacinamide; 10 mg vitamin B-6; 5 mg pantothenic acid; 50 mg magnesium; 0.15 mg iodine; 35 mg iron (as ferrous fumarate); 2 mg copper and 15 mg zinc; or group 2 received 5000 µg (5 mg) folic acid 5 mg (PregVit-folic 5®); 2700 IU b-carotene; 30 IU vitamin E; 12 µg vitamin B_{12}; 120 mg vitamin C; 250 IU vitamin D; 3 mg thiamine; 300 mg calcium; 3.4 mg riboflavin; 20 mg niacinamide; 10 mg vitamin B6; 5 mg pantothenic acid; 50 mg magnesium; 0.15 mg iodine; 35 mg iron (as ferrous fumarate) ; 2 mg copper and 15 mg zinc. The women were instructed to take the supplement for 30 weeks. Plasma and red blood cell (RBC) folate concentrations were measured at baseline and at weeks 2, 4, 6, 12, and 30. The use of 5 mg folic acid among women of childbearing age produced higher blood folate concentrations, with a faster rate of folate accumulation, compared with 1.1 mg folic acid. The type of participants and comparisons are out of the scope of this review.
Pitkin 2007	This is not an intervention trial. This review includes prospective and retrospective studies. In the clinical trials section the conclusion is that folic acid supplementation was useful to prevent occurrence (only 1 trial) and recurrence of NTD.

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Pritchard 1991	This paper describes the causes of abruptio placentae. It was excluded because is out of the scope of this review.
Ramakrishnan 2003	A randomised, double-blind clinical trial in semi-rural Mexico to compare the effects of multiple micronutrients supplements with those of iron supplements during pregnancy on birth size. 873 pregnant women were recruited before 13 weeks of gestation and randomly assigned to 1 of 2 groups: group 1 received supplements containing 2150 IU vitamin A; 309 IU vitamin D_3; 5.73 IU vitamin E; 0.93 mg thiamine; 1.87 mg riboflavin; 15.5 mg niacin; 215 µg (0.21 mg) folic acid; 1.94 mg vitamin B_6; 2.04 µg vitamin B_{12}; 66.5 mg vitamin C; 12.9 mg zinc; 62.4 mg elemental iron (as ferrous sulfate) and 252 mg magnesium; group 2 received 60 mg elemental iron (as ferrous sulfate). The supplements were provided 6 days a week at home. Routine antenatal care was provided to both groups until delivery. These findings suggest that multiple micronutrient supplementation during pregnancy does not lead to greater infant birth size than iron-only supplementation. The study was excluded because supplementation time surpassed 12 weeks of pregnancy.
Ray 2007	A population-based case-control study in Ontario, Canada (89 NTD cases and 422 controls). The outcome was serum holotranscobalamin (holoTC) at 15 to 20 weeks' gestation. There was a trend of increasing risk with lower levels of holoTC. The type of study and intervention are out of the scope of this review.
Ray 2008	A cross-sectional study among 10,622 women in Ontario, Canada. Authors determined the prevalence of biochemical B_{12} deficiency and found that 1 in 20 women may be deficient in B_{12} in early pregnancy. The type of study is out of the scope of this review.
Robbins 2005	232 non-pregnant women from 2 clinics in Arkansas, United States were assigned randomly to receive brief folic acid counselling, a reminder phone call, and 30 folic acid tablets (n = 162 women; intervention group) or to receive counselling about other preventive health behaviours and a folic acid informational pamphlet (n = 160 women; control group). Self-reported folic acid use was compared at baseline and at 2 months. Weekly folic acid intake increased in the intervention group by 68%, compared with 20% in the control group. No significant differences were found in daily intake. The type of participants and interventions are out of the scope of this review.
Rolschau 1999	8184 Danish female citizens resident in the county of Funen, Denmark planning a pregnancy or already pregnant were offered a free supplement of folic acid of either 100 µg (1 mg) folic acid or 250 µg (2.5 mg) folic acid in a double-blind randomised study to determine whether a supplement of folic acid given preconceptionally or early in pregnancy had any influence on birthweight, incidence of preterm labour, low birthweight and small-for-gestational age. Folic acid given preconceptionally or in the first half of pregnancy slightly increased birthweight and a decreased the incidence of preterm labour, infants with low birthweight and small for gestational age. The type of interventions is out of the scope of this review.
Sayers 1997	A cross-sectional community-based survey was conducted in Dublin, Ireland to document the knowledge and behavior of 335 women of childbearing age to periconceptional folic acid. Approximately two-thirds (213/ 335, 63.6%) had heard of folic acid. Knowledge was significantly associated with higher social class and higher education; few were advised to take folic acid before pregnancy. The type of study, participants and interventions are out of the scope of this review.
Schorah 1993	The authors studied the impact of folate fortification of food on folate intake in women of childbearing age. Folic acid intake was measured by a 7-day weighed procedure from 1986 to 1988. The results show that the consumption of fortified cereals considerably increased the intake of folic acid in women. The type of study, participants and interventions are out of the scope of this review.

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Schwarz 2008	446 English-speaking women, aged 18 to 45 years from 2 urgent clinics in San Francisco, United States were randomly assigned received computerised counselling about periconceptional folate supplements, while women in the control group received computerized counselling about emergency contraception. After 6 months the intervention group were more likely to know that folate prevents birth defects, that folate is most important in early pregnancy, and to report the recent use of a folate supplement. The type of participants is out of the scope of this review.
Shaw 1995	Case-control study to investigate if periconceptional use of multivitamins containing folic acid was associated with a reduced risk of orofacial clefts (n = 734 per group). Women who used multivitamins containing periconceptional folic acid had a 25% to 50% reduction in risk for offspring with orofacial clefts compared to women who did not use such vitamins. Maternal daily consumption of cereal containing folic acid produced similar results. The type of study is out of the scope of this review.
Shaw 2006	Population-based case-control study investigating whether periconceptional intakes of supplemental folic acid, dietary folate, and several other nutrients were associated with orofacial clefts. There was no association detected. The type of study is out of the scope of this review.
Shrimpton 2002	Commentary about the advantages of supplementation in different age groups. This is not an intervention trial.
van der Put 1998a	Case-control study which suggests that the combined heterozygosity for the 2 methylenetetrahydrofolate reductase (MTHFR) gene common mutations accounts for a proportion of folate-related NTDs. The type of study, participants, and interventions are out of the scope of this review.
van Rooij 2004	Case-control study (n = 174) to investigate the association between maternal folate intake by supplement and food and the risk of cleft lip with or without CLP. Dietary folate intake reduced CLP risk independently in a dose-response manner. The largest risk reductions were found in those mothers who had a diet of more than 200 μg folate per day in combination with a folic acid supplement. The type of study is out of the scope of this review.
Wald 2004	A letter to the editor addressing the expediency of folic acid fortification to prevent NTDs versus masking vitamin B12 status. The letter was excluded because does not contain results from clinical trials.
Walsh 2007	Cross-sectional population study looking at blood folate status of over 400 sequential primigravid Caucasian women with a singleton pregnancy, booking at less than or equal to 20 weeks' gestation. The type of study is out of the scope of the review.
Watson 1999	This study explores different methods of communicating information to increase folate awareness in women of childbearing age, who participated in a community randomised trial. The type of interventions is out of the scope of this review.
Wen 2005	Review of 65 studies to examine the biological basis of why folic acid may have health effects beyond its proven effect of reducing NTDs; and to explore controversial policies of folic acid supplementation and food fortification. This is not an intervention trial.
Westphal 2004	A double-blind control study to determine the effects of a commercial blend that includes folic acid on progesterone level, basal body temperature, menstrual cycle and pregnancy rate. The type of interventions is out of the scope of this review.

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Wilcox 2007	National population based case-control study to measure the possible association of facial clefts with maternal intake of folic acid supplements, multivitamins, and folates in diet. Results show that 400 µg (0.4 mg) folic acid supplementation daily during early pregnancy was associated with a reduced risk of isolated cleft lip with or without cleft palate, especially among women with folate rich diets who also took folic acid supplements and multivitamins. Folic acid provided no protection against cleft palate. The type of study is out of the scope of the review.
Zeng 2008	5828 pregnant women in 2 rural counties in Shaanxi Province, in north west China. Villages were randomly assigned to 1 of 3 groups: group 1 received supplements containing 400 µg (0.4 mg) folic acid; group 2 received 30 mg elemental iron, 400 µg (0.4 mg) folic acid; and group 3 received 30 mg elemental iron, 400 µg (0.4 mg) folic acid; 15 mg zinc; 2 mg copper; 65 µg selenium; 150 µg iodine; 800 µg vitamin A; 1.4 mg vitamin B_1 (thiamine); 1.4 mg vitamin B_2 (riboflavin); 1.9 mg vitamin B_6; 2.6 µg vitamin B_{12}; 5 µg vitamin D; 70 mg vitamin C; 10 mg vitamin E; and 18 mg niacin. Authors found that antenatal supplementation with iron-folic acid was associated with longer gestation and a reduction in early neonatal mortality compared with folic acid. Multiple micronutrients supplements were associated with modest increase in birthweight compared with folic acid. All women received folic acid. The type of interventions is out of the scope of this review.

CLP: cleft palate
MM: multiple micronutrient
NTD: neural tube defect

Characteristics of ongoing studies *[ordered by study ID]*

Javois 2006

Trial name or title	Oral cleft prevention trial in Brazil.
Methods	Randomised, double-blind, dose comparison, parallel assignment, efficacy study.
Participants	Women 16 to 45 years. Inclusion criteria: all women must reside in the state where the clinic is located. Can include women with non-syndromic cleft lip with or without clef palate (NSCL/P) who attend the craniofacial clinics, and women who have at least one natural child of any age with NSCL/P.
Interventions	The hypothesis was that folic acid supplementation of 4 mg/day at preconception and during the first 3 months of pregnancy will decrease the recurrence of non-syndromic cleft lip with or without cleft palate (NSCL/P) in a high-risk group of women when compared to women taking 0.4 mg per day of folic acid. The total sample will include 2000 women (that either have NSCL/P or that have at least 1 child with NSCL/P) randomly assigned to the 4 mg versus the 0.4 mg folic acid study groups. The study will also compare the recurrence rates of NSCL/P in the total sample of subjects as well as the 2 study groups (4 mg, 0.4 mg) to that of a historical control group.
Outcomes	The primary outcome is the recurrence of non-syndromic cleft lip with or without cleft palate (NSCL/P) in offspring of trial mothers. Secondary outcomes include: recurrence of NSCL/P compared to a historical control group; overall and high versus low dose, serum and red blood cell folate levels, severity of NSCL/

Javois 2006 (*Continued*)

	P in offspring of trial mothers, twinning rate, miscarriage rate, pre-eclampsia, rates of other birth defects, birthweight, gestational age at delivery.
Starting date	January 2004.
Contact information	Lorette Javois, Ph.D. javoisl@mail.nih.gov
Notes	Recruitment has recently finished (2009). http://clinicaltrials.gov/ct2/show/NCT00098319

DATA AND ANALYSES

Comparison 1. Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Neural tube defects (ALL)	5	6105	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.15, 0.52]
2 Neural tube defects (by subgroups)	5		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Daily supplementation scheme	5	6105	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.15, 0.52]
2.2 Dose of 400 µg or less	1	364	Risk Ratio (M-H, Fixed, 95% CI)	0.12 [0.01, 2.29]
2.3 Dose of more than 400 µg	4	5741	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.56]
2.4 Started pre-pregnancy and continued during the first trimester	5	6105	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.15, 0.52]
2.5 History of NTDs	4	1949	Risk Ratio (M-H, Fixed, 95% CI)	0.32 [0.17, 0.60]
2.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	0.08 [0.00, 1.33]
3 Cleft palate (ALL)	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.11, 3.92]
4 Cleft palate (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Daily supplementation scheme	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.11, 3.92]
4.2 Dose of 400 µg or less	1	364	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.3 Dose of more than 400 µg	2	5351	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.11, 3.92]
4.4 Started pre-pregnancy and continued during first trimester	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.11, 3.92]
4.5 History of NTDs	2	1559	Risk Ratio (M-H, Fixed, 95% CI)	3.05 [0.12, 74.61]
4.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	0.20 [0.01, 4.06]
5 Cleft lip (ALL)	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.27, 3.74]
6 Cleft lip (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
6.1 Daily supplementation scheme	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.27, 3.74]
6.2 Dose of 400 µg or less	1	364	Risk Ratio (M-H, Fixed, 95% CI)	0.37 [0.02, 9.07]
6.3 Dose of more than 400 µg	2	5351	Risk Ratio (M-H, Fixed, 95% CI)	1.30 [0.29, 5.80]
6.4 Started pre-pregnancy and continued during first trimester	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.27, 3.74]
6.5 History of NTDs	2	1559	Risk Ratio (M-H, Fixed, 95% CI)	0.37 [0.02, 9.07]
6.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	1.30 [0.29, 5.80]
7 Congenital cardiovascular defects (ALL)	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	0.55 [0.27, 1.14]
8 Congenital cardiovascular defects (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
8.1 Daily supplementation scheme	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	0.55 [0.27, 1.14]
8.2 Dose of more than 400 µg	2	5351	Risk Ratio (M-H, Fixed, 95% CI)	0.60 [0.28, 1.26]

8.3 Dose of 400 µg or less	1	364	Risk Ratio (M-H, Fixed, 95% CI)	0.22 [0.01, 4.62]
8.4 Started pre-pregnancy and continued	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	0.55 [0.27, 1.14]
8.5 History of NTDs	2	1559	Risk Ratio (M-H, Fixed, 95% CI)	0.46 [0.07, 3.14]
8.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	0.57 [0.26, 1.25]
9 Other birth defects (any) (ALL)	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	0.72 [0.48, 1.07]
10 Other birth defects (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
10.1 Daily supplementation scheme	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	0.72 [0.48, 1.07]
10.2 Dose of 400 µg or less	1	364	Risk Ratio (M-H, Fixed, 95% CI)	0.84 [0.19, 3.69]
10.3 Dose of more than 400 µg	2	5351	Risk Ratio (M-H, Fixed, 95% CI)	0.71 [0.47, 1.07]
10.4 Started pre-pregnancy and continued during the first trimester	3	5715	Risk Ratio (M-H, Fixed, 95% CI)	0.72 [0.48, 1.07]
10.5 History of NTDs	2	1559	Risk Ratio (M-H, Fixed, 95% CI)	1.29 [0.67, 2.48]
10.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	0.50 [0.30, 0.84]
11 Miscarriage (ALL)	5	7618	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [0.97, 1.26]
12 Miscarriage (by subgroups)	5		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
12.1 Daily supplementation scheme	5	7618	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [0.97, 1.26]
12.2 Dose of 400 µg or less	1	364	Risk Ratio (M-H, Fixed, 95% CI)	1.37 [0.68, 2.77]
12.3 Dose of more than 400 µg	4	7254	Risk Ratio (M-H, Fixed, 95% CI)	1.09 [0.96, 1.25]
12.4 Started pre-pregnancy and continued during first trimester	5	7618	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [0.97, 1.26]
12.5 History of NTDs	4	2116	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.79, 1.30]
12.6 No history of NTDs or unknown	1	5502	Risk Ratio (M-H, Fixed, 95% CI)	1.14 [0.97, 1.34]
13 Stillbirth (ALL)	4	5994	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.51, 1.83]
14 Stillbirth (by subgroups)	4		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
14.1 Daily supplementation	4	5994	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.51, 1.83]
14.2 Dose of 400 μg or less	1	364	Risk Ratio (M-H, Fixed, 95% CI)	0.09 [0.00, 1.51]
14.3 Dose of more than 400 μg	3	5630	Risk Ratio (M-H, Fixed, 95% CI)	1.38 [0.68, 2.81]
14.4 Started pre-pregnancy and continued	4	5994	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.51, 1.83]
14.5 History of NTDs	3	1838	Risk Ratio (M-H, Fixed, 95% CI)	0.63 [0.25, 1.57]
14.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	1.53 [0.60, 3.95]
15 Multiple pregnancy (ALL)	3	6239	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.88, 1.98]
16 Multiple pregnancy (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
16.1 Daily supplementation scheme	3	6239	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.88, 1.98]
16.2 Dose of 400 μg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable

16.3 Dose of more than 400 μg	3	6239	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.88, 1.98]
16.4 Started pre-pregnancy and continued during first trimester	3	6239	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.88, 1.98]
16.5 History of NTDs	2	1472	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.38, 2.70]
16.6 No history of NTDs or unknown	1	4767	Risk Ratio (M-H, Fixed, 95% CI)	1.39 [0.89, 2.18]
17 Pregnancy termination for fetal abnormality (ALL)	4	5908	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.54]
18 Pregnancy termination for fetal abnormality (by subgroups)	4		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
18.1 Daily supplementation scheme	4	5908	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.54]
18.2 Dose of 400 μg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
18.3 Dose of more than 400 μg	4	5908	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.54]
18.4 Started pre-pregnancy and continued during first trimester	4	5908	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.54]
18.5 History of NTDs	3	1752	Risk Ratio (M-H, Fixed, 95% CI)	0.33 [0.16, 0.65]
18.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	0.23 [0.06, 0.79]
19 Low birthweight (ALL)	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]
20 Low birthweight (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
20.1 Daily supplementation scheme	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]
20.2 Dose of 400 μg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.3 Dose of more than 400 μg	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]
20.4 Started pre-pregnancy and continue during first trimester	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]
20.5 History of NTDs	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]
20.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable

Comparison 2. Supplementation with folic acid alone versus no treatment/placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Neural tube defects (ALL)	2	299	Risk Ratio (M-H, Fixed, 95% CI)	0.32 [0.08, 1.34]
2 Neural tube defects (by subgroups)	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Daily supplementation scheme	2	299	Risk Ratio (M-H, Fixed, 95% CI)	0.32 [0.08, 1.34]
2.2 Dose of 400 μg or less	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.17 [0.01, 3.30]

2.3 Dose of more than 400 µg	1	111	Risk Ratio (M-H, Fixed, 95% CI)	0.43 [0.08, 2.23]
2.4 Started pre-pregnancy and continued during the first trimester	2	299	Risk Ratio (M-H, Fixed, 95% CI)	0.32 [0.08, 1.34]
2.5 History of NTDs	2	299	Risk Ratio (M-H, Fixed, 95% CI)	0.32 [0.08, 1.34]
2.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
3 Cleft palate (ALL)	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4 Cleft palate (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Daily supplementation scheme	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.2 Dose of 400 µg or less	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.4 Started pre-pregnancy and continued during first trimester	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.5 History of NTDs	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
5 Cleft lip (ALL)	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6 Cleft lip (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
6.1 Daily supplementation scheme	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.2 Dose of 400 µg or less	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.4 Started pre-pregnancy and continued during first trimester	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.5 History of NTDs	1	188	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
7 Congenital cardiovascular defects (ALL)	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.40 [0.02, 9.77]
8 Congenital cardiovascular defects (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
8.1 Daily supplementation scheme	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.40 [0.02, 9.77]
8.2 Dose of 400 µg or less	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.40 [0.02, 9.77]
8.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
8.4 Started pre-pregnancy and continued	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.40 [0.02, 9.77]
8.5 History of NTDs	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.40 [0.02, 9.77]
8.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
9 Other birth defects (any) (ALL)	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.61 [0.06, 6.57]
10 Other birth defects (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
10.1 Daily supplementation scheme	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.61 [0.06, 6.57]
10.2 Dose of 400 µg or less	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.61 [0.06, 6.57]
10.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable

10.4 Started pre-pregnancy and continued during the first trimester	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.61 [0.06, 6.57]
10.5 History of NTDs	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.61 [0.06, 6.57]
10.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
11 Miscarriage (ALL)	2	299	Risk Ratio (M-H, Fixed, 95% CI)	1.66 [0.66, 4.18]
12 Miscarriage (by subgroups)	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
12.1 Daily supplementation scheme	2	299	Risk Ratio (M-H, Fixed, 95% CI)	1.66 [0.66, 4.18]
12.2 Dose of 400 µg or less	1	188	Risk Ratio (M-H, Fixed, 95% CI)	1.82 [0.67, 4.90]
12.3 Dose of more than 400 µg	1	111	Risk Ratio (M-H, Fixed, 95% CI)	0.85 [0.05, 13.25]
12.4 Started pre-pregnancy and continued during first trimester	2	299	Risk Ratio (M-H, Fixed, 95% CI)	1.66 [0.66, 4.18]
12.5 History of NTDs	2	299	Risk Ratio (M-H, Fixed, 95% CI)	1.66 [0.66, 4.18]
12.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
13 Stillbirth (ALL)	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.46]
14 Stillbirth (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
14.1 Daily supplementation	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.46]
14.2 Dose of 400 μg or less	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.46]
14.3 Dose of more than 400 μg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
14.4 Started pre-pregnancy and continued during first trimester	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.46]
14.5 History of NTDs	1	188	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.46]
14.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
15 Multiple pregnancy (ALL)	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16 Multiple pregnancy (by subgroups)	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.1 Daily supplementation scheme	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.2 Dose of 400 μg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.3 Dose of more than 400 μg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.4 Started pre-pregnancy and continued during first trimester	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.5 History of NTDs	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
17 Pregnancy termination for fetal abnormality (ALL)	1	111	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.01, 6.83]
18 Pregnancy termination for fetal abnormality (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
18.1 Daily supplementation scheme	1	111	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.01, 6.83]

18.2 Dose of 400 μg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
18.3 Dose of more than 400 μg	1	111	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.01, 6.83]
18.4 Started pre-pregnancy and continued during first trimester	1	111	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.01, 6.83]
18.5 History of NTDs	1	111	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.01, 6.83]
18.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
19 Low birthweight (ALL)	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20 Low birthweight (by subgroups)	0		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
20.1 Daily supplementation scheme	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.2 Dose of 400 μg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.3 Dose of more than 400 μg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.4 Started pre-pregnancy and continued during first trimester	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.5 History of NTDs	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable

Comparison 3. Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Neural tube defects (ALL)	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.17 [0.01, 3.22]
2 Neural tube defects (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Daily supplementation scheme	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.17 [0.01, 3.22]
2.2 Dose of 400 µg or less	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.17 [0.01, 3.22]
2.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
2.4 Started pre-pregnancy and continued during first trimester	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.17 [0.01, 3.22]
2.5 History of NTDs	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.17 [0.01, 3.22]
2.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
3 Cleft palate (ALL)	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4 Cleft palate (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Daily supplementation scheme	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.2 Dose of 400 µg or less	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable

4.4 Started pre-pregnancy and continued during the first trimester	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.5 History of NTDs	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
5 Cleft lip (ALL)	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6 Cleft lip (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
6.1 Daily supplementation scheme	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.2 Dose of 400 µg or less	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.4 Started pre-pregnancy and continued during the first trimester	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.5 History of NTDs	1	190	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
7 Congenital cardiovascular defects (ALL)	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.39 [0.02, 9.55]
8 Congenital cardiovascular defects (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
8.1 Daily supplementation scheme	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.39 [0.02, 9.55]
8.2 Dose of 400 µg or less	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.39 [0.02, 9.55]
8.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
8.4 Started pre-pregnancy and continued during the first trimester	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.39 [0.02, 9.55]
8.5 History of NTDs	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.39 [0.02, 9.55]
8.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
9 Other birth defects (any) (ALL)	1	190	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [0.17, 8.23]
10 Other birth defects (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
10.1 Daily supplementation scheme	1	190	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [0.17, 8.23]
10.2 Dose of 400 µg or less	1	190	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [0.17, 8.23]
10.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
10.4 Started pre-pregnancy and continued during the first trimester	1	190	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [0.17, 8.23]
10.5 History of NTDs	1	190	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [0.17, 8.23]
10.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
11 Miscarriage (ALL)	1	190	Risk Ratio (M-H, Fixed, 95% CI)	5.91 [0.29, 121.46]
12 Miscarriage (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
12.1 Daily supplementation scheme	1	190	Risk Ratio (M-H, Fixed, 95% CI)	5.91 [0.29, 121.46]
12.2 Dose of 400 µg or less	1	190	Risk Ratio (M-H, Fixed, 95% CI)	5.91 [0.29, 121.46]

12.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
12.4 Started pre-pregnancy and continued during the first trimester	1	190	Risk Ratio (M-H, Fixed, 95% CI)	5.91 [0.29, 121.46]
12.5 History of NTDs	1	190	Risk Ratio (M-H, Fixed, 95% CI)	5.91 [0.29, 121.46]
12.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
13 Stillbirth (ALL)	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.41]
14 Stillbirth (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
14.1 Daily supplementation	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.41]
14.2 Dose of 400 µg or less	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.41]
14.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
14.4 Started pre-pregnancy and continued	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.41]
14.5 History of NTDs	1	190	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.01, 2.41]
14.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
15 Multiple pregnancy (ALL)	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16 Multiple pregnancy (by subgroups)	0		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
16.1 Daily supplementation scheme	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.2 Dose of 400 µg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.4 Started pre-pregnancy and continued during first trimester	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.5 History of NTDs	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
17 Pregnancy termination for fetal abnormality (ALL)	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
18 Pregnancy termination for fetal abnormality (by subgroups)	0		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
18.1 Daily supplementation scheme	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
18.2 Dose of 400 µg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
18.3 Dose of more than 400 µg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
18.4 Started pre-pregnancy and continued during first trimester	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
18.5 History of NTDs	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
18.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
19 Low birthweight (ALL)	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20 Low birthweight (by subgroups)	0		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only

20.1 Daily supplementation scheme	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.2 Dose of 400 μg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.3 Dose of more than 400 μg	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.4 Started pre-pregnancy and continued during first trimester	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.5 History of NTDs	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable

Comparison 4. Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Neural tube defects (ALL)	4	5806	Risk Ratio (M-H, Fixed, 95% CI)	0.29 [0.15, 0.56]
2 Neural tube defects (by subgroups)	4		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Daily supplementation scheme	4	5806	Risk Ratio (M-H, Fixed, 95% CI)	0.29 [0.15, 0.56]
2.2 Dose of 400 µg or less	1	176	Risk Ratio (M-H, Fixed, 95% CI)	0.34 [0.01, 8.26]
2.3 Dose of more than 400 µg	3	5630	Risk Ratio (M-H, Fixed, 95% CI)	0.28 [0.14, 0.56]
2.4 Started pre-pregnancy and continued during the first trimester	4	5806	Risk Ratio (M-H, Fixed, 95% CI)	0.29 [0.15, 0.56]
2.5 History of NTDs	3	1650	Risk Ratio (M-H, Fixed, 95% CI)	0.33 [0.17, 0.66]
2.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	0.08 [0.00, 1.33]
3 Cleft palate (ALL)	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.11, 3.92]
4 Cleft palate (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Daily supplementation scheme	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.11, 3.92]
4.2 Dose of 400 µg or less	1	176	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
4.3 Dose of more than 400 µg	2	5351	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.11, 3.92]
4.4 Started pre-pregnancy and continued during the first trimester	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.66 [0.11, 3.92]
4.5 History of NTDs	2	1371	Risk Ratio (M-H, Fixed, 95% CI)	3.05 [0.12, 74.61]
4.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	0.20 [0.01, 4.06]
5 Cleft lip (ALL)	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.27, 3.65]
6 Cleft lip (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
6.1 Daily supplementation scheme	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.27, 3.65]
6.2 Dose of 400 µg or less	1	176	Risk Ratio (M-H, Fixed, 95% CI)	0.34 [0.01, 8.26]
6.3 Dose of more than 400 µg	2	5351	Risk Ratio (M-H, Fixed, 95% CI)	1.30 [0.29, 5.80]

6.4 Started pre-pregnancy and continued during the first trimester	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.27, 3.65]
6.5 History of NTDs	2	1371	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
6.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	1.30 [0.29, 5.80]
7 Congenital cardiovascular defects (ALL)	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.43, 2.22]
8 Congenital cardiovascular defects (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
8.1 Daily supplementation scheme	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.43, 2.22]
8.2 Dose of 400 µg or less	1	176	Risk Ratio (M-H, Fixed, 95% CI)	0.34 [0.01, 8.26]
8.3 Dose of more than 400 µg	2	5351	Risk Ratio (M-H, Fixed, 95% CI)	1.08 [0.46, 2.53]
8.4 Started pre-pregnancy and continued during the first trimester	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.43, 2.22]
8.5 History of NTDs	2	1371	Risk Ratio (M-H, Fixed, 95% CI)	0.61 [0.08, 4.61]
8.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	1.08 [0.44, 2.66]
9 Other birth defects (any) (ALL)	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.75 [0.50, 1.12]
10 Other birth defects (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
10.1 Daily supplementation scheme	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.75 [0.50, 1.12]
10.2 Dose of 400 µg or less	1	176	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.15, 7.10]
10.3 Dose of more than 400 µg	2	5351	Risk Ratio (M-H, Fixed, 95% CI)	0.74 [0.49, 1.12]
10.4 Started pre-pregnancy and continued during the first trimester	3	5527	Risk Ratio (M-H, Fixed, 95% CI)	0.75 [0.50, 1.12]
10.5 History of NTDs	2	1371	Risk Ratio (M-H, Fixed, 95% CI)	1.38 [0.70, 2.73]
10.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	0.53 [0.31, 0.89]
11 Miscarriage (ALL)	4	7319	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [0.96, 1.26]
12 Miscarriage (by subgroups)	4		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
12.1 Daily supplementation scheme	4	7319	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [0.96, 1.26]
12.2 Dose of 400 µg or less	1	176	Risk Ratio (M-H, Fixed, 95% CI)	5.11 [0.25, 105.01]
12.3 Dose of more than 400 µg	3	7143	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [0.96, 1.26]
12.4 Started pre-pregnancy and continued during the first trimester	4	7319	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [0.96, 1.26]
12.5 History of NTDs	3	1817	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.76, 1.30]
12.6 No history of NTDs or unknown	1	5502	Risk Ratio (M-H, Fixed, 95% CI)	1.14 [0.97, 1.34]
13 Stillbirth (ALL)	4	5806	Risk Ratio (M-H, Fixed, 95% CI)	1.36 [0.68, 2.75]
14 Stillbirth (by subgroups)	4		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
14.1 Daily supplementation scheme	4	5806	Risk Ratio (M-H, Fixed, 95% CI)	1.36 [0.68, 2.75]

14.2 Dose of 400 μg or less	1	176	Risk Ratio (M-H, Fixed, 95% CI)	0.20 [0.01, 4.20]
14.3 Dose of more than 400 μg	3	5630	Risk Ratio (M-H, Fixed, 95% CI)	1.62 [0.77, 3.43]
14.4 Started pre-pregnancy and continued	4	5806	Risk Ratio (M-H, Fixed, 95% CI)	1.36 [0.68, 2.75]
14.5 History of NTDs	3	1650	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [0.41, 3.36]
14.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	1.53 [0.60, 3.95]
15 Multiple pregnancy (ALL)	3	6239	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.88, 1.98]
16 Multiple pregnancy (by subgroups)	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
16.1 Daily supplementation scheme	3	6239	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.88, 1.98]
16.2 Dose of 400 μg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
16.3 Dose of more than 400 μg	3	6239	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.88, 1.98]
16.4 Started pre-pregnancy and continued during first trimester	3	6239	Risk Ratio (M-H, Fixed, 95% CI)	1.32 [0.88, 1.98]
16.5 History of NTDs	2	1472	Risk Ratio (M-H, Fixed, 95% CI)	1.02 [0.38, 2.70]
16.6 No history of NTDs or unknown	1	4767	Risk Ratio (M-H, Fixed, 95% CI)	1.39 [0.89, 2.18]
17 Pregnancy termination for fetal abnormality (ALL)	3	5797	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.55]
18 Pregnancy termination for fetal abnormality (by subgroups)	3	23188	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.22, 0.40]
18.1 Daily supplementation scheme	3	5797	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.55]
18.2 Dose of 400 μg or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
18.3 Dose of more than 400 μg	3	5797	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.55]
18.4 Started pre-pregnancy and continued during first trimester	3	5797	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.16, 0.55]
18.5 History of NTDs	2	1641	Risk Ratio (M-H, Fixed, 95% CI)	0.33 [0.16, 0.67]
18.6 No history of NTDs or unknown	1	4156	Risk Ratio (M-H, Fixed, 95% CI)	0.23 [0.06, 0.79]
19 Low birthweight (ALL)	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]
20 Low birthweight (by subgroups)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
20.1 Daily supplementation scheme	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]
20.2 Dose of 400 É¥g or less	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable
20.3 Dose of more than 400 μg	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]
20.4 Started pre-pregnancy and continued during first trimester	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]
20.5 History of NTDs	1	186	Risk Ratio (M-H, Fixed, 95% CI)	0.80 [0.39, 1.64]

20.6 No history of NTDs or unknown	0	0	Risk Ratio (M-H, Fixed, 95% CI)	Not estimable

Analysis 1.1. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 1 Neural tube defects (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

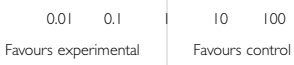
Outcome: 1 Neural tube defects (ALL)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	0/2104	6/2052		14.4 %	0.08 [0.00, 1.33]
ICMR 2000	4/137	10/142		21.4 %	0.41 [0.13, 1.29]
Kirke 1992	0/172	4/192		9.3 %	0.12 [0.01, 2.29]
Laurence 1981	2/60	4/51		9.4 %	0.43 [0.08, 2.23]
MRC 1991	6/593	21/602		45.5 %	0.29 [0.12, 0.71]
Total (95% CI)	**3066**	**3039**		**100.0 %**	**0.28 [0.15, 0.52]**

Total events: 12 (Folic acid), 45 (No treat/other MN/placebo)

Heterogeneity: Chi^2 = 1.79, df = 4 (P = 0.77); I^2 =0.0%

Test for overall effect: Z = 4.05 (P = 0.000051)



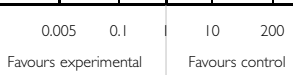
Analysis 1.2. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 2 Neural tube defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 2 Neural tube defects (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	0/2104	6/2052		14.4 %	0.08 [0.00, 1.33]
ICMR 2000	4/137	10/142		21.4 %	0.41 [0.13, 1.29]
Kirke 1992	0/172	4/192		9.3 %	0.12 [0.01, 2.29]
Laurence 1981	2/60	4/51		9.4 %	0.43 [0.08, 2.23]
MRC 1991	6/593	21/602		45.5 %	0.29 [0.12, 0.71]
Subtotal (95% CI)	**3066**	**3039**		**100.0 %**	**0.28 [0.15, 0.52]**
Total events: 12 (Folic acid), 45 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 1.79, df = 4 (P = 0.77); I^2 =0.0%					
Test for overall effect: Z = 4.05 (P = 0.000051)					
2 Dose of 400 g or less					
Kirke 1992	0/172	4/192		100.0 %	0.12 [0.01, 2.29]
Subtotal (95% CI)	**172**	**192**		**100.0 %**	**0.12 [0.01, 2.29]**
Total events: 0 (Folic acid), 4 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.40 (P = 0.16)					
3 Dose of more than 400 g					
Czeizel 1994	0/2104	6/2052		15.8 %	0.08 [0.00, 1.33]
ICMR 2000	4/137	10/142		23.6 %	0.41 [0.13, 1.29]
Laurence 1981	2/60	4/51		10.4 %	0.43 [0.08, 2.23]
MRC 1991	6/593	21/602		50.1 %	0.29 [0.12, 0.71]
Subtotal (95% CI)	**2894**	**2847**		**100.0 %**	**0.30 [0.16, 0.56]**
Total events: 12 (Folic acid), 41 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 1.38, df = 3 (P = 0.71); I^2 =0.0%					
Test for overall effect: Z = 3.78 (P = 0.00016)					
4 Started pre-pregnancy and continued during the first trimester					
Czeizel 1994	0/2104	6/2052		14.4 %	0.08 [0.00, 1.33]
ICMR 2000	4/137	10/142		21.4 %	0.41 [0.13, 1.29]
Kirke 1992	0/172	4/192		9.3 %	0.12 [0.01, 2.29]
Laurence 1981	2/60	4/51		9.4 %	0.43 [0.08, 2.23]



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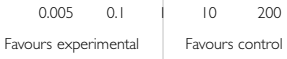
Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
MRC 1991	6/593	21/602		45.5 %	0.29 [0.12, 0.71]
Subtotal (95% CI)	**3066**	**3039**		**100.0 %**	**0.28 [0.15, 0.52]**
Total events: 12 (Folic acid), 45 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 1.79, df = 4 (P = 0.77); I^2 =0.0%					
Test for overall effect: Z = 4.05 (P = 0.000051)					
5 History of NTDs					
ICMR 2000	4/137	10/142		25.0 %	0.41 [0.13, 1.29]
Kirke 1992	0/172	4/192		10.8 %	0.12 [0.01, 2.29]
Laurence 1981	2/60	4/51		11.0 %	0.43 [0.08, 2.23]
MRC 1991	6/593	21/602		53.1 %	0.29 [0.12, 0.71]
Subtotal (95% CI)	**962**	**987**		**100.0 %**	**0.32 [0.17, 0.60]**
Total events: 12 (Folic acid), 39 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.77, df = 3 (P = 0.86); I^2 =0.0%					
Test for overall effect: Z = 3.56 (P = 0.00037)					
6 No history of NTDs or unknown					
Czeizel 1994	0/2104	6/2052		100.0 %	0.08 [0.00, 1.33]
Subtotal (95% CI)	**2104**	**2052**		**100.0 %**	**0.08 [0.00, 1.33]**
Total events: 0 (Folic acid), 6 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.77 (P = 0.078)					



Analysis 1.3. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 3 Cleft palate (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

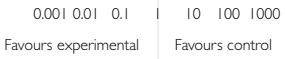
Outcome: 3 Cleft palate (ALL)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	0/2104	2/2052		0.20 [0.01, 4.06]
Kirke 1992	0/172	0/192		0.0 [0.0, 0.0]
MRC 1991	1/593	0/602		3.05 [0.12, 74.61]
Total (95% CI)	**2869**	**2846**		**0.66 [0.11, 3.92]**

Total events: 1 (Folic acid), 2 (No treat/other MN/placebo)

Heterogeneity: Chi^2 = 1.50, df = 1 (P = 0.22); I^2 =33%

Test for overall effect: Z = 0.45 (P = 0.65)



Analysis 1.4. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 4 Cleft palate (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 4 Cleft palate (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme				
Czeizel 1994	0/2104	2/2052		0.20 [0.01, 4.06]
Kirke 1992	0/172	0/192		0.0 [0.0, 0.0]
MRC 1991	1/593	0/602		3.05 [0.12, 74.61]
Subtotal (95% CI)	**2869**	**2846**		**0.66 [0.11, 3.92]**
Total events: 1 (Folic acid), 2 (No treat/other MN/placebo)				
Heterogeneity: Chi² = 1.50, df = 1 (P = 0.22); I² =33%				
Test for overall effect: Z = 0.45 (P = 0.65)				
2 Dose of 400 g or less				
Kirke 1992	0/172	0/192		0.0 [0.0, 0.0]
Subtotal (95% CI)	**172**	**192**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treat/other MN/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
3 Dose of more than 400 g				
Czeizel 1994	0/2104	2/2052		0.20 [0.01, 4.06]
MRC 1991	1/593	0/602		3.05 [0.12, 74.61]
Subtotal (95% CI)	**2697**	**2654**		**0.66 [0.11, 3.92]**
Total events: 1 (Folic acid), 2 (No treat/other MN/placebo)				
Heterogeneity: Chi² = 1.50, df = 1 (P = 0.22); I² =33%				
Test for overall effect: Z = 0.45 (P = 0.65)				
4 Started pre-pregnancy and continued during first trimester				
Czeizel 1994	0/2104	2/2052		0.20 [0.01, 4.06]
Kirke 1992	0/172	0/192		0.0 [0.0, 0.0]
MRC 1991	1/593	0/602		3.05 [0.12, 74.61]
Subtotal (95% CI)	**2869**	**2846**		**0.66 [0.11, 3.92]**
Total events: 1 (Folic acid), 2 (No treat/other MN/placebo)				
Heterogeneity: Chi² = 1.50, df = 1 (P = 0.22); I² =33%				
Test for overall effect: Z = 0.45 (P = 0.65)				
5 History of NTDs				
Kirke 1992	0/172	0/192		0.0 [0.0, 0.0]

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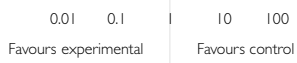
Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
MRC 1991	1/593	0/602		3.05 [0.12, 74.61]
Subtotal (95% CI)	**765**	**794**		**3.05 [0.12, 74.61]**
Total events: 1 (Folic acid), 0 (No treat/other MN/placebo)				
Heterogeneity: Chi^2 = 0.0, df = 0 (P = 1.00); I^2 =0.0%				
Test for overall effect: Z = 0.68 (P = 0.49)				
6 No history of NTDs or unknown				
Czeizel 1994	0/2104	2/2052		0.20 [0.01, 4.06]
Subtotal (95% CI)	**2104**	**2052**		**0.20 [0.01, 4.06]**
Total events: 0 (Folic acid), 2 (No treat/other MN/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 1.06 (P = 0.29)				



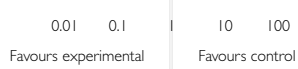
Analysis 1.5. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 5 Cleft lip (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 5 Cleft lip (ALL)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
Kirke 1992	0/172	1/192		0.37 [0.02, 9.07]
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Total (95% CI)	**2869**	**2846**		**1.00 [0.27, 3.74]**
Total events: 4 (Folic acid), 4 (No treat/other MN/placebo)				
Heterogeneity: Chi^2 = 0.49, df = 1 (P = 0.49); I^2 =0.0%				
Test for overall effect: Z = 0.01 (P = 0.99)				



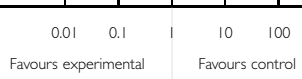
Analysis 1.6. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 6 Cleft lip (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 6 Cleft lip (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme				
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
Kirke 1992	0/172	1/192		0.37 [0.02, 9.07]
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Subtotal (95% CI)	**2869**	**2846**		**1.00 [0.27, 3.74]**
Total events: 4 (Folic acid), 4 (No treat/other MN/placebo)				
Heterogeneity: Chi^2 = 0.49, df = 1 (P = 0.49); I^2 =0.0%				
Test for overall effect: Z = 0.01 (P = 0.99)				
2 Dose of 400 g or less				
Kirke 1992	0/172	1/192		0.37 [0.02, 9.07]
Subtotal (95% CI)	**172**	**192**		**0.37 [0.02, 9.07]**
Total events: 0 (Folic acid), 1 (No treat/other MN/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.61 (P = 0.54)				
3 Dose of more than 400 g				
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Subtotal (95% CI)	**2697**	**2654**		**1.30 [0.29, 5.80]**
Total events: 4 (Folic acid), 3 (No treat/other MN/placebo)				
Heterogeneity: Chi^2 = 0.0, df = 0 (P = 1.00); I^2 =0.0%				
Test for overall effect: Z = 0.34 (P = 0.73)				
4 Started pre-pregnancy and continued during first trimester				
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
Kirke 1992	0/172	1/192		0.37 [0.02, 9.07]
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Subtotal (95% CI)	**2869**	**2846**		**1.00 [0.27, 3.74]**
Total events: 4 (Folic acid), 4 (No treat/other MN/placebo)				
Heterogeneity: Chi^2 = 0.49, df = 1 (P = 0.49); I^2 =0.0%				
Test for overall effect: Z = 0.01 (P = 0.99)				
5 History of NTDs				
Kirke 1992	0/172	1/192		0.37 [0.02, 9.07]



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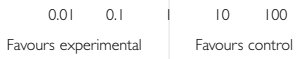
Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Subtotal (95% CI)	**765**	**794**		**0.37 [0.02, 9.07]**
Total events: 0 (Folic acid), 1 (No treat/other MN/placebo)				
Heterogeneity: Chi^2 = 0.00, df = 0 (P<0.00001); I^2 =100%				
Test for overall effect: Z = 0.61 (P = 0.54)				
6 No history of NTDs or unknown				
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
Subtotal (95% CI)	**2104**	**2052**		**1.30 [0.29, 5.80]**
Total events: 4 (Folic acid), 3 (No treat/other MN/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.34 (P = 0.73)				



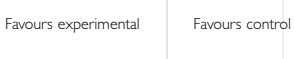
Analysis 1.7. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 7 Congenital cardiovascular defects (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 7 Congenital cardiovascular defects (ALL)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	10/2104	17/2052		83.7 %	0.57 [0.26, 1.25]
Kirke 1992	0/172	2/192		11.5 %	0.22 [0.01, 4.62]
MRC 1991	1/593	1/602		4.8 %	1.02 [0.06, 16.19]
Total (95% CI)	**2869**	**2846**		**100.0 %**	**0.55 [0.27, 1.14]**
Total events: 11 (Folic acid), 20 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.54, df = 2 (P = 0.76); I^2 =0.0%					
Test for overall effect: Z = 1.60 (P = 0.11)					



Analysis 1.8. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 8 Congenital cardiovascular defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 8 Congenital cardiovascular defects (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	10/2104	17/2052		83.7 %	0.57 [0.26, 1.25]
Kirke 1992	0/172	2/192		11.5 %	0.22 [0.01, 4.62]
MRC 1991	1/593	1/602		4.8 %	1.02 [0.06, 16.19]
Subtotal (95% CI)	**2869**	**2846**		**100.0 %**	**0.55 [0.27, 1.14]**
Total events: 11 (Folic acid), 20 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.54, df = 2 (P = 0.76); I^2 =0.0%					
Test for overall effect: Z = 1.60 (P = 0.11)					
2 Dose of more than 400 g					
Czeizel 1994	10/2104	17/2052		94.5 %	0.57 [0.26, 1.25]
MRC 1991	1/593	1/602		5.5 %	1.02 [0.06, 16.19]
Subtotal (95% CI)	**2697**	**2654**		**100.0 %**	**0.60 [0.28, 1.26]**
Total events: 11 (Folic acid), 18 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.15, df = 1 (P = 0.70); I^2 =0.0%					
Test for overall effect: Z = 1.35 (P = 0.18)					
3 Dose of 400 g or less					
Kirke 1992	0/172	2/192		100.0 %	0.22 [0.01, 4.62]
Subtotal (95% CI)	**172**	**192**		**100.0 %**	**0.22 [0.01, 4.62]**
Total events: 0 (Folic acid), 2 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.97 (P = 0.33)					
4 Started pre-pregnancy and continued					
Czeizel 1994	10/2104	17/2052		83.7 %	0.57 [0.26, 1.25]
Kirke 1992	0/172	2/192		11.5 %	0.22 [0.01, 4.62]
MRC 1991	1/593	1/602		4.8 %	1.02 [0.06, 16.19]
Subtotal (95% CI)	**2869**	**2846**		**100.0 %**	**0.55 [0.27, 1.14]**
Total events: 11 (Folic acid), 20 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.54, df = 2 (P = 0.76); I^2 =0.0%					
Test for overall effect: Z = 1.60 (P = 0.11)					
5 History of NTDs					
Kirke 1992	0/172	2/192		70.4 %	0.22 [0.01, 4.62]

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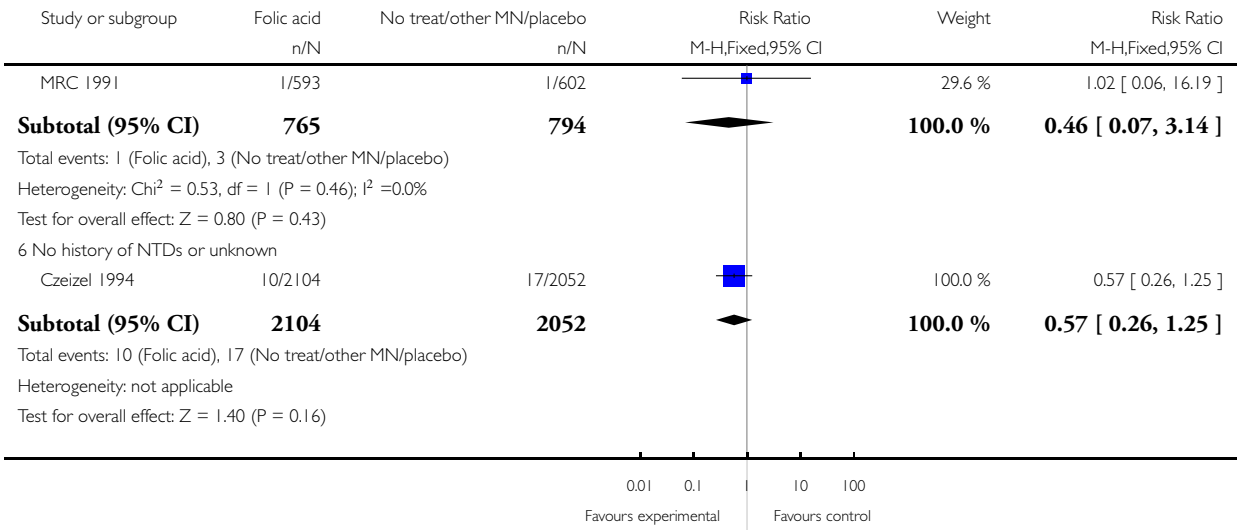
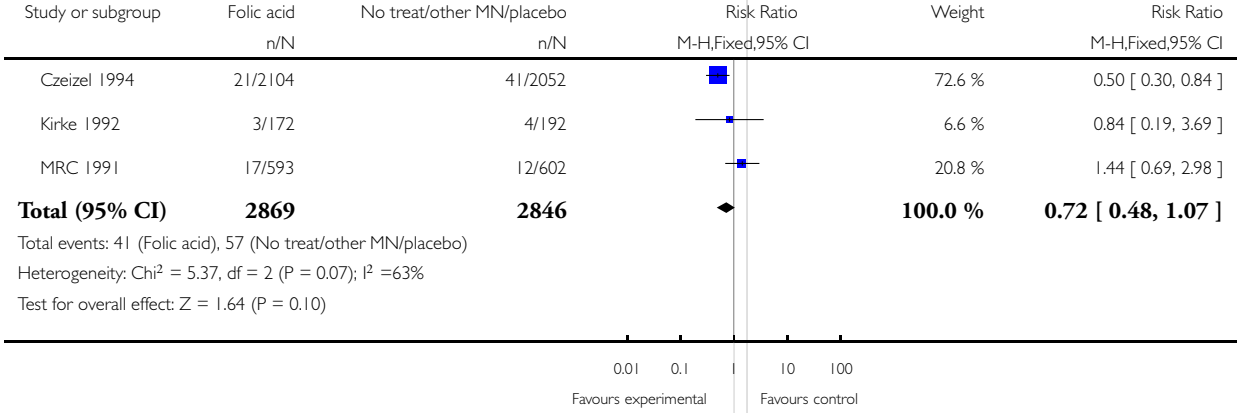


Analysis 1.9. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 9 Other birth defects (any) (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 9 Other birth defects (any) (ALL)



Analysis 1.10. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 10 Other birth defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 10 Other birth defects (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	21/2104	41/2052		72.6 %	0.50 [0.30, 0.84]
Kirke 1992	3/172	4/192		6.6 %	0.84 [0.19, 3.69]
MRC 1991	17/593	12/602		20.8 %	1.44 [0.69, 2.98]
Subtotal (95% CI)	**2869**	**2846**		**100.0 %**	**0.72 [0.48, 1.07]**
Total events: 41 (Folic acid), 57 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 5.37, df = 2 (P = 0.07); I^2 =63%					
Test for overall effect: Z = 1.64 (P = 0.10)					
2 Dose of 400 g or less					
Kirke 1992	3/172	4/192		100.0 %	0.84 [0.19, 3.69]
Subtotal (95% CI)	**172**	**192**		**100.0 %**	**0.84 [0.19, 3.69]**
Total events: 3 (Folic acid), 4 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.23 (P = 0.81)					
3 Dose of more than 400 g					
Czeizel 1994	21/2104	41/2052		77.7 %	0.50 [0.30, 0.84]
MRC 1991	17/593	12/602		22.3 %	1.44 [0.69, 2.98]
Subtotal (95% CI)	**2697**	**2654**		**100.0 %**	**0.71 [0.47, 1.07]**
Total events: 38 (Folic acid), 53 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 5.33, df = 1 (P = 0.02); I^2 =81%					
Test for overall effect: Z = 1.64 (P = 0.10)					
4 Started pre-pregnancy and continued during the first trimester					
Czeizel 1994	21/2104	41/2052		72.6 %	0.50 [0.30, 0.84]
Kirke 1992	3/172	4/192		6.6 %	0.84 [0.19, 3.69]
MRC 1991	17/593	12/602		20.8 %	1.44 [0.69, 2.98]
Subtotal (95% CI)	**2869**	**2846**		**100.0 %**	**0.72 [0.48, 1.07]**
Total events: 41 (Folic acid), 57 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 5.37, df = 2 (P = 0.07); I^2 =63%					
Test for overall effect: Z = 1.64 (P = 0.10)					
5 History of NTDs					
Kirke 1992	3/172	4/192		24.1 %	0.84 [0.19, 3.69]

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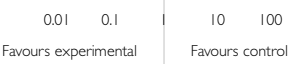
Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
MRC 1991	17/593	12/602		75.9 %	1.44 [0.69, 2.98]
Subtotal (95% CI)	**765**	**794**		**100.0 %**	**1.29 [0.67, 2.48]**
Total events: 20 (Folic acid), 16 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.41, df = 1 (P = 0.52); I^2 =0.0%					
Test for overall effect: Z = 0.77 (P = 0.44)					
6 No history of NTDs or unknown					
Czeizel 1994	21/2104	41/2052		100.0 %	0.50 [0.30, 0.84]
Subtotal (95% CI)	**2104**	**2052**		**100.0 %**	**0.50 [0.30, 0.84]**
Total events: 21 (Folic acid), 41 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 2.60 (P = 0.0092)					



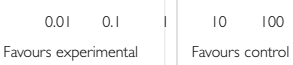
Analysis 1.11. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 11 Miscarriage (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 11 Miscarriage (ALL)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	301/2819	251/2683		70.1 %	1.14 [0.97, 1.34]
ICMR 2000	6/137	14/142		3.7 %	0.44 [0.18, 1.12]
Kirke 1992	16/172	13/192		3.3 %	1.37 [0.68, 2.77]
Laurence 1981	1/60	1/51		0.3 %	0.85 [0.05, 13.25]
MRC 1991	87/677	83/685		22.5 %	1.06 [0.80, 1.41]
Total (95% CI)	**3865**	**3753**		**100.0 %**	**1.10 [0.97, 1.26]**
Total events: 411 (Folic acid), 362 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 4.36, df = 4 (P = 0.36); I^2 =8%					
Test for overall effect: Z = 1.45 (P = 0.15)					



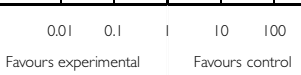
Analysis 1.12. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 12 Miscarriage (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 12 Miscarriage (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	301/2819	251/2683		70.1 %	1.14 [0.97, 1.34]
ICMR 2000	6/137	14/142		3.7 %	0.44 [0.18, 1.12]
Kirke 1992	16/172	13/192		3.3 %	1.37 [0.68, 2.77]
Laurence 1981	1/60	1/51		0.3 %	0.85 [0.05, 13.25]
MRC 1991	87/677	83/685		22.5 %	1.06 [0.80, 1.41]
Subtotal (95% CI)	**3865**	**3753**		**100.0 %**	**1.10 [0.97, 1.26]**
Total events: 411 (Folic acid), 362 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 4.36, df = 4 (P = 0.36); I^2 =8%					
Test for overall effect: Z = 1.45 (P = 0.15)					
2 Dose of 400 g or less					
Kirke 1992	16/172	13/192		100.0 %	1.37 [0.68, 2.77]
Subtotal (95% CI)	**172**	**192**		**100.0 %**	**1.37 [0.68, 2.77]**
Total events: 16 (Folic acid), 13 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.89 (P = 0.38)					
3 Dose of more than 400 g					
Czeizel 1994	301/2819	251/2683		72.5 %	1.14 [0.97, 1.34]
ICMR 2000	6/137	14/142		3.9 %	0.44 [0.18, 1.12]
Laurence 1981	1/60	1/51		0.3 %	0.85 [0.05, 13.25]
MRC 1991	87/677	83/685		23.3 %	1.06 [0.80, 1.41]
Subtotal (95% CI)	**3693**	**3561**		**100.0 %**	**1.09 [0.96, 1.25]**
Total events: 395 (Folic acid), 349 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 3.98, df = 3 (P = 0.26); I^2 =25%					
Test for overall effect: Z = 1.30 (P = 0.19)					
4 Started pre-pregnancy and continued during first trimester					
Czeizel 1994	301/2819	251/2683		70.1 %	1.14 [0.97, 1.34]
ICMR 2000	6/137	14/142		3.7 %	0.44 [0.18, 1.12]
Kirke 1992	16/172	13/192		3.3 %	1.37 [0.68, 2.77]
Laurence 1981	1/60	1/51		0.3 %	0.85 [0.05, 13.25]



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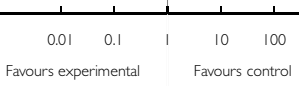
Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
MRC 1991	87/677	83/685		22.5 %	1.06 [0.80, 1.41]
Subtotal (95% CI)	**3865**	**3753**		**100.0 %**	**1.10 [0.97, 1.26]**
Total events: 411 (Folic acid), 362 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 4.36, df = 4 (P = 0.36); I^2 =8%					
Test for overall effect: Z = 1.45 (P = 0.15)					
5 History of NTDs					
ICMR 2000	6/137	14/142		12.5 %	0.44 [0.18, 1.12]
Kirke 1992	16/172	13/192		11.2 %	1.37 [0.68, 2.77]
Laurence 1981	1/60	1/51		1.0 %	0.85 [0.05, 13.25]
MRC 1991	87/677	83/685		75.3 %	1.06 [0.80, 1.41]
Subtotal (95% CI)	**1046**	**1070**		**100.0 %**	**1.02 [0.79, 1.30]**
Total events: 110 (Folic acid), 111 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 3.87, df = 3 (P = 0.28); I^2 =23%					
Test for overall effect: Z = 0.13 (P = 0.90)					
6 No history of NTDs or unknown					
Czeizel 1994	301/2819	251/2683		100.0 %	1.14 [0.97, 1.34]
Subtotal (95% CI)	**2819**	**2683**		**100.0 %**	**1.14 [0.97, 1.34]**
Total events: 301 (Folic acid), 251 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.63 (P = 0.10)					



Analysis 1.13. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 13 Stillbirth (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 13 Stillbirth (ALL)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	11/2104	7/2052		37.0 %	1.53 [0.60, 3.95]
ICMR 2000	3/137	3/142		15.4 %	1.04 [0.21, 5.05]
Kirke 1992	0/172	6/192		32.1 %	0.09 [0.00, 1.51]
MRC 1991	4/593	3/602		15.5 %	1.35 [0.30, 6.02]
Total (95% CI)	**3006**	**2988**		**100.0 %**	**0.96 [0.51, 1.83]**

Total events: 18 (Folic acid), 19 (No treat/other MN/placebo)

Heterogeneity: $Chi^2 = 3.86$, df = 3 (P = 0.28); $I^2 = 22\%$

Test for overall effect: Z = 0.11 (P = 0.91)

Analysis 1.14. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 14 Stillbirth (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 14 Stillbirth (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation					
Czeizel 1994	11/2104	7/2052		37.0 %	1.53 [0.60, 3.95]
ICMR 2000	3/137	3/142		15.4 %	1.04 [0.21, 5.05]
Kirke 1992	0/172	6/192		32.1 %	0.09 [0.00, 1.51]
MRC 1991	4/593	3/602		15.5 %	1.35 [0.30, 6.02]
Subtotal (95% CI)	**3006**	**2988**		**100.0 %**	**0.96 [0.51, 1.83]**
Total events: 18 (Folic acid), 19 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 3.86, df = 3 (P = 0.28); I^2 =22%					
Test for overall effect: Z = 0.11 (P = 0.91)					
2 Dose of 400 g or less					
Kirke 1992	0/172	6/192		100.0 %	0.09 [0.00, 1.51]
Subtotal (95% CI)	**172**	**192**		**100.0 %**	**0.09 [0.00, 1.51]**
Total events: 0 (Folic acid), 6 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.68 (P = 0.093)					
3 Dose of more than 400 g					
Czeizel 1994	11/2104	7/2052		54.5 %	1.53 [0.60, 3.95]
ICMR 2000	3/137	3/142		22.6 %	1.04 [0.21, 5.05]
MRC 1991	4/593	3/602		22.9 %	1.35 [0.30, 6.02]
Subtotal (95% CI)	**2834**	**2796**		**100.0 %**	**1.38 [0.68, 2.81]**
Total events: 18 (Folic acid), 13 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.17, df = 2 (P = 0.92); I^2 =0.0%					
Test for overall effect: Z = 0.89 (P = 0.38)					
4 Started pre-pregnancy and continued					
Czeizel 1994	11/2104	7/2052		37.0 %	1.53 [0.60, 3.95]
ICMR 2000	3/137	3/142		15.4 %	1.04 [0.21, 5.05]
Kirke 1992	0/172	6/192		32.1 %	0.09 [0.00, 1.51]
MRC 1991	4/593	3/602		15.5 %	1.35 [0.30, 6.02]
Subtotal (95% CI)	**3006**	**2988**		**100.0 %**	**0.96 [0.51, 1.83]**
Total events: 18 (Folic acid), 19 (No treat/other MN/placebo)					

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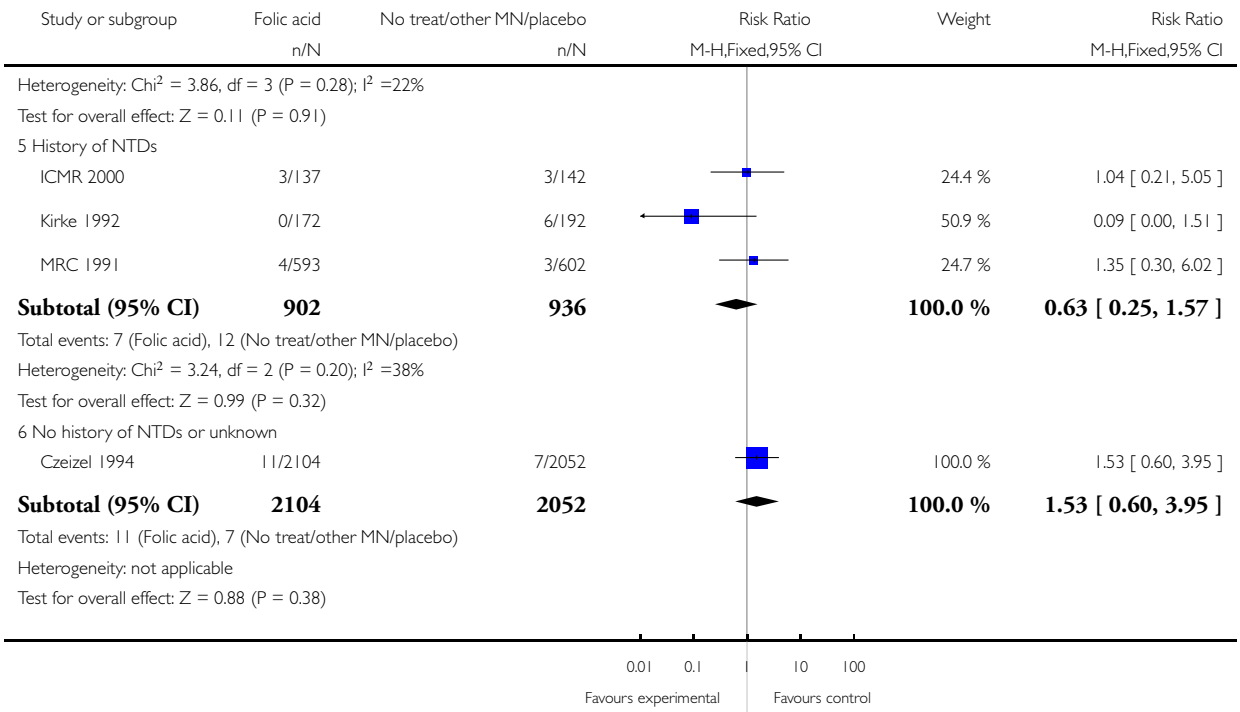
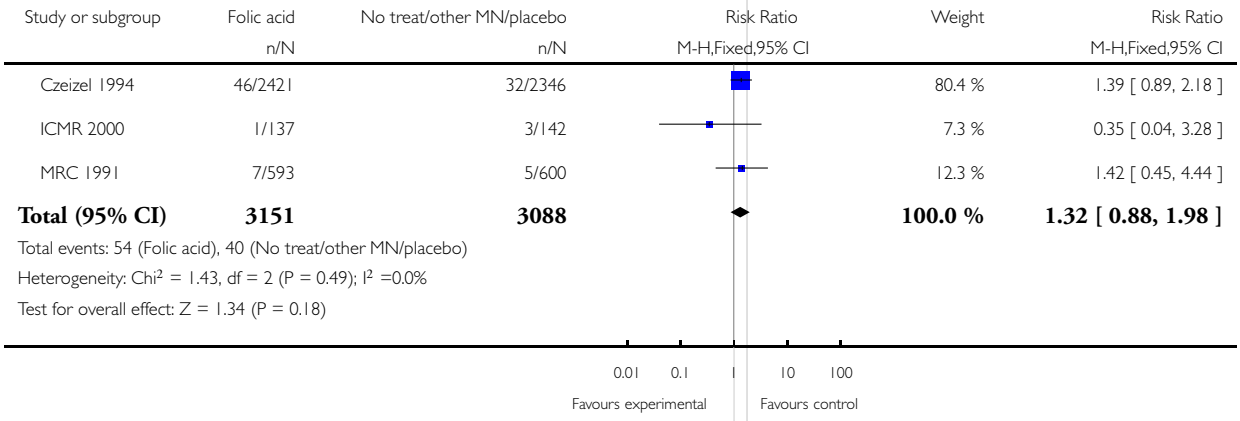


Analysis 1.15. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 15 Multiple pregnancy (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 15 Multiple pregnancy (ALL)



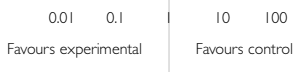
Analysis 1.16. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 16 Multiple pregnancy (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 16 Multiple pregnancy (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	46/2421	32/2346		80.4 %	1.39 [0.89, 2.18]
ICMR 2000	1/137	3/142		7.3 %	0.35 [0.04, 3.28]
MRC 1991	7/593	5/600		12.3 %	1.42 [0.45, 4.44]
Subtotal (95% CI)	**3151**	**3088**		**100.0 %**	**1.32 [0.88, 1.98]**
Total events: 54 (Folic acid), 40 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 1.43, df = 2 (P = 0.49); I^2 =0.0%					
Test for overall effect: Z = 1.34 (P = 0.18)					
2 Dose of 400 g or less					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
3 Dose of more than 400 g					
Czeizel 1994	46/2421	32/2346		80.4 %	1.39 [0.89, 2.18]
ICMR 2000	1/137	3/142		7.3 %	0.35 [0.04, 3.28]
MRC 1991	7/593	5/600		12.3 %	1.42 [0.45, 4.44]
Subtotal (95% CI)	**3151**	**3088**		**100.0 %**	**1.32 [0.88, 1.98]**
Total events: 54 (Folic acid), 40 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 1.43, df = 2 (P = 0.49); I^2 =0.0%					
Test for overall effect: Z = 1.34 (P = 0.18)					
4 Started pre-pregnancy and continued during first trimester					
Czeizel 1994	46/2421	32/2346		80.4 %	1.39 [0.89, 2.18]
ICMR 2000	1/137	3/142		7.3 %	0.35 [0.04, 3.28]
MRC 1991	7/593	5/600		12.3 %	1.42 [0.45, 4.44]
Subtotal (95% CI)	**3151**	**3088**		**100.0 %**	**1.32 [0.88, 1.98]**
Total events: 54 (Folic acid), 40 (No treat/other MN/placebo)					



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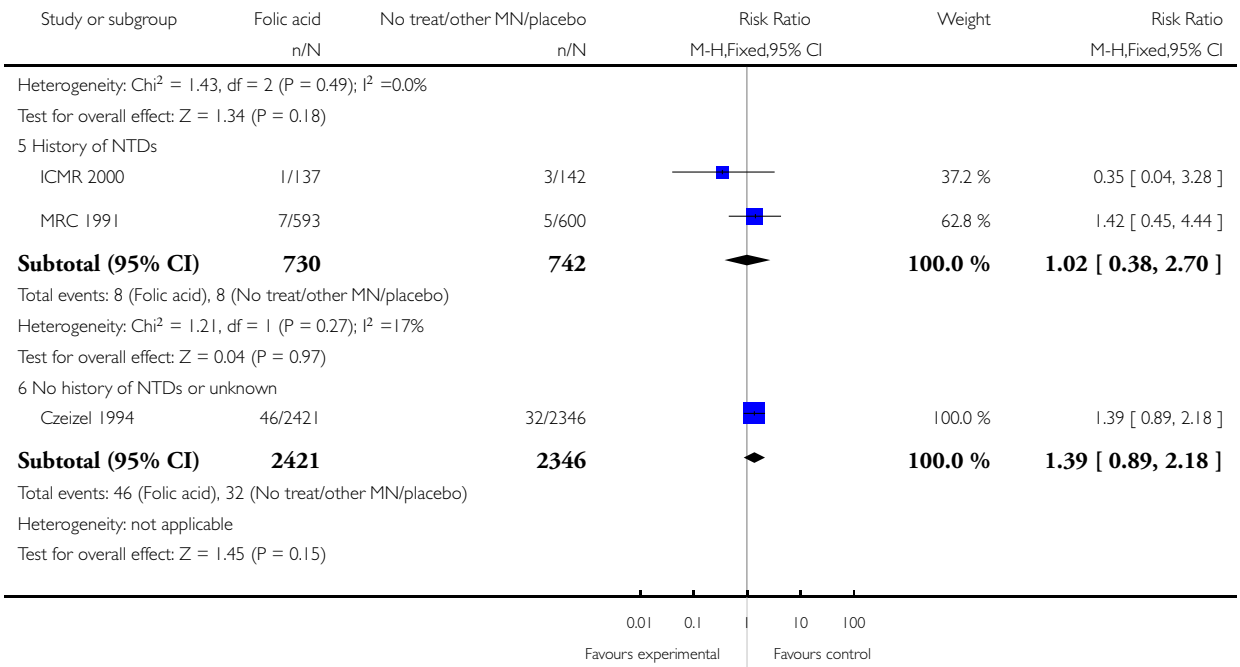
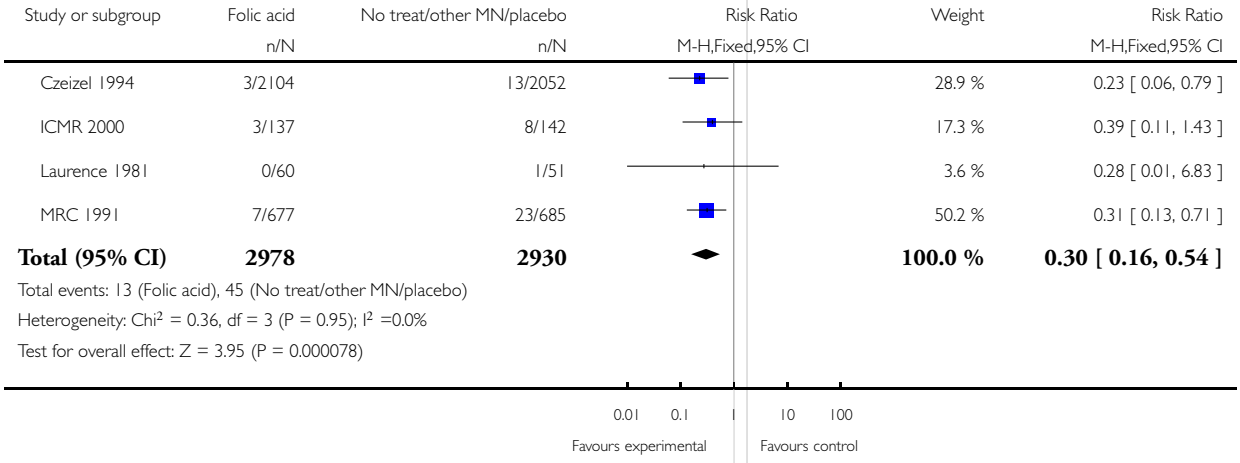


Analysis 1.17. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 17 Pregnancy termination for fetal abnormality (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 17 Pregnancy termination for fetal abnormality (ALL)



Analysis 1.18. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 18 Pregnancy termination for fetal abnormality (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 18 Pregnancy termination for fetal abnormality (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	3/2104	13/2052		28.9 %	0.23 [0.06, 0.79]
ICMR 2000	3/137	8/142		17.3 %	0.39 [0.11, 1.43]
Laurence 1981	0/60	1/51		3.6 %	0.28 [0.01, 6.83]
MRC 1991	7/677	23/685		50.2 %	0.31 [0.13, 0.71]
Subtotal (95% CI)	**2978**	**2930**		**100.0 %**	**0.30 [0.16, 0.54]**
Total events: 13 (Folic acid), 45 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.36, df = 3 (P = 0.95); I^2 =0.0%					
Test for overall effect: Z = 3.95 (P = 0.000078)					
2 Dose of 400 g or less					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
3 Dose of more than 400 g					
Czeizel 1994	3/2104	13/2052		28.9 %	0.23 [0.06, 0.79]
ICMR 2000	3/137	8/142		17.3 %	0.39 [0.11, 1.43]
Laurence 1981	0/60	1/51		3.6 %	0.28 [0.01, 6.83]
MRC 1991	7/677	23/685		50.2 %	0.31 [0.13, 0.71]
Subtotal (95% CI)	**2978**	**2930**		**100.0 %**	**0.30 [0.16, 0.54]**
Total events: 13 (Folic acid), 45 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.36, df = 3 (P = 0.95); I^2 =0.0%					
Test for overall effect: Z = 3.95 (P = 0.000078)					
4 Started pre-pregnancy and continued during first trimester					
Czeizel 1994	3/2104	13/2052		28.9 %	0.23 [0.06, 0.79]
ICMR 2000	3/137	8/142		17.3 %	0.39 [0.11, 1.43]
Laurence 1981	0/60	1/51		3.6 %	0.28 [0.01, 6.83]

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Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
MRC 1991	7/677	23/685		50.2 %	0.31 [0.13, 0.71]
Subtotal (95% CI)	**2978**	**2930**		**100.0 %**	**0.30 [0.16, 0.54]**
Total events: 13 (Folic acid), 45 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.36, df = 3 (P = 0.95); I^2 =0.0%					
Test for overall effect: Z = 3.95 (P = 0.000078)					
5 History of NTDs					
ICMR 2000	3/137	8/142		24.3 %	0.39 [0.11, 1.43]
Laurence 1981	0/60	1/51		5.0 %	0.28 [0.01, 6.83]
MRC 1991	7/677	23/685		70.7 %	0.31 [0.13, 0.71]
Subtotal (95% CI)	**874**	**878**		**100.0 %**	**0.33 [0.16, 0.65]**
Total events: 10 (Folic acid), 32 (No treat/other MN/placebo)					
Heterogeneity: Chi^2 = 0.09, df = 2 (P = 0.95); I^2 =0.0%					
Test for overall effect: Z = 3.19 (P = 0.0014)					
6 No history of NTDs or unknown					
Czeizel 1994	3/2104	13/2052		100.0 %	0.23 [0.06, 0.79]
Subtotal (95% CI)	**2104**	**2052**		**100.0 %**	**0.23 [0.06, 0.79]**
Total events: 3 (Folic acid), 13 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 2.33 (P = 0.020)					

Analysis 1.19. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 19 Low birthweight (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 19 Low birthweight (ALL)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Total (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid), 14 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					

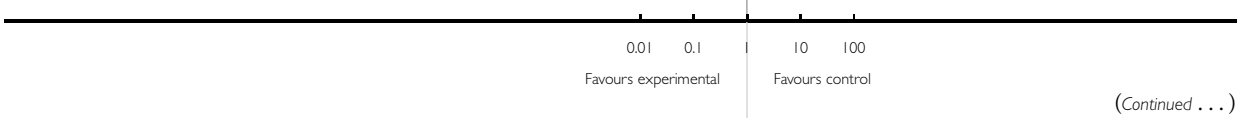
Analysis 1.20. Comparison 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo, Outcome 20 Low birthweight (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 1 Supplementation with folic acid versus no treatment/other micronutrients/placebo

Outcome: 20 Low birthweight (by subgroups)

Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Subtotal (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid), 14 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					
2 Dose of 400 g or less					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
3 Dose of more than 400 g					
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Subtotal (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid), 14 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					
4 Started pre-pregnancy and continue during first trimester					
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Subtotal (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid), 14 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					
5 History of NTDs					
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Subtotal (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid), 14 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					
6 No history of NTDs or unknown					



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Study or subgroup	Folic acid n/N	No treat/other MN/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treat/other MN/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					

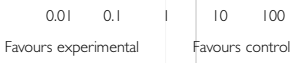
Analysis 2.1. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 1 Neural tube defects (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 1 Neural tube defects (ALL)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/85	3/103		42.3 %	0.17 [0.01, 3.30]
Laurence 1981	2/60	4/51		57.7 %	0.43 [0.08, 2.23]
Total (95% CI)	**145**	**154**		**100.0 %**	**0.32 [0.08, 1.34]**
Total events: 2 (Folic acid), 7 (No treatment/placebo)					
Heterogeneity: Chi^2 = 0.28, df = 1 (P = 0.60); I^2 =0.0%					
Test for overall effect: Z = 1.56 (P = 0.12)					

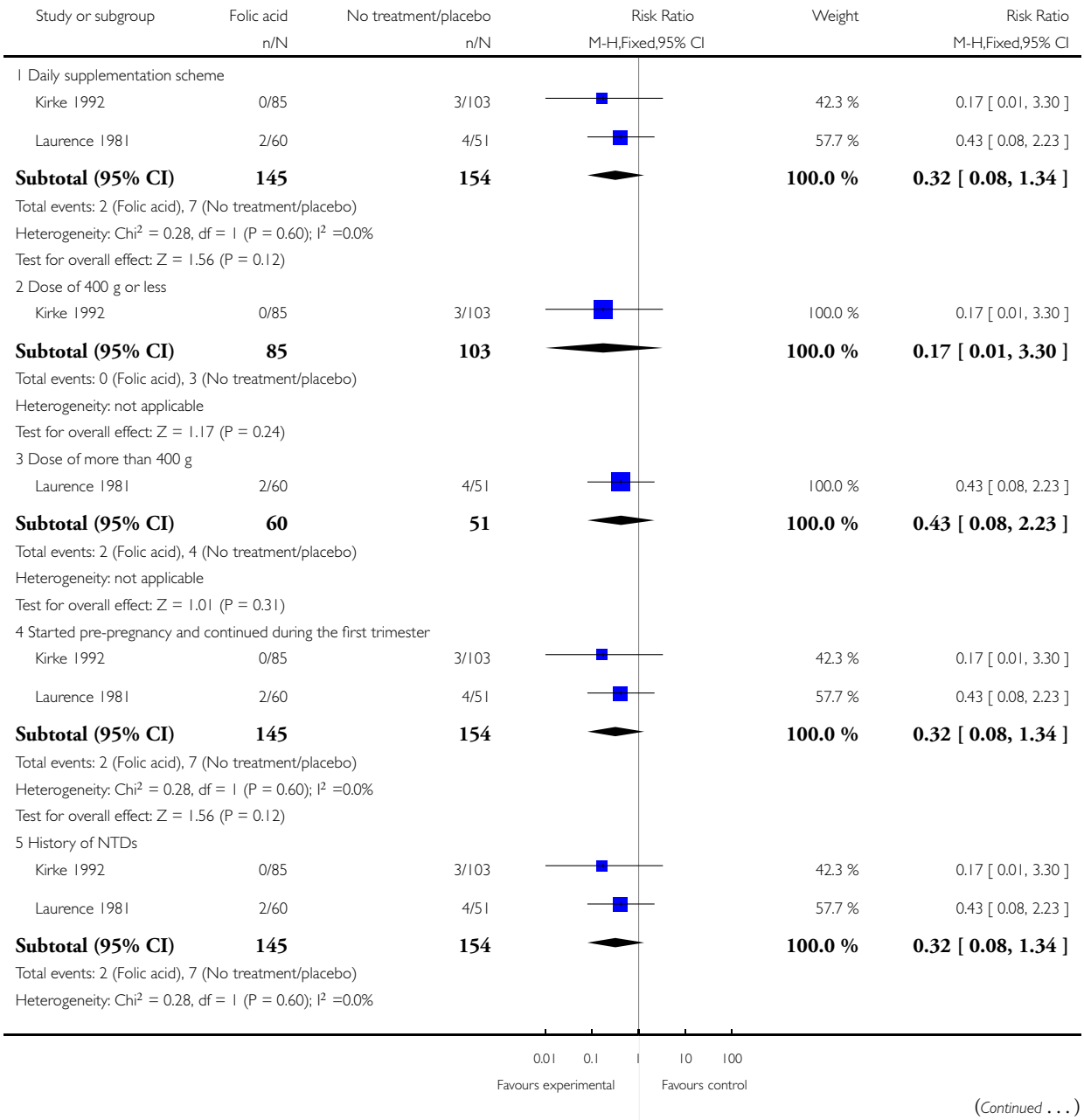


Analysis 2.2. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 2 Neural tube defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 2 Neural tube defects (by subgroups)



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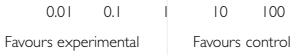
Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 1.56 (P = 0.12)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					



Analysis 2.3. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 3 Cleft palate (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 3 Cleft palate (ALL)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Total (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				

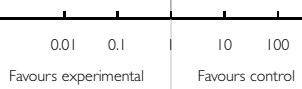
Analysis 2.4. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 4 Cleft palate (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 4 Cleft palate (by subgroups)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme				
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
2 Dose of 400 g or less				
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
3 Dose of more than 400 g				
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: not applicable				
4 Started pre-pregnancy and continued during first trimester				
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
5 History of NTDs				
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
6 No history of NTDs or unknown				
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: not applicable				



Analysis 2.5. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 5 Cleft lip (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 5 Cleft lip (ALL)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Total (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				

Analysis 2.6. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 6 Cleft lip (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 6 Cleft lip (by subgroups)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme				
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
2 Dose of 400 g or less				
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				

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Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 0.0 (P < 0.00001)				
3 Dose of more than 400 g				
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: not applicable				
4 Started pre-pregnancy and continued during first trimester				
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
5 History of NTDs				
Kirke 1992	0/85	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**85**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
6 No history of NTDs or unknown				
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: not applicable				

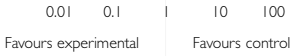
Analysis 2.7. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 7 Congenital cardiovascular defects (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 7 Congenital cardiovascular defects (ALL)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/85	1/103		100.0 %	0.40 [0.02, 9.77]
Total (95% CI)	**85**	**103**		**100.0 %**	**0.40 [0.02, 9.77]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.56 (P = 0.58)					



Analysis 2.8. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 8 Congenital cardiovascular defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 8 Congenital cardiovascular defects (by subgroups)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Kirke 1992	0/85	1/103		100.0 %	0.40 [0.02, 9.77]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.40 [0.02, 9.77]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.56 (P = 0.58)					
2 Dose of 400 g or less					
Kirke 1992	0/85	1/103		100.0 %	0.40 [0.02, 9.77]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.40 [0.02, 9.77]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					

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Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 0.56 (P = 0.58)					
3 Dose of more than 400 g					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
4 Started pre-pregnancy and continued					
Kirke 1992	0/85	1/103		100.0 %	0.40 [0.02, 9.77]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.40 [0.02, 9.77]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.56 (P = 0.58)					
5 History of NTDs					
Kirke 1992	0/85	1/103		100.0 %	0.40 [0.02, 9.77]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.40 [0.02, 9.77]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.56 (P = 0.58)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					

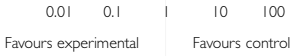
Analysis 2.9. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 9 Other birth defects (any) (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 9 Other birth defects (any) (ALL)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	1/85	2/103		100.0 %	0.61 [0.06, 6.57]
Total (95% CI)	**85**	**103**		**100.0 %**	**0.61 [0.06, 6.57]**
Total events: 1 (Folic acid), 2 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.41 (P = 0.68)					



Analysis 2.10. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 10 Other birth defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 10 Other birth defects (by subgroups)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Kirke 1992	1/85	2/103		100.0 %	0.61 [0.06, 6.57]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.61 [0.06, 6.57]**
Total events: 1 (Folic acid), 2 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.41 (P = 0.68)					
2 Dose of 400 g or less					
Kirke 1992	1/85	2/103		100.0 %	0.61 [0.06, 6.57]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.61 [0.06, 6.57]**
Total events: 1 (Folic acid), 2 (No treatment/placebo)					
Heterogeneity: not applicable					

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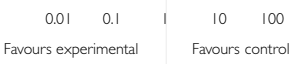
Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 0.41 (P = 0.68)					
3 Dose of more than 400 g					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
4 Started pre-pregnancy and continued during the first trimester					
Kirke 1992	1/85	2/103		100.0 %	0.61 [0.06, 6.57]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.61 [0.06, 6.57]**
Total events: 1 (Folic acid), 2 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.41 (P = 0.68)					
5 History of NTDs					
Kirke 1992	1/85	2/103		100.0 %	0.61 [0.06, 6.57]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.61 [0.06, 6.57]**
Total events: 1 (Folic acid), 2 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.41 (P = 0.68)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					

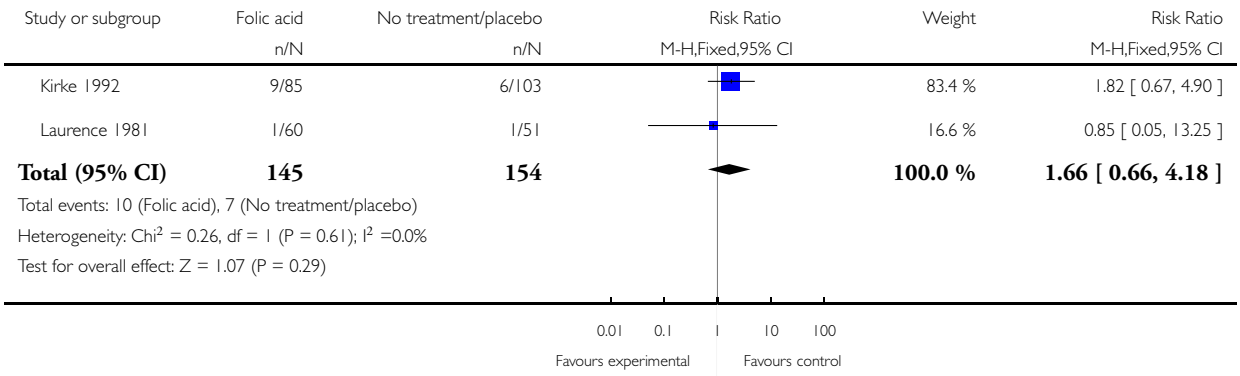


Analysis 2.11. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 11 Miscarriage (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 11 Miscarriage (ALL)

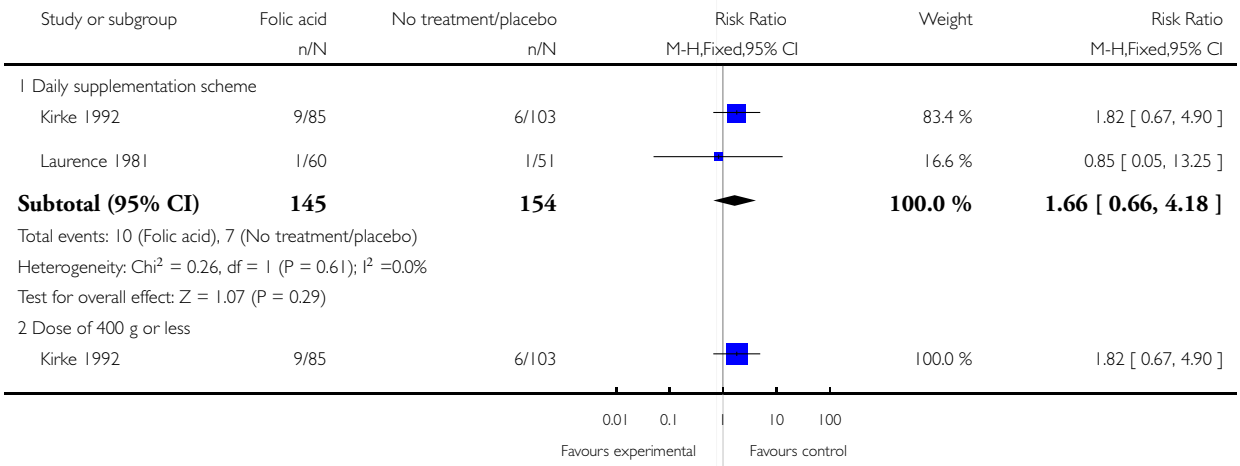


Analysis 2.12. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 12 Miscarriage (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 12 Miscarriage (by subgroups)



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Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**1.82 [0.67, 4.90]**
Total events: 9 (Folic acid), 6 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.18 (P = 0.24)					
3 Dose of more than 400 g					
Laurence 1981	1/60	1/51		100.0 %	0.85 [0.05, 13.25]
Subtotal (95% CI)	**60**	**51**		**100.0 %**	**0.85 [0.05, 13.25]**
Total events: 1 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.12 (P = 0.91)					
4 Started pre-pregnancy and continued during first trimester					
Kirke 1992	9/85	6/103		83.4 %	1.82 [0.67, 4.90]
Laurence 1981	1/60	1/51		16.6 %	0.85 [0.05, 13.25]
Subtotal (95% CI)	**145**	**154**		**100.0 %**	**1.66 [0.66, 4.18]**
Total events: 10 (Folic acid), 7 (No treatment/placebo)					
Heterogeneity: Chi^2 = 0.26, df = 1 (P = 0.61); I^2 =0.0%					
Test for overall effect: Z = 1.07 (P = 0.29)					
5 History of NTDs					
Kirke 1992	9/85	6/103		83.4 %	1.82 [0.67, 4.90]
Laurence 1981	1/60	1/51		16.6 %	0.85 [0.05, 13.25]
Subtotal (95% CI)	**145**	**154**		**100.0 %**	**1.66 [0.66, 4.18]**
Total events: 10 (Folic acid), 7 (No treatment/placebo)					
Heterogeneity: Chi^2 = 0.26, df = 1 (P = 0.61); I^2 =0.0%					
Test for overall effect: Z = 1.07 (P = 0.29)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					

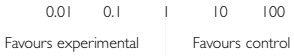
Analysis 2.13. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 13 Stillbirth (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 13 Stillbirth (ALL)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/85	4/103		100.0 %	0.13 [0.01, 2.46]
Total (95% CI)	**85**	**103**		**100.0 %**	**0.13 [0.01, 2.46]**
Total events: 0 (Folic acid), 4 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.35 (P = 0.18)					



Analysis 2.14. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 14 Stillbirth (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 14 Stillbirth (by subgroups)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation					
Kirke 1992	0/85	4/103		100.0 %	0.13 [0.01, 2.46]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.13 [0.01, 2.46]**
Total events: 0 (Folic acid), 4 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.35 (P = 0.18)					
2 Dose of 400 g or less					
Kirke 1992	0/85	4/103		100.0 %	0.13 [0.01, 2.46]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.13 [0.01, 2.46]**
Total events: 0 (Folic acid), 4 (No treatment/placebo)					
Heterogeneity: not applicable					

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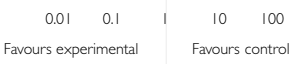
Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 1.35 (P = 0.18)					
3 Dose of more than 400 g					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
4 Started pre-pregnancy and continued during first trimester					
Kirke 1992	0/85	4/103		100.0 %	0.13 [0.01, 2.46]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.13 [0.01, 2.46]**
Total events: 0 (Folic acid), 4 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.35 (P = 0.18)					
5 History of NTDs					
Kirke 1992	0/85	4/103		100.0 %	0.13 [0.01, 2.46]
Subtotal (95% CI)	**85**	**103**		**100.0 %**	**0.13 [0.01, 2.46]**
Total events: 0 (Folic acid), 4 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.35 (P = 0.18)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					



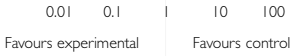
Analysis 2.17. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 17 Pregnancy termination for fetal abnormality (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 17 Pregnancy termination for fetal abnormality (ALL)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Laurence 1981	0/60	1/51		100.0 %	0.28 [0.01, 6.83]
Total (95% CI)	**60**	**51**		**100.0 %**	**0.28 [0.01, 6.83]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.78 (P = 0.44)					



Analysis 2.18. Comparison 2 Supplementation with folic acid alone versus no treatment/placebo, Outcome 18 Pregnancy termination for fetal abnormality (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 2 Supplementation with folic acid alone versus no treatment/placebo

Outcome: 18 Pregnancy termination for fetal abnormality (by subgroups)

Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Laurence 1981	0/60	1/51		100.0 %	0.28 [0.01, 6.83]
Subtotal (95% CI)	**60**	**51**		**100.0 %**	**0.28 [0.01, 6.83]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.78 (P = 0.44)					
2 Dose of 400 g or less					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					

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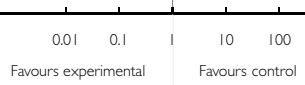
Study or subgroup	Folic acid n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
3 Dose of more than 400 g					
Laurence 1981	0/60	1/51		100.0 %	0.28 [0.01, 6.83]
Subtotal (95% CI)	**60**	**51**		**100.0 %**	**0.28 [0.01, 6.83]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.78 (P = 0.44)					
4 Started pre-pregnancy and continued during first trimester					
Laurence 1981	0/60	1/51		100.0 %	0.28 [0.01, 6.83]
Subtotal (95% CI)	**60**	**51**		**100.0 %**	**0.28 [0.01, 6.83]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.78 (P = 0.44)					
5 History of NTDs					
Laurence 1981	0/60	1/51		100.0 %	0.28 [0.01, 6.83]
Subtotal (95% CI)	**60**	**51**		**100.0 %**	**0.28 [0.01, 6.83]**
Total events: 0 (Folic acid), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.78 (P = 0.44)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					



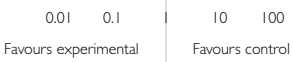
Analysis 3.1. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 1 Neural tube defects (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 1 Neural tube defects (ALL)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/87	3/103		100.0 %	0.17 [0.01, 3.22]
Total (95% CI)	**87**	**103**		**100.0 %**	**0.17 [0.01, 3.22]**
Total events: 0 (Folic acid+ MN), 3 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.18 (P = 0.24)					



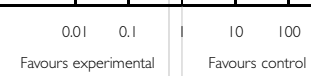
Analysis 3.2. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 2 Neural tube defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 2 Neural tube defects (by subgroups)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Kirke 1992	0/87	3/103		100.0 %	0.17 [0.01, 3.22]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.17 [0.01, 3.22]**
Total events: 0 (Folic acid+ MN), 3 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.18 (P = 0.24)					
2 Dose of 400 g or less					
Kirke 1992	0/87	3/103		100.0 %	0.17 [0.01, 3.22]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.17 [0.01, 3.22]**
Total events: 0 (Folic acid+ MN), 3 (No treatment/placebo)					
Heterogeneity: not applicable					



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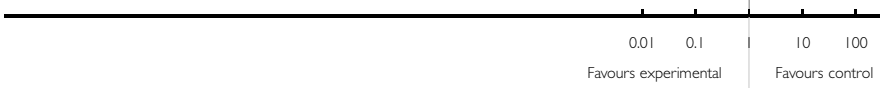
Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 1.18 (P = 0.24)					
3 Dose of more than 400 g					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
4 Started pre-pregnancy and continued during first trimester					
Kirke 1992	0/87	3/103		100.0 %	0.17 [0.01, 3.22]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.17 [0.01, 3.22]**
Total events: 0 (Folic acid+ MN), 3 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.18 (P = 0.24)					
5 History of NTDs					
Kirke 1992	0/87	3/103		100.0 %	0.17 [0.01, 3.22]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.17 [0.01, 3.22]**
Total events: 0 (Folic acid+ MN), 3 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.18 (P = 0.24)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					



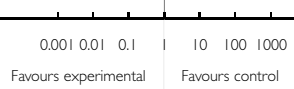
Analysis 3.3. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 3 Cleft palate (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 3 Cleft palate (ALL)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Total (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				



Analysis 3.4. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 4 Cleft palate (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 4 Cleft palate (by subgroups)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme				
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
2 Dose of 400 g or less				
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				

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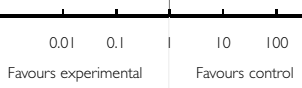
Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 0.0 (P < 0.00001)				
3 Dose of more than 400 g				
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: not applicable				
4 Started pre-pregnancy and continued during the first trimester				
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
5 History of NTDs				
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
6 No history of NTDs or unknown				
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: not applicable				



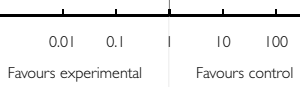
Analysis 3.5. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 5 Cleft lip (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 5 Cleft lip (ALL)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Total (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				



Analysis 3.6. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 6 Cleft lip (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 6 Cleft lip (by subgroups)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme				
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
2 Dose of 400 g or less				
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				

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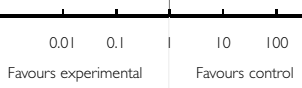
Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 0.0 (P < 0.00001)				
3 Dose of more than 400 g				
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: not applicable				
4 Started pre-pregnancy and continued during the first trimester				
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
5 History of NTDs				
Kirke 1992	0/87	0/103		0.0 [0.0, 0.0]
Subtotal (95% CI)	**87**	**103**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
6 No history of NTDs or unknown				
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)				
Heterogeneity: not applicable				
Test for overall effect: not applicable				



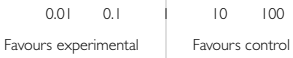
Analysis 3.7. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 7 Congenital cardiovascular defects (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 7 Congenital cardiovascular defects (ALL)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/87	1/103		100.0 %	0.39 [0.02, 9.55]
Total (95% CI)	**87**	**103**		**100.0 %**	**0.39 [0.02, 9.55]**
Total events: 0 (Folic acid+ MN), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.57 (P = 0.57)					



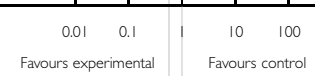
Analysis 3.8. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 8 Congenital cardiovascular defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 8 Congenital cardiovascular defects (by subgroups)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Kirke 1992	0/87	1/103		100.0 %	0.39 [0.02, 9.55]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.39 [0.02, 9.55]**
Total events: 0 (Folic acid+ MN), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.57 (P = 0.57)					
2 Dose of 400 g or less					
Kirke 1992	0/87	1/103		100.0 %	0.39 [0.02, 9.55]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.39 [0.02, 9.55]**
Total events: 0 (Folic acid+ MN), 1 (No treatment/placebo)					
Heterogeneity: not applicable					



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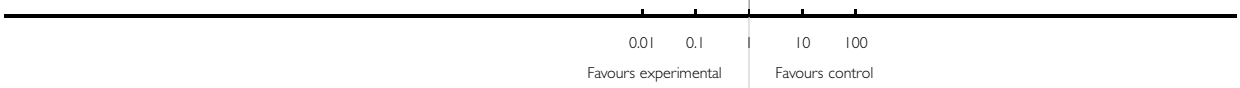
Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 0.57 (P = 0.57)					
3 Dose of more than 400 g					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
4 Started pre-pregnancy and continued during the first trimester					
Kirke 1992	0/87	1/103		100.0 %	0.39 [0.02, 9.55]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.39 [0.02, 9.55]**
Total events: 0 (Folic acid+ MN), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.57 (P = 0.57)					
5 History of NTDs					
Kirke 1992	0/87	1/103		100.0 %	0.39 [0.02, 9.55]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.39 [0.02, 9.55]**
Total events: 0 (Folic acid+ MN), 1 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.57 (P = 0.57)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					



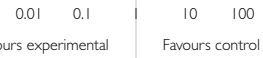
Analysis 3.9. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 9 Other birth defects (any) (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 9 Other birth defects (any) (ALL)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	2/87	2/103		100.0 %	1.18 [0.17, 8.23]
Total (95% CI)	**87**	**103**		**100.0 %**	**1.18 [0.17, 8.23]**
Total events: 2 (Folic acid+ MN), 2 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.17 (P = 0.86)					



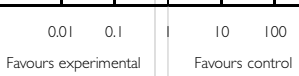
Analysis 3.10. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 10 Other birth defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 10 Other birth defects (by subgroups)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Kirke 1992	2/87	2/103		100.0 %	1.18 [0.17, 8.23]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**1.18 [0.17, 8.23]**
Total events: 2 (Folic acid+ MN), 2 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.17 (P = 0.86)					
2 Dose of 400 g or less					
Kirke 1992	2/87	2/103		100.0 %	1.18 [0.17, 8.23]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**1.18 [0.17, 8.23]**
Total events: 2 (Folic acid+ MN), 2 (No treatment/placebo)					
Heterogeneity: not applicable					



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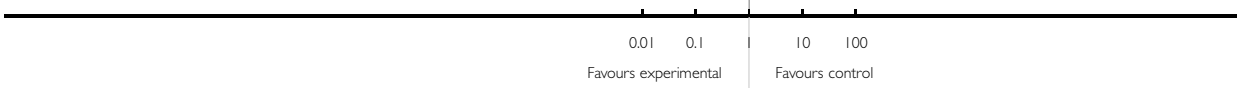
Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 0.17 (P = 0.86)					
3 Dose of more than 400 g					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
4 Started pre-pregnancy and continued during the first trimester					
Kirke 1992	2/87	2/103		100.0 %	1.18 [0.17, 8.23]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**1.18 [0.17, 8.23]**
Total events: 2 (Folic acid+ MN), 2 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.17 (P = 0.86)					
5 History of NTDs					
Kirke 1992	2/87	2/103		100.0 %	1.18 [0.17, 8.23]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**1.18 [0.17, 8.23]**
Total events: 2 (Folic acid+ MN), 2 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.17 (P = 0.86)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					



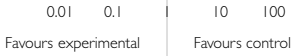
Analysis 3.11. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 11 Miscarriage (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 11 Miscarriage (ALL)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	2/87	0/103		100.0 %	5.91 [0.29, 121.46]
Total (95% CI)	**87**	**103**		**100.0 %**	**5.91 [0.29, 121.46]**
Total events: 2 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.15 (P = 0.25)					



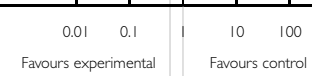
Analysis 3.12. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 12 Miscarriage (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 12 Miscarriage (by subgroups)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Kirke 1992	2/87	0/103		100.0 %	5.91 [0.29, 121.46]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**5.91 [0.29, 121.46]**
Total events: 2 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.15 (P = 0.25)					
2 Dose of 400 g or less					
Kirke 1992	2/87	0/103		100.0 %	5.91 [0.29, 121.46]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**5.91 [0.29, 121.46]**
Total events: 2 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					



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Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 1.15 (P = 0.25)					
3 Dose of more than 400 g					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
4 Started pre-pregnancy and continued during the first trimester					
Kirke 1992	2/87	0/103		100.0 %	5.91 [0.29, 121.46]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**5.91 [0.29, 121.46]**
Total events: 2 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.15 (P = 0.25)					
5 History of NTDs					
Kirke 1992	2/87	0/103		100.0 %	5.91 [0.29, 121.46]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**5.91 [0.29, 121.46]**
Total events: 2 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.15 (P = 0.25)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					

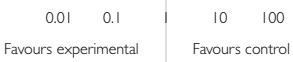
Analysis 3.13. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 13 Stillbirth (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 13 Stillbirth (ALL)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Kirke 1992	0/87	4/103		100.0 %	0.13 [0.01, 2.41]
Total (95% CI)	**87**	**103**		**100.0 %**	**0.13 [0.01, 2.41]**
Total events: 0 (Folic acid+ MN), 4 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.37 (P = 0.17)					



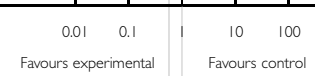
Analysis 3.14. Comparison 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo, Outcome 14 Stillbirth (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 3 Supplementation with folic acid + other micronutrients versus no treatment/placebo

Outcome: 14 Stillbirth (by subgroups)

Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation					
Kirke 1992	0/87	4/103		100.0 %	0.13 [0.01, 2.41]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.13 [0.01, 2.41]**
Total events: 0 (Folic acid+ MN), 4 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.37 (P = 0.17)					
2 Dose of 400 g or less					
Kirke 1992	0/87	4/103		100.0 %	0.13 [0.01, 2.41]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.13 [0.01, 2.41]**
Total events: 0 (Folic acid+ MN), 4 (No treatment/placebo)					
Heterogeneity: not applicable					



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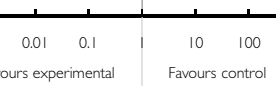
Study or subgroup	Folic acid+ MN n/N	No treatment/placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 1.37 (P = 0.17)					
3 Dose of more than 400 g					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
4 Started pre-pregnancy and continued					
Kirke 1992	0/87	4/103		100.0 %	0.13 [0.01, 2.41]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.13 [0.01, 2.41]**
Total events: 0 (Folic acid+ MN), 4 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.37 (P = 0.17)					
5 History of NTDs					
Kirke 1992	0/87	4/103		100.0 %	0.13 [0.01, 2.41]
Subtotal (95% CI)	**87**	**103**		**100.0 %**	**0.13 [0.01, 2.41]**
Total events: 0 (Folic acid+ MN), 4 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.37 (P = 0.17)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (No treatment/placebo)					
Heterogeneity: not applicable					
Test for overall effect: not applicable					



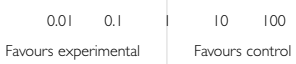
Analysis 4.1. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 1 Neural tube defects (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 1 Neural tube defects (ALL)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	0/2104	6/2052		17.0 %	0.08 [0.00, 1.33]
ICMR 2000	4/137	10/142		25.4 %	0.41 [0.13, 1.29]
Kirke 1992	0/87	1/89		3.8 %	0.34 [0.01, 8.26]
MRC 1991	6/593	21/602		53.8 %	0.29 [0.12, 0.71]
Total (95% CI)	**2921**	**2885**		**100.0 %**	**0.29 [0.15, 0.56]**
Total events: 10 (Folic acid+ MN), 38 (MN (without FA))					
Heterogeneity: Chi^2 = 1.25, df = 3 (P = 0.74); I^2 =0.0%					
Test for overall effect: Z = 3.70 (P = 0.00022)					



Analysis 4.2. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 2 Neural tube defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 2 Neural tube defects (by subgroups)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	0/2104	6/2052		17.0 %	0.08 [0.00, 1.33]
ICMR 2000	4/137	10/142		25.4 %	0.41 [0.13, 1.29]
Kirke 1992	0/87	1/89		3.8 %	0.34 [0.01, 8.26]
MRC 1991	6/593	21/602		53.8 %	0.29 [0.12, 0.71]
Subtotal (95% CI)	**2921**	**2885**		**100.0 %**	**0.29 [0.15, 0.56]**
Total events: 10 (Folic acid+ MN), 38 (MN (without FA))					
Heterogeneity: Chi^2 = 1.25, df = 3 (P = 0.74); I^2 =0.0%					
Test for overall effect: Z = 3.70 (P = 0.00022)					
2 Dose of 400 g or less					
Kirke 1992	0/87	1/89		100.0 %	0.34 [0.01, 8.26]
Subtotal (95% CI)	**87**	**89**		**100.0 %**	**0.34 [0.01, 8.26]**
Total events: 0 (Folic acid+ MN), 1 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.66 (P = 0.51)					
3 Dose of more than 400 g					
Czeizel 1994	0/2104	6/2052		17.7 %	0.08 [0.00, 1.33]
ICMR 2000	4/137	10/142		26.4 %	0.41 [0.13, 1.29]
MRC 1991	6/593	21/602		56.0 %	0.29 [0.12, 0.71]
Subtotal (95% CI)	**2834**	**2796**		**100.0 %**	**0.28 [0.14, 0.56]**
Total events: 10 (Folic acid+ MN), 37 (MN (without FA))					
Heterogeneity: Chi^2 = 1.25, df = 2 (P = 0.54); I^2 =0.0%					
Test for overall effect: Z = 3.64 (P = 0.00027)					
4 Started pre-pregnancy and continued during the first trimester					
Czeizel 1994	0/2104	6/2052		17.0 %	0.08 [0.00, 1.33]
ICMR 2000	4/137	10/142		25.4 %	0.41 [0.13, 1.29]
Kirke 1992	0/87	1/89		3.8 %	0.34 [0.01, 8.26]
MRC 1991	6/593	21/602		53.8 %	0.29 [0.12, 0.71]
Subtotal (95% CI)	**2921**	**2885**		**100.0 %**	**0.29 [0.15, 0.56]**
Total events: 10 (Folic acid+ MN), 38 (MN (without FA))					

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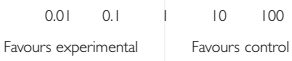
Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Heterogeneity: Chi² = 1.25, df = 3 (P = 0.74); I² =0.0%					
Test for overall effect: Z = 3.70 (P = 0.00022)					
5 History of NTDs					
ICMR 2000	4/137	10/142		30.6 %	0.41 [0.13, 1.29]
Kirke 1992	0/87	1/89		4.6 %	0.34 [0.01, 8.26]
MRC 1991	6/593	21/602		64.8 %	0.29 [0.12, 0.71]
Subtotal (95% CI)	**817**	**833**		**100.0 %**	**0.33 [0.17, 0.66]**
Total events: 10 (Folic acid+ MN), 32 (MN (without FA))					
Heterogeneity: Chi² = 0.23, df = 2 (P = 0.89); I² =0.0%					
Test for overall effect: Z = 3.16 (P = 0.0016)					
6 No history of NTDs or unknown					
Czeizel 1994	0/2104	6/2052		100.0 %	0.08 [0.00, 1.33]
Subtotal (95% CI)	**2104**	**2052**		**100.0 %**	**0.08 [0.00, 1.33]**
Total events: 0 (Folic acid+ MN), 6 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.77 (P = 0.078)					



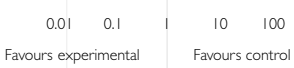
Analysis 4.3. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 3 Cleft palate (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 3 Cleft palate (ALL)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	0/2104	2/2052		0.20 [0.01, 4.06]
Kirke 1992	0/87	0/89		0.0 [0.0, 0.0]
MRC 1991	1/593	0/602		3.05 [0.12, 74.61]
Total (95% CI)	**2784**	**2743**		**0.66 [0.11, 3.92]**
Total events: 1 (Folic acid+ MN), 2 (MN (without FA))				
Heterogeneity: Chi² = 1.50, df = 1 (P = 0.22); I² =33%				
Test for overall effect: Z = 0.45 (P = 0.65)				

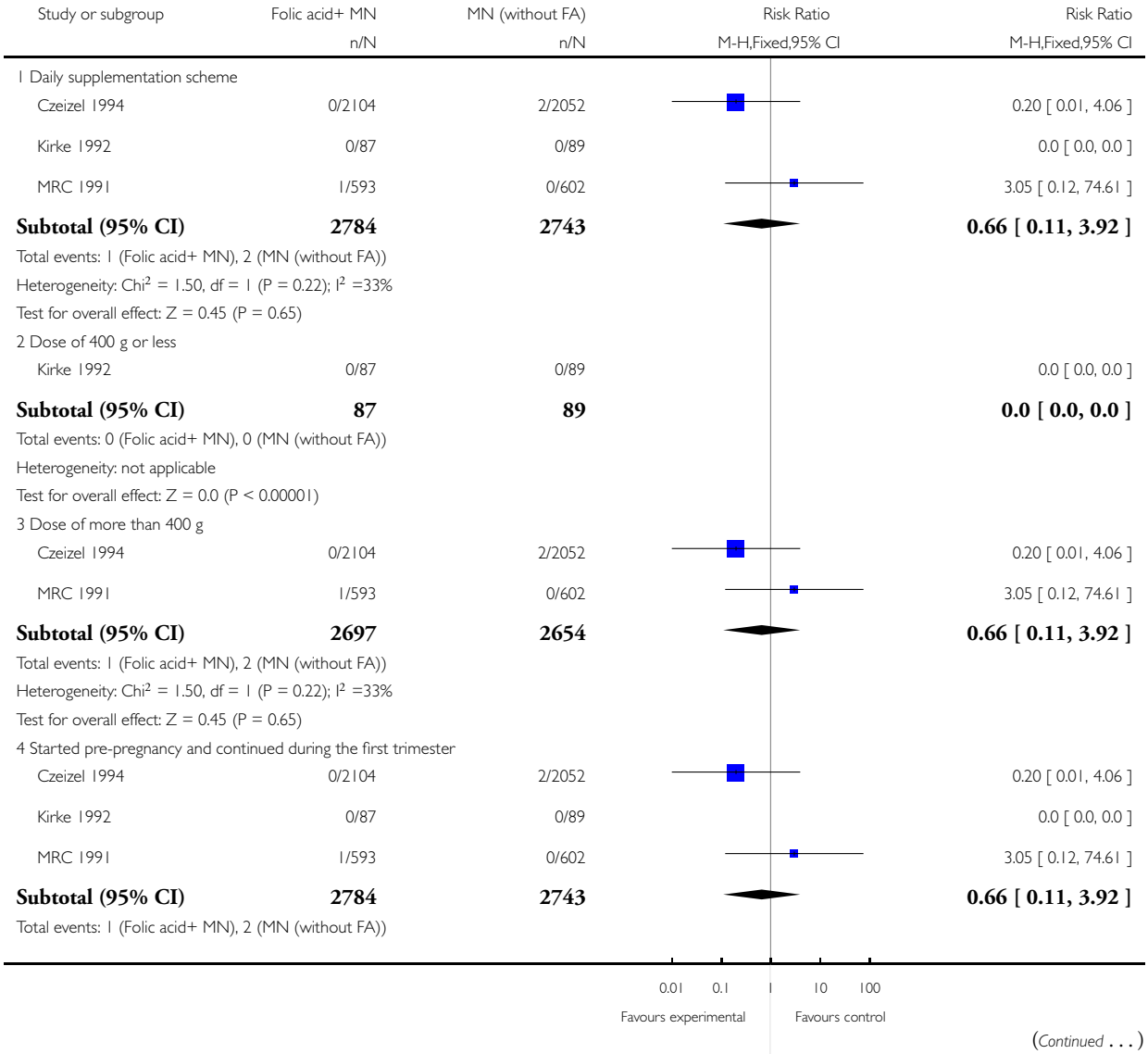


Analysis 4.4. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 4 Cleft palate (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 4 Cleft palate (by subgroups)



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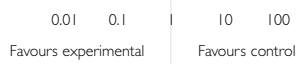
Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Heterogeneity: Chi² = 1.50, df = 1 (P = 0.22); I² =33%				
Test for overall effect: Z = 0.45 (P = 0.65)				
5 History of NTDs				
Kirke 1992	0/87	0/89		0.0 [0.0, 0.0]
MRC 1991	1/593	0/602		3.05 [0.12, 74.61]
Subtotal (95% CI)	**680**	**691**		**3.05 [0.12, 74.61]**
Total events: 1 (Folic acid+ MN), 0 (MN (without FA))				
Heterogeneity: Chi² = 0.0, df = 0 (P = 1.00); I² =0.0%				
Test for overall effect: Z = 0.68 (P = 0.49)				
6 No history of NTDs or unknown				
Czeizel 1994	0/2104	2/2052		0.20 [0.01, 4.06]
Subtotal (95% CI)	**2104**	**2052**		**0.20 [0.01, 4.06]**
Total events: 0 (Folic acid+ MN), 2 (MN (without FA))				
Heterogeneity: not applicable				
Test for overall effect: Z = 1.06 (P = 0.29)				



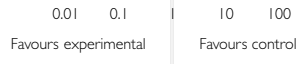
Analysis 4.5. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 5 Cleft lip (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 5 Cleft lip (ALL)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
Kirke 1992	0/87	1/89		0.34 [0.01, 8.26]
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Total (95% CI)	**2784**	**2743**		**0.99 [0.27, 3.65]**
Total events: 4 (Folic acid+ MN), 4 (MN (without FA))				
Heterogeneity: Chi² = 0.56, df = 1 (P = 0.46); I² =0.0%				
Test for overall effect: Z = 0.02 (P = 0.98)				



Analysis 4.6. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 6 Cleft lip (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 6 Cleft lip (by subgroups)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme				
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
Kirke 1992	0/87	1/89		0.34 [0.01, 8.26]
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Subtotal (95% CI)	**2784**	**2743**		**0.99 [0.27, 3.65]**
Total events: 4 (Folic acid+ MN), 4 (MN (without FA))				
Heterogeneity: Chi^2 = 0.56, df = 1 (P = 0.46); I^2 =0.0%				
Test for overall effect: Z = 0.02 (P = 0.98)				
2 Dose of 400 g or less				
Kirke 1992	0/87	1/89		0.34 [0.01, 8.26]
Subtotal (95% CI)	**87**	**89**		**0.34 [0.01, 8.26]**
Total events: 0 (Folic acid+ MN), 1 (MN (without FA))				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.66 (P = 0.51)				
3 Dose of more than 400 g				
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Subtotal (95% CI)	**2697**	**2654**		**1.30 [0.29, 5.80]**
Total events: 4 (Folic acid+ MN), 3 (MN (without FA))				
Heterogeneity: Chi^2 = 0.0, df = 0 (P = 1.00); I^2 =0.0%				
Test for overall effect: Z = 0.34 (P = 0.73)				
4 Started pre-pregnancy and continued during the first trimester				
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
Kirke 1992	0/87	1/89		0.34 [0.01, 8.26]
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Subtotal (95% CI)	**2784**	**2743**		**0.99 [0.27, 3.65]**
Total events: 4 (Folic acid+ MN), 4 (MN (without FA))				
Heterogeneity: Chi^2 = 0.56, df = 1 (P = 0.46); I^2 =0.0%				
Test for overall effect: Z = 0.02 (P = 0.98)				

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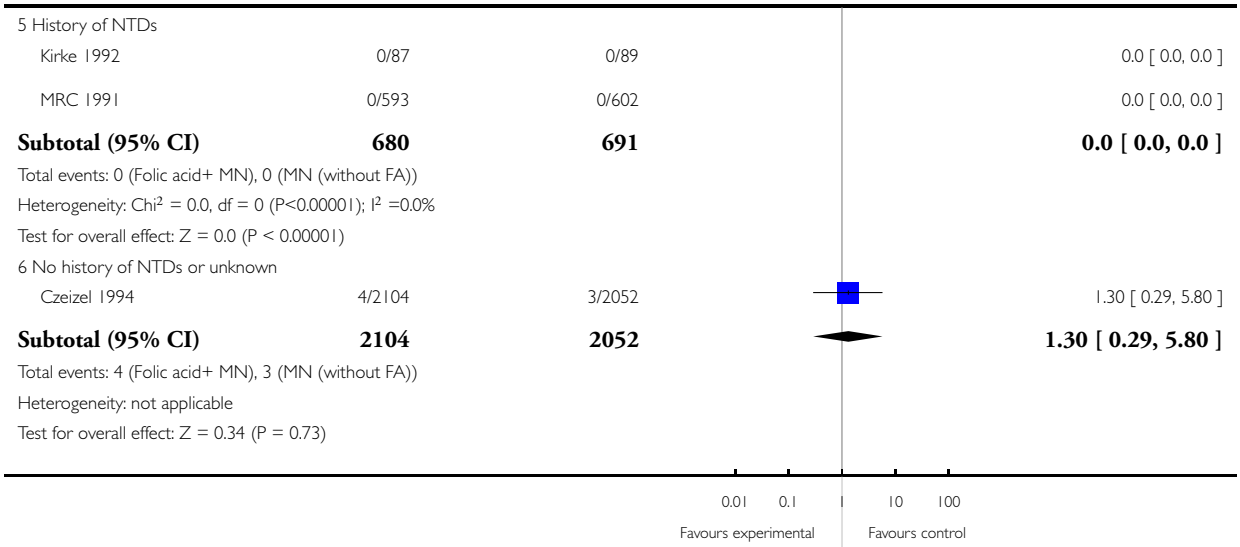
Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
5 History of NTDs				
Kirke 1992	0/87	0/89		0.0 [0.0, 0.0]
MRC 1991	0/593	0/602		0.0 [0.0, 0.0]
Subtotal (95% CI)	**680**	**691**		**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (MN (without FA))				
Heterogeneity: Chi^2 = 0.0, df = 0 (P<0.00001); I^2 =0.0%				
Test for overall effect: Z = 0.0 (P < 0.00001)				
6 No history of NTDs or unknown				
Czeizel 1994	4/2104	3/2052		1.30 [0.29, 5.80]
Subtotal (95% CI)	**2104**	**2052**		**1.30 [0.29, 5.80]**
Total events: 4 (Folic acid+ MN), 3 (MN (without FA))				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.34 (P = 0.73)				



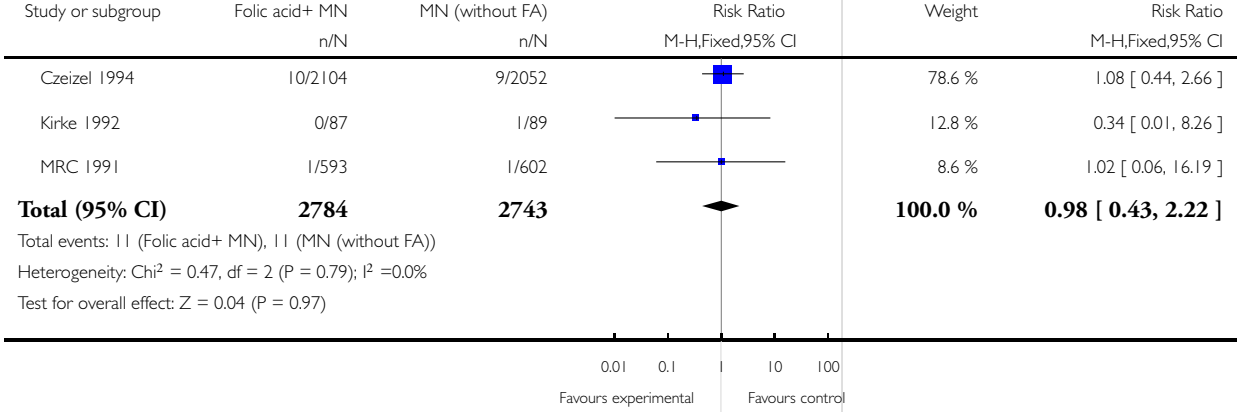
Analysis 4.7. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 7 Congenital cardiovascular defects (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 7 Congenital cardiovascular defects (ALL)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	10/2104	9/2052		78.6 %	1.08 [0.44, 2.66]
Kirke 1992	0/87	1/89		12.8 %	0.34 [0.01, 8.26]
MRC 1991	1/593	1/602		8.6 %	1.02 [0.06, 16.19]
Total (95% CI)	**2784**	**2743**		**100.0 %**	**0.98 [0.43, 2.22]**
Total events: 11 (Folic acid+ MN), 11 (MN (without FA))					
Heterogeneity: Chi^2 = 0.47, df = 2 (P = 0.79); I^2 =0.0%					
Test for overall effect: Z = 0.04 (P = 0.97)					



Analysis 4.8. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 8 Congenital cardiovascular defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 8 Congenital cardiovascular defects (by subgroups)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	10/2104	9/2052		78.6 %	1.08 [0.44, 2.66]
Kirke 1992	0/87	1/89		12.8 %	0.34 [0.01, 8.26]
MRC 1991	1/593	1/602		8.6 %	1.02 [0.06, 16.19]
Subtotal (95% CI)	**2784**	**2743**		**100.0 %**	**0.98 [0.43, 2.22]**
Total events: 11 (Folic acid+ MN), 11 (MN (without FA))					
Heterogeneity: Chi² = 0.47, df = 2 (P = 0.79); I² =0.0%					
Test for overall effect: Z = 0.04 (P = 0.97)					
2 Dose of 400 g or less					
Kirke 1992	0/87	1/89		100.0 %	0.34 [0.01, 8.26]
Subtotal (95% CI)	**87**	**89**		**100.0 %**	**0.34 [0.01, 8.26]**
Total events: 0 (Folic acid+ MN), 1 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.66 (P = 0.51)					
3 Dose of more than 400 g					
Czeizel 1994	10/2104	9/2052		90.2 %	1.08 [0.44, 2.66]
MRC 1991	1/593	1/602		9.8 %	1.02 [0.06, 16.19]
Subtotal (95% CI)	**2697**	**2654**		**100.0 %**	**1.08 [0.46, 2.53]**
Total events: 11 (Folic acid+ MN), 10 (MN (without FA))					
Heterogeneity: Chi² = 0.00, df = 1 (P = 0.96); I² =0.0%					
Test for overall effect: Z = 0.17 (P = 0.87)					
4 Started pre-pregnancy and continued during the first trimester					
Czeizel 1994	10/2104	9/2052		78.6 %	1.08 [0.44, 2.66]
Kirke 1992	0/87	1/89		12.8 %	0.34 [0.01, 8.26]
MRC 1991	1/593	1/602		8.6 %	1.02 [0.06, 16.19]
Subtotal (95% CI)	**2784**	**2743**		**100.0 %**	**0.98 [0.43, 2.22]**
Total events: 11 (Folic acid+ MN), 11 (MN (without FA))					
Heterogeneity: Chi² = 0.47, df = 2 (P = 0.79); I² =0.0%					
Test for overall effect: Z = 0.04 (P = 0.97)					
5 History of NTDs					
Kirke 1992	0/87	1/89		59.9 %	0.34 [0.01, 8.26]

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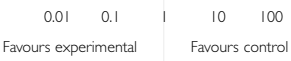
Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
MRC 1991	1/593	1/602		40.1 %	1.02 [0.06, 16.19]
Subtotal (95% CI)	**680**	**691**		**100.0 %**	**0.61 [0.08, 4.61]**
Total events: 1 (Folic acid+ MN), 2 (MN (without FA))					
Heterogeneity: Chi^2 = 0.26, df = 1 (P = 0.61); I^2 =0.0%					
Test for overall effect: Z = 0.48 (P = 0.63)					
6 No history of NTDs or unknown					
Czeizel 1994	10/2104	9/2052		100.0 %	1.08 [0.44, 2.66]
Subtotal (95% CI)	**2104**	**2052**		**100.0 %**	**1.08 [0.44, 2.66]**
Total events: 10 (Folic acid+ MN), 9 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.18 (P = 0.86)					



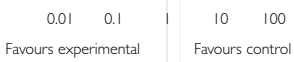
Analysis 4.9. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 9 Other birth defects (any) (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 9 Other birth defects (any) (ALL)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	21/2104	39/2052		74.0 %	0.53 [0.31, 0.89]
Kirke 1992	2/87	2/89		3.7 %	1.02 [0.15, 7.10]
MRC 1991	17/593	12/602		22.3 %	1.44 [0.69, 2.98]
Total (95% CI)	**2784**	**2743**		**100.0 %**	**0.75 [0.50, 1.12]**
Total events: 40 (Folic acid+ MN), 53 (MN (without FA))					
Heterogeneity: Chi^2 = 4.91, df = 2 (P = 0.09); I^2 =59%					
Test for overall effect: Z = 1.41 (P = 0.16)					



Analysis 4.10. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 10 Other birth defects (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 10 Other birth defects (by subgroups)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	21/2104	39/2052		74.0 %	0.53 [0.31, 0.89]
Kirke 1992	2/87	2/89		3.7 %	1.02 [0.15, 7.10]
MRC 1991	17/593	12/602		22.3 %	1.44 [0.69, 2.98]
Subtotal (95% CI)	**2784**	**2743**		**100.0 %**	**0.75 [0.50, 1.12]**
Total events: 40 (Folic acid+ MN), 53 (MN (without FA))					
Heterogeneity: Chi^2 = 4.91, df = 2 (P = 0.09); I^2 =59%					
Test for overall effect: Z = 1.41 (P = 0.16)					
2 Dose of 400 g or less					
Kirke 1992	2/87	2/89		100.0 %	1.02 [0.15, 7.10]
Subtotal (95% CI)	**87**	**89**		**100.0 %**	**1.02 [0.15, 7.10]**
Total events: 2 (Folic acid+ MN), 2 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.02 (P = 0.98)					
3 Dose of more than 400 g					
Czeizel 1994	21/2104	39/2052		76.8 %	0.53 [0.31, 0.89]
MRC 1991	17/593	12/602		23.2 %	1.44 [0.69, 2.98]
Subtotal (95% CI)	**2697**	**2654**		**100.0 %**	**0.74 [0.49, 1.12]**
Total events: 38 (Folic acid+ MN), 51 (MN (without FA))					
Heterogeneity: Chi^2 = 4.81, df = 1 (P = 0.03); I^2 =79%					
Test for overall effect: Z = 1.44 (P = 0.15)					
4 Started pre-pregnancy and continued during the first trimester					
Czeizel 1994	21/2104	39/2052		74.0 %	0.53 [0.31, 0.89]
Kirke 1992	2/87	2/89		3.7 %	1.02 [0.15, 7.10]
MRC 1991	17/593	12/602		22.3 %	1.44 [0.69, 2.98]
Subtotal (95% CI)	**2784**	**2743**		**100.0 %**	**0.75 [0.50, 1.12]**
Total events: 40 (Folic acid+ MN), 53 (MN (without FA))					
Heterogeneity: Chi^2 = 4.91, df = 2 (P = 0.09); I^2 =59%					
Test for overall effect: Z = 1.41 (P = 0.16)					
5 History of NTDs					
Kirke 1992	2/87	2/89		14.2 %	1.02 [0.15, 7.10]

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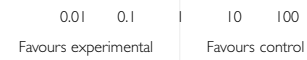
Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
MRC 1991	17/593	12/602		85.8 %	1.44 [0.69, 2.98]
Subtotal (95% CI)	**680**	**691**		**100.0 %**	**1.38 [0.70, 2.73]**
Total events: 19 (Folic acid+ MN), 14 (MN (without FA))					
Heterogeneity: Chi^2 = 0.10, df = 1 (P = 0.75); I^2 =0.0%					
Test for overall effect: Z = 0.92 (P = 0.36)					
6 No history of NTDs or unknown					
Czeizel 1994	21/2104	39/2052		100.0 %	0.53 [0.31, 0.89]
Subtotal (95% CI)	**2104**	**2052**		**100.0 %**	**0.53 [0.31, 0.89]**
Total events: 21 (Folic acid+ MN), 39 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 2.40 (P = 0.017)					



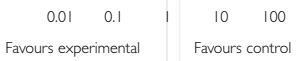
Analysis 4.11. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 11 Miscarriage (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 11 Miscarriage (ALL)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	301/2819	251/2683		72.7 %	1.14 [0.97, 1.34]
ICMR 2000	6/137	14/142		3.9 %	0.44 [0.18, 1.12]
Kirke 1992	2/87	0/89		0.1 %	5.11 [0.25, 105.01]
MRC 1991	87/677	83/685		23.3 %	1.06 [0.80, 1.41]
Total (95% CI)	**3720**	**3599**		**100.0 %**	**1.10 [0.96, 1.26]**
Total events: 396 (Folic acid+ MN), 348 (MN (without FA))					
Heterogeneity: Chi^2 = 4.94, df = 3 (P = 0.18); I^2 =39%					
Test for overall effect: Z = 1.38 (P = 0.17)					



Analysis 4.12. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 12 Miscarriage (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 12 Miscarriage (by subgroups)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	301/2819	251/2683		72.7 %	1.14 [0.97, 1.34]
ICMR 2000	6/137	14/142		3.9 %	0.44 [0.18, 1.12]
Kirke 1992	2/87	0/89		0.1 %	5.11 [0.25, 105.01]
MRC 1991	87/677	83/685		23.3 %	1.06 [0.80, 1.41]
Subtotal (95% CI)	**3720**	**3599**		**100.0 %**	**1.10 [0.96, 1.26]**
Total events: 396 (Folic acid+ MN), 348 (MN (without FA))					
Heterogeneity: Chi^2 = 4.94, df = 3 (P = 0.18); I^2 =39%					
Test for overall effect: Z = 1.38 (P = 0.17)					
2 Dose of 400 g or less					
Kirke 1992	2/87	0/89		100.0 %	5.11 [0.25, 105.01]
Subtotal (95% CI)	**87**	**89**		**100.0 %**	**5.11 [0.25, 105.01]**
Total events: 2 (Folic acid+ MN), 0 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.06 (P = 0.29)					
3 Dose of more than 400 g					
Czeizel 1994	301/2819	251/2683		72.8 %	1.14 [0.97, 1.34]
ICMR 2000	6/137	14/142		3.9 %	0.44 [0.18, 1.12]
MRC 1991	87/677	83/685		23.3 %	1.06 [0.80, 1.41]
Subtotal (95% CI)	**3633**	**3510**		**100.0 %**	**1.10 [0.96, 1.26]**
Total events: 394 (Folic acid+ MN), 348 (MN (without FA))					
Heterogeneity: Chi^2 = 3.95, df = 2 (P = 0.14); I^2 =49%					
Test for overall effect: Z = 1.31 (P = 0.19)					
4 Started pre-pregnancy and continued during the first trimester					
Czeizel 1994	301/2819	251/2683		72.7 %	1.14 [0.97, 1.34]
ICMR 2000	6/137	14/142		3.9 %	0.44 [0.18, 1.12]
Kirke 1992	2/87	0/89		0.1 %	5.11 [0.25, 105.01]
MRC 1991	87/677	83/685		23.3 %	1.06 [0.80, 1.41]
Subtotal (95% CI)	**3720**	**3599**		**100.0 %**	**1.10 [0.96, 1.26]**
Total events: 396 (Folic acid+ MN), 348 (MN (without FA))					

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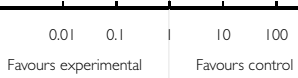
Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Heterogeneity: Chi² = 4.94, df = 3 (P = 0.18); I² =39%					
Test for overall effect: Z = 1.38 (P = 0.17)					
5 History of NTDs					
ICMR 2000	6/137	14/142		14.2 %	0.44 [0.18, 1.12]
Kirke 1992	2/87	0/89		0.5 %	5.11 [0.25, 105.01]
MRC 1991	87/677	83/685		85.3 %	1.06 [0.80, 1.41]
Subtotal (95% CI)	**901**	**916**		**100.0 %**	**0.99 [0.76, 1.30]**
Total events: 95 (Folic acid+ MN), 97 (MN (without FA))					
Heterogeneity: Chi² = 4.23, df = 2 (P = 0.12); I² =53%					
Test for overall effect: Z = 0.05 (P = 0.96)					
6 No history of NTDs or unknown					
Czeizel 1994	301/2819	251/2683		100.0 %	1.14 [0.97, 1.34]
Subtotal (95% CI)	**2819**	**2683**		**100.0 %**	**1.14 [0.97, 1.34]**
Total events: 301 (Folic acid+ MN), 251 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.63 (P = 0.10)					



Analysis 4.13. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 13 Stillbirth (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

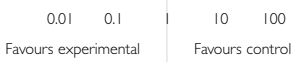
Outcome: 13 Stillbirth (ALL)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	11/2104	7/2052		52.4 %	1.53 [0.60, 3.95]
ICMR 2000	3/137	1/142		7.3 %	3.11 [0.33, 29.53]
Kirke 1992	0/87	2/89		18.3 %	0.20 [0.01, 4.20]
MRC 1991	4/593	3/602		22.0 %	1.35 [0.30, 6.02]
Total (95% CI)	**2921**	**2885**		**100.0 %**	**1.36 [0.68, 2.75]**

Total events: 18 (Folic acid+ MN), 13 (MN (without FA))

Heterogeneity: $Chi^2 = 2.09$, df = 3 (P = 0.55); $I^2 = 0.0\%$

Test for overall effect: Z = 0.87 (P = 0.38)

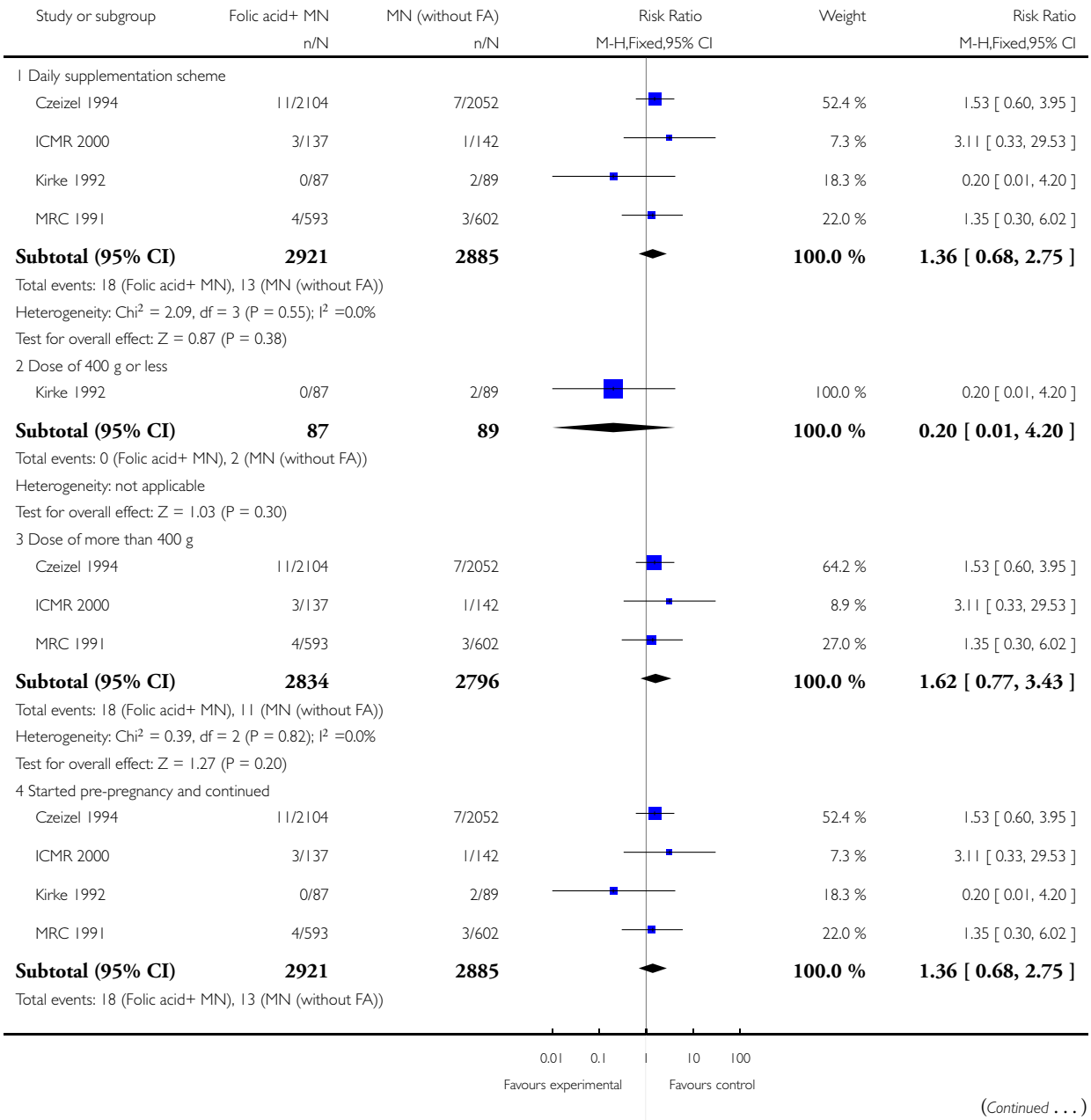


Analysis 4.14. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 14 Stillbirth (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 14 Stillbirth (by subgroups)



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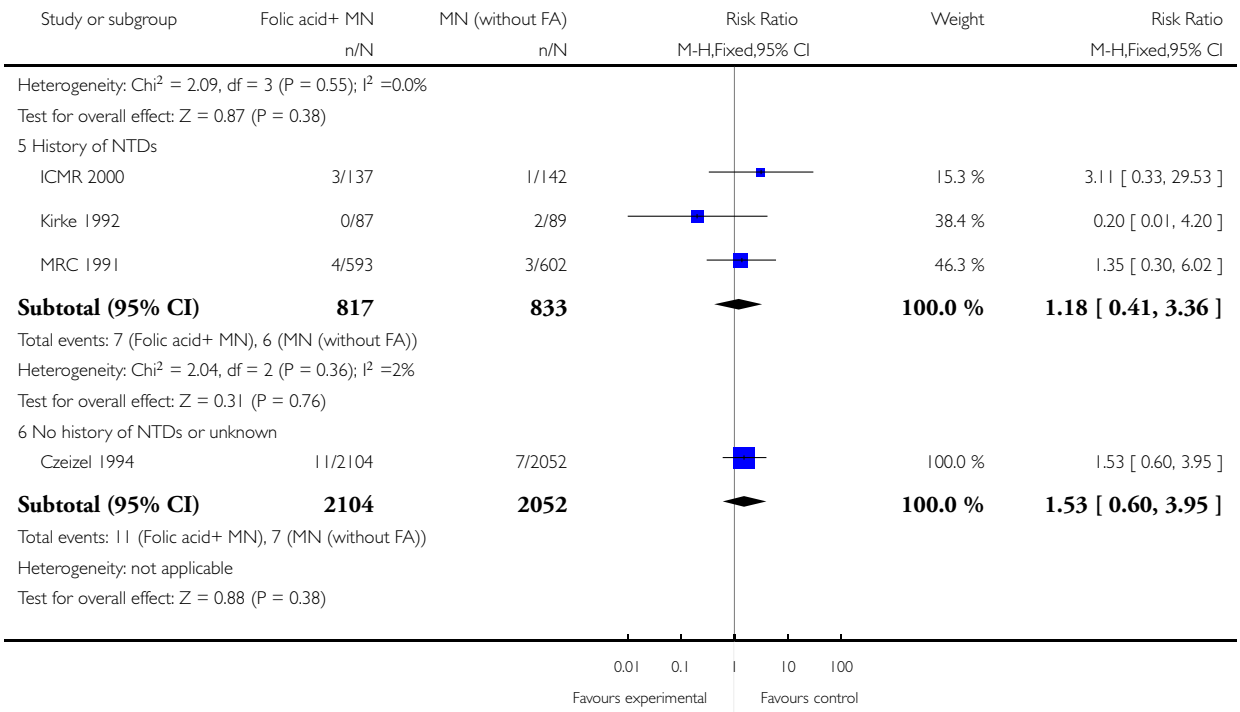
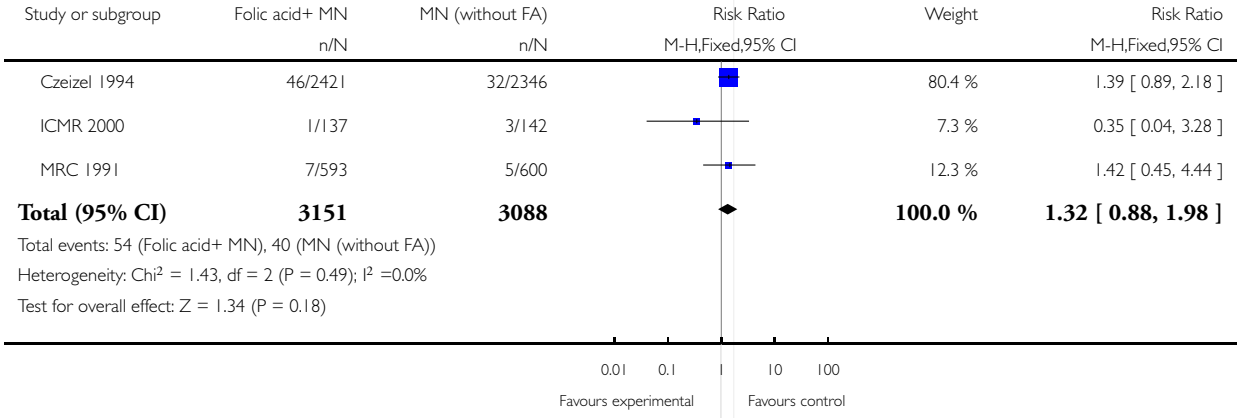


Analysis 4.15. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 15 Multiple pregnancy (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 15 Multiple pregnancy (ALL)



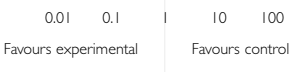
Analysis 4.16. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 16 Multiple pregnancy (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 16 Multiple pregnancy (by subgroups)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	46/2421	32/2346		80.4 %	1.39 [0.89, 2.18]
ICMR 2000	1/137	3/142		7.3 %	0.35 [0.04, 3.28]
MRC 1991	7/593	5/600		12.3 %	1.42 [0.45, 4.44]
Subtotal (95% CI)	**3151**	**3088**		**100.0 %**	**1.32 [0.88, 1.98]**
Total events: 54 (Folic acid+ MN), 40 (MN (without FA))					
Heterogeneity: Chi^2 = 1.43, df = 2 (P = 0.49); I^2 =0.0%					
Test for overall effect: Z = 1.34 (P = 0.18)					
2 Dose of 400 g or less					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
3 Dose of more than 400 g					
Czeizel 1994	46/2421	32/2346		80.4 %	1.39 [0.89, 2.18]
ICMR 2000	1/137	3/142		7.3 %	0.35 [0.04, 3.28]
MRC 1991	7/593	5/600		12.3 %	1.42 [0.45, 4.44]
Subtotal (95% CI)	**3151**	**3088**		**100.0 %**	**1.32 [0.88, 1.98]**
Total events: 54 (Folic acid+ MN), 40 (MN (without FA))					
Heterogeneity: Chi^2 = 1.43, df = 2 (P = 0.49); I^2 =0.0%					
Test for overall effect: Z = 1.34 (P = 0.18)					
4 Started pre-pregnancy and continued during first trimester					
Czeizel 1994	46/2421	32/2346		80.4 %	1.39 [0.89, 2.18]
ICMR 2000	1/137	3/142		7.3 %	0.35 [0.04, 3.28]
MRC 1991	7/593	5/600		12.3 %	1.42 [0.45, 4.44]
Subtotal (95% CI)	**3151**	**3088**		**100.0 %**	**1.32 [0.88, 1.98]**
Total events: 54 (Folic acid+ MN), 40 (MN (without FA))					



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Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Heterogeneity: Chi² = 1.43, df = 2 (P = 0.49); I² =0.0%					
Test for overall effect: Z = 1.34 (P = 0.18)					
5 History of NTDs					
ICMR 2000	1/137	3/142		37.2 %	0.35 [0.04, 3.28]
MRC 1991	7/593	5/600		62.8 %	1.42 [0.45, 4.44]
Subtotal (95% CI)	**730**	**742**		**100.0 %**	**1.02 [0.38, 2.70]**
Total events: 8 (Folic acid+ MN), 8 (MN (without FA))					
Heterogeneity: Chi² = 1.21, df = 1 (P = 0.27); I² =17%					
Test for overall effect: Z = 0.04 (P = 0.97)					
6 No history of NTDs or unknown					
Czeizel 1994	46/2421	32/2346		100.0 %	1.39 [0.89, 2.18]
Subtotal (95% CI)	**2421**	**2346**		**100.0 %**	**1.39 [0.89, 2.18]**
Total events: 46 (Folic acid+ MN), 32 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.45 (P = 0.15)					

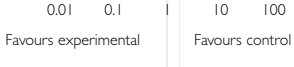
Analysis 4.17. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 17 Pregnancy termination for fetal abnormality (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 17 Pregnancy termination for fetal abnormality (ALL)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Czeizel 1994	3/2104	13/2052		30.0 %	0.23 [0.06, 0.79]
ICMR 2000	3/137	8/142		17.9 %	0.39 [0.11, 1.43]
MRC 1991	7/677	23/685		52.1 %	0.31 [0.13, 0.71]
Total (95% CI)	**2918**	**2879**		**100.0 %**	**0.30 [0.16, 0.55]**
Total events: 13 (Folic acid+ MN), 44 (MN (without FA))					
Heterogeneity: Chi² = 0.36, df = 2 (P = 0.84); I² =0.0%					
Test for overall effect: Z = 3.87 (P = 0.00011)					



Analysis 4.18. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 18 Pregnancy termination for fetal abnormality (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 18 Pregnancy termination for fetal abnormality (by subgroups)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
Czeizel 1994	3/2104	13/2052		7.5 %	0.23 [0.06, 0.79]
ICMR 2000	3/137	8/142		4.5 %	0.39 [0.11, 1.43]
MRC 1991	7/677	23/685		13.0 %	0.31 [0.13, 0.71]
Subtotal (95% CI)	**2918**	**2879**		**25.0 %**	**0.30 [0.16, 0.55]**
Total events: 13 (Folic acid+ MN), 44 (MN (without FA))					
Heterogeneity: Chi² = 0.36, df = 2 (P = 0.84); I² =0.0%					
Test for overall effect: Z = 3.87 (P = 0.00011)					
2 Dose of 400 g or less					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
3 Dose of more than 400 g					
Czeizel 1994	3/2104	13/2052		7.5 %	0.23 [0.06, 0.79]
ICMR 2000	3/137	8/142		4.5 %	0.39 [0.11, 1.43]
MRC 1991	7/677	23/685		13.0 %	0.31 [0.13, 0.71]
Subtotal (95% CI)	**2918**	**2879**		**25.0 %**	**0.30 [0.16, 0.55]**
Total events: 13 (Folic acid+ MN), 44 (MN (without FA))					
Heterogeneity: Chi² = 0.36, df = 2 (P = 0.84); I² =0.0%					
Test for overall effect: Z = 3.87 (P = 0.00011)					
4 Started pre-pregnancy and continued during first trimester					
Czeizel 1994	3/2104	13/2052		7.5 %	0.23 [0.06, 0.79]
ICMR 2000	3/137	8/142		4.5 %	0.39 [0.11, 1.43]
MRC 1991	7/677	23/685		13.0 %	0.31 [0.13, 0.71]
Subtotal (95% CI)	**2918**	**2879**		**25.0 %**	**0.30 [0.16, 0.55]**
Total events: 13 (Folic acid+ MN), 44 (MN (without FA))					
Heterogeneity: Chi² = 0.36, df = 2 (P = 0.84); I² =0.0%					
Test for overall effect: Z = 3.87 (P = 0.00011)					

(*Continued . . .*)

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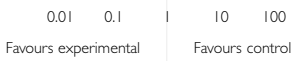
Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
5 History of NTDs					
ICMR 2000	3/137	8/142		4.5 %	0.39 [0.11, 1.43]
MRC 1991	7/677	23/685		13.0 %	0.31 [0.13, 0.71]
Subtotal (95% CI)	**814**	**827**		**17.5 %**	**0.33 [0.16, 0.67]**
Total events: 10 (Folic acid+ MN), 31 (MN (without FA))					
Heterogeneity: Chi^2 = 0.09, df = 1 (P = 0.77); I^2 =0.0%					
Test for overall effect: Z = 3.09 (P = 0.0020)					
6 No history of NTDs or unknown					
Czeizel 1994	3/2104	13/2052		7.5 %	0.23 [0.06, 0.79]
Subtotal (95% CI)	**2104**	**2052**		**7.5 %**	**0.23 [0.06, 0.79]**
Total events: 3 (Folic acid+ MN), 13 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 2.33 (P = 0.020)					
Total (95% CI)	**11672**	**11516**		**100.0 %**	**0.30 [0.22, 0.40]**
Total events: 52 (Folic acid+ MN), 176 (MN (without FA))					
Heterogeneity: Chi^2 = 1.43, df = 11 (P = 1.00); I^2 =0.0%					
Test for overall effect: Z = 7.75 (P < 0.00001)					



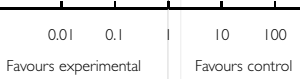
Analysis 4.19. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 19 Low birthweight (ALL).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 19 Low birthweight (ALL)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Total (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid+ MN), 14 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					



Analysis 4.20. Comparison 4 Supplementation with folic acid + other micronutrients versus other micronutrients (without folic acid), Outcome 20 Low birthweight (by subgroups).

Review: Effects and safety of periconceptional folate supplementation for preventing birth defects

Comparison: 4 Supplementation with folic acid+ other micronutrientsversusother micronutrients (without folic acid)

Outcome: 20 Low birthweight (by subgroups)

Study or subgroup	Folic acid+ MN n/N	MN (without FA) n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Daily supplementation scheme					
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Subtotal (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid+ MN), 14 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					
2 Dose of 400 É¥g or less					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: not applicable					
3 Dose of more than 400 g					
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Subtotal (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid+ MN), 14 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					
4 Started pre-pregnancy and continued during first trimester					
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Subtotal (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid+ MN), 14 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					
5 History of NTDs					
ICMR 2000	12/96	14/90		100.0 %	0.80 [0.39, 1.64]
Subtotal (95% CI)	**96**	**90**		**100.0 %**	**0.80 [0.39, 1.64]**
Total events: 12 (Folic acid+ MN), 14 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.60 (P = 0.55)					
6 No history of NTDs or unknown					
Subtotal (95% CI)	**0**	**0**		**0.0 %**	**0.0 [0.0, 0.0]**
Total events: 0 (Folic acid+ MN), 0 (MN (without FA))					
Heterogeneity: not applicable					
Test for overall effect: not applicable					

HISTORY

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CONTRIBUTIONS OF AUTHORS

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Disclaimer: Luz Maria de Regil and Juan Pablo Pena-Rosas are currently staff members of the World Health Organization. The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the World Health Organization.

DECLARATIONS OF INTEREST

There are no known conflicts of interest.

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