

Folic acid and risk of twinning

A report for Food Standards Australia New Zealand

May 10, 2005

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1. Executive summary

- A Cochrane review published in 2001 confirmed that periconceptional folic acid (FA) intake reduced the prevalence of neural tube defects. A secondary meta analysis of data from three randomised controlled trials in the review provided some evidence for a non-significant increase in the likelihood (1.40; 95% CI 0.93-2.11) of a twin pregnancy. This result was heavily weighted by one study where the dose of FA was 0.8mg, included in a multivitamin tablet.
- Also in 2001, the same authors published a paper highlighting the potential negative impact of a proposed 40% increase in multiple births in a hypothetical cohort of 100,000 women all having FA supplementation. An increase of 5.7 twin gestations per 1000 confinements was modelled.
- This report critiques 10 studies that have examined the association of FA and twinning that have been published in the peer-reviewed literature since 2001.
- Important confounders in an analysis of twinning rates are maternal age and use of infertility treatments and considerable weight was placed on these issues when examining the studies.
- The inclusion of fetal deaths in the study population and differentiation between dizygous and monozygous twins were also noted.
- The most convincing of five supplementation studies was a 2005 publication from Norway because it accounted for under-reporting of both infertility treatment use and 0.4mg daily FA supplementation use. After adjusting for these sources of bias, there was no increased risk of twinning associated with FA supplementation.
- Another high quality supplementation study came from a prospective intervention trial in China. However, genetic influences may be causing low baseline twinning rates in China and these in turn may interact with the effect of FA on twinning. This genetic interaction is speculative and the data from this study do not support any increase in twinning associated with 0.4mg daily FA supplementation.
- The other three supplementation studies had serious flaws in methodology and interpretation of data.

- No data from five fortification studies in the US since 2001 support an annual increase in twinning rates of over 5% (approx 7 twin pairs per 10,000 pregnancies in a population with a twinning rate of 15/1000).
- A possible dose effect has not been adequately accounted for to date and further studies would help to resolve this issue, by recording red blood cell (or serum) levels of FA, in conjunction with accurate monitoring of twin livebirths and fetal deaths, and use of infertility treatments.
- This report concludes that, using the WHO 2003 classification for assessing evidence, there is a possible association between FA and rates of twinning. This conclusion is based on findings from several high quality studies that showed a small percent increase in twinning (<5%), and the biological plausibility that FA could support fetal growth and development. However, the public health impact of this small increase is far less than that associated with the 40% increase in twinning proposed in the 2001 meta analysis.

2. Introduction

The primary preventive capability of folic acid (FA) with regards to neural tube defects (NTDs) is well recognised and is documented in a Cochrane review, [1] but there is controversy about the purported relationship between FA and twinning. This concern was highlighted in a submission responding to the FSANZ Initial Assessment Report, “Proposal P295: Consideration of Mandatory Fortification with FA”. Specifically, authors make the comment that “no evidence of effect is not necessarily evidence of no effect”. This comment refers to a hypothesis originating from a meta analysis of three randomised trials, two in a high risk population looking at frequency of recurrent NTDs and one reporting on occurrent NTD events in the general maternity population. [1] This meta-analysis found an association that was “not inconsequential” and “approaching statistical significance” between twinning and FA. In their submission to FSANZ, the authors also refer to three observational studies published since their review, which they believe support their concern. A number of other studies have examined twinning rates associated with either FA supplementation or food fortification.

This report brings together all ten primary studies published in English, since 2001. It begins by briefly reviewing data on twinning prevalence, causes of twinning, public health implications of twinning and a brief comment on the possible mechanism by which FA could enhance survival of twins. The main aim of this report is to critically review literature on the topic of twinning and FA and the remaining sections present these results and accompanying discussion.

2.1 Twinning prevalence and causes of twinning

Rising rates of multiple pregnancies have been observed all over the Western world with the prevalence in the UK increasing from 9.5/1000 pregnancies (maternities) in 1976 to 14.2/1000 in 2000. In France the rise has been from 8.9/1000 in 1972 to 14.4/1000 in 1998, [2] and, in Australia, from 9.0/1000 in 1977 to 15.1/1000 in 2000. [3] Twinning is particularly increased in women aged 35 – 39 years and one quarter to one third of the rise is attributed to the increasing maternal age of populations. [4] The remaining increase has been attributed to ovarian stimulation and assisted reproductive technologies (ART). In some places the prevalence of multiple pregnancies is thought to be beginning to fall as the ability to transfer a single embryo is being maximised. [5] However, it is unlikely that a substantial decrease in

infertility related twinning rates will be achieved in the near future because of the contribution of ovulation inducing drugs. [4]

Overall, two-thirds of twins are dizygotic (DZ) and one third monozygotic (MZ). The prevalence of spontaneous DZ twinning varies and is associated with increased concentrations of follicle stimulating hormone which is in turn associated with geography, season, ethnic origin (1 in 100 livebirths in North America and 1 in 250 in Japan), increasing parity, [6] and is raised in tall, heavy and older mothers. A peak in this risk appears when women are around 37 years of age and declines after this due to 'ovarian follicular exhaustion'. [7]

DZ twinning runs in families and studies from Australia and Belgium show that a dominantly inherited gene could be carried by 7-15% of their study populations. [8, 9] There was also a variant of the methylenetetrahydrofolate reductase (MTHFR) gene (C677T allele) described as having an inverse association with DZ twinning. [10] As this mutation is common in the Chinese population, it was postulated that this could explain the low prevalence of DZ twins seen in China. [11] However, in 2003, a more recent study of twins [12] found no association of any MTHFR haplotypes with twinning, so this hypothesis now seems unlikely.

It is primarily DZ twinning that is seen as a direct result of ART and ovarian stimulation, with MZ twinning also increased, but on a much smaller scale (2-5 times). [13] Generally, the prevalence of MZ twins is steady (1 in 250 pregnancies), being a random genetic event unaffected by the factors that influence DZ twinning rates. [5]

2.2 Public health implications of twinning

There are obvious public health implications of the increasing prevalence of twins, related to both infant and maternal mortality and morbidity. [2, 5] An increase in MZ twins is of greater public health significance than DZ twins because MZ twins have a much higher rate of mortality and morbidity mainly because of twin-twin transfusion syndrome [14] and also the presence of birth defects in at least 10% of them. Some data are available on monochorionic (MC) and dichorionic (DC) twins in terms of perinatal mortality with MC twins miscarrying at a rate of 12% between 10-14 and 24 weeks compared with a rate of 1.8% in DC twins. [15] However, reporting of perinatal outcomes does not usually differentiate between DZ and MZ twins. Local routinely collected data on all twins show that preterm delivery occurred in 53% of them in 2001-2002 [16] and a systematic review also reported this figure of 53%. [17] In 2000, low birth weight (less than 2500gram) was recorded in approximately 50% of all twins,

(24% were less than 2000g), compared with 5% in singletons. [3] As well as the neurological sequelae in children, the consequences of prematurity and low birth weight, there are psychological and economic effects on families associated with having twins, all of which have the potential to impact on public health in the long term. [14]

2.3 How folic acid may influence twinning rates

The mechanism that FA may play in twinning relates to the fact that twin embryos have extra requirements for micronutrients and vitamins, including FA, to facilitate epigenetic modifications e.g. DNA methylation, necessary for cell differentiation, [18] and to prevent DNA strand breakage. [19] If FA levels are low, this could reduce the survival chances of twins which are known to miscarry early in pregnancy. [14, 20-22] FA and other micronutrients may ‘rescue’ twins about to abort by establishing methylation patterns to enhance cell differentiation and DNA repair.

3. Review of the literature

The research questions and methodology of our electronic database search are presented in Appendix A. This database search identified 10 primary studies pertinent to our research questions that had been published since January 2001, the same year as the publication of the Cochrane review on periconceptional FA intake and risk of neural tube defects. [1] Four studies were retrospective population-based cohort studies and one study a prospective cohort investigating the likelihood of twin births following periconceptional FA supplementation. Another five were ecological studies from the United States investigating a possible association between multiple births following mandatory fortification of foodstuffs in 1998. Full text copies of all of these publications were obtained and critically appraised as to their methodology, results, interpretation of findings and generalisability to other populations. Detailed summaries of each study are presented in Appendix B (supplementation studies) and C (fortification studies). Table 1 is a summary of the five studies of populations where FA intake is in the form of supplements. Key features of the five studies on twinning rates following fortification are presented in Table 2.

The important factors to consider when examining and comparing trends in twinning rates are:

- 1) The increasing age of women having babies
- 2) The increasing use of ART and other treatments for infertility
- 3) The increasing ascertainment of twins amongst fetal losses identified through prenatal screening programs which are becoming more widespread
- 4) Whether any measure of zygosity has been made.

This report places considerable importance on whether or not suitable adjustments have been made for the first two confounders. Maternal age is almost always taken into account, but there are inherent difficulties in accounting for infertility treatments and this has influenced our interpretation of results in many studies. The third point cannot be measured, but the studies that only count livebirths cannot be compared directly with those that also count fetal deaths. Zygosity is sometimes modelled using the Weinberg difference method in twin epidemiology, while at other times zygosity was based on whether the twins were the same sex (MZ) or unlike sex (DZ).

3.1 Supplementation studies

Five studies (Table 1) have assessed the rate of twinning in populations where supplementation with FA was the exposure since 2001. Three of these studies [23-25] have major problems with regard to quality of the data, interpretation and presentation of the results, and conclusions drawn. We have placed little value on these studies for the reasons outlined in the detailed review of each of them (Appendix B). However, some of their results are discussed below.

3.1.1 Exposure – intake of FA from supplementation

There was some variation in the dose that women could have been exposed to, with one Hungarian study* [25] indicating that women could have from 3- 9mg/day, * but in all other studies the tablets had the recommended dose of 0.4mg/day. There was also variation in the background preconception use of FA in the different populations studied. The two studies

* We have included the Hungarian study despite the dose apparently being higher than 1.0mg/day, because it is referred to in the submission to FSANZ

from Sweden reported extremely low use – 0.6% in the first study [23] examining births in 1995-1999, and 1.2% overall in the later study [26] which also included births in 2000 and 2001. The study in Hungary [25] was on births from 1995 to 1996 and data presented indicated 5% preconception use. It was 6% in the Norwegian study [27] and, because of the prospective design of the Chinese study, [11] was 52.5% there. This impacted on sample size, especially in the study from Hungary where there were only 20 twin pairs whose mothers had taken FA before pregnancy. It might also have contributed to bias because in times of low population awareness and use of FA, women planning a pregnancy who were taking fertility drugs would probably have taking FA as well. Kallen [26] accounted for this by excluding women who reported using infertility treatment and involuntary childlessness.

The method of determining how many women took supplements in four of the studies included asking women to remember what they had taken (with a memory aid) [25], examination of medical records, and birth registry forms. [23, 26, 27] It was shown in the most recent study [27] that recording of FA supplementation was extremely inaccurate (45% misclassification), and this finding tends to undermine confidence placed on conclusions of studies where there was not a compliance check. Again, because of the prospective design of the Chinese intervention trial, the reported frequency of supplementation use was known to be accurate, another reason to place considerable weight on the results from that study

Two studies [25, 27] examined the independent contribution of multivitamins to twinning rates and both showed more of an effect than that seen with FA alone. This raises further the point that the original Hungarian trial included in the meta analysis of the impact of FA on twinning, [1] and which weighed heavily (84%) on their overall odds ratio, was based on a supplement that had a dose of 0.8mg as part of a multivitamin tablet.

3.1.2 Maternal age

All supplementation studies either stratified or adjusted for maternal age in their analyses, with no particular age groups targeted in their methodology.

Table 1 Cohort studies examining rates of twinning following periconceptional folic acid (FA) supplementation

Authors, year, place	Study population	Dose, ascertainment and preconception use of FA	Specific issues addressed	Relevant findings	Comments
Vollset et al [27] 2005 Norway	176,042 women giving birth from December 1, 1998 to December 31, 2001. 3154 twin pregnancies (1.79%)	0.2 or 0.4mg tablets available, recommended dose 0.4mg/day Ascertainment: Birth certificate Preconceptional FA use: 6%	Provided estimates of under reporting: 45% misclassification of FA intake 12.7% misclassification of IVF use	Adjusted for age, parity: likelihood of a twin birth with FA was not significantly increased (OR 1.13 (0.97 to 1.33)) and for unlike sex twins the OR was 1.43 (1.12 to 1.83). After further adjusting for under reporting of FA and IVF use there were no significant associations between FA intake and risk of twinning. (OR 1.02 (0.85-1.24)) Non-significant differential effect seen for same-sex (classified as MZ) and unlike-sex (classified as DZ) twins.	Important study with a number of adjustments highlighting the importance of ART confounding. Independent effect of multivitamins also studied.
Czeizel et al [25] 2004 Hungary	38,151 women who gave birth to a child w/o congenital abnormalities, between 1980 and 1996. 395 twin pregnancies (1.04%)	Estimated dose of 3, 6 or 9mg /day Ascertainment: Birth certificate Reported overall preconception use said to be 32%, but data presented indicate only 5% use	Differentiation between pre- and postconceptional use of FA Clomiphene use.	(Surprisingly) use of clomiphene was not increased in mothers of twins. Adjusted likelihood of a twin birth with preconceptional use of FA was not significantly increased (OR 1.60 (0.95-2.69)) and with postconceptional use there was a weak association (OR 1.38 (1.04 to 1.82)). ART not accounted for satisfactorily.	Weak study, based on small numbers of twins. Very high doses of FA. No data on zygosity. Independent effect of multivitamins also studied.
Kallen [26] 2004 Sweden	July 1, 1995 to December 31, 2001. 6953 women who reported use of FA and 8676 women who had unlike-sex twins of whom 232 reported use of FA, compared to 576,873 women who gave birth	0.4mg FA tablets available, recommended dose 0.4mg/day Ascertainment: Birth certificate From data in tables, we estimate 1.2% FA use in early pregnancy	Years of involuntary childlessness recorded on birth certificate as surrogate for infertility. Excluded these women and those who reported use of ovulation induction or marked by midwife as having had ART	Adjusted for age, parity, smoking OR for DZ twinning (unlike sex) = 1.71 (1.21 – 2.42) in 2001-2002. Exact data used to obtain this result not shown in paper	This very weak study is based on inconsistent methodology, obscure statistical comparisons and flawed data and interpretation of results. Findings of this study must be presented together with its limitations. [28]

Table 1 Cohort studies examining rates of twinning following periconceptional folic acid (FA) supplementation (continued)

Authors, year, place	Study population	Dose, ascertainment and preconception use of FA	Specific issues addressed	Relevant findings	Comments
Li et al, [11] 2003 China	240,519 singletons and 1496 multiple births to women registered in the FA community intervention program between October 1993 and September 1995, who delivered before December 31, 1996.	Women were asked to take 0.4mg of FA/day, starting at time of registration (even if not pregnant) until end of first trimester. Intake was recorded monthly. 52.5% of women took FA and 47.5% did not.	No access to ART or over the counter multivitamins. Young population with a mean age of women around 25 years. 3 time periods of supplementation (starting before ovulation, during fertility and after conception).	There was no association between use of FA starting before ovulation, around fertility and after conception with risk of a multiple pregnancy (overall RR 0.91 (0.82, 1.00)).	A convincing study because women had no access to ART, a major confounder, and there was good FA exposure data. A limitation is that Asian populations have low rates of twinning, esp. DZ.
Ericson et al, [23] 2001 Sweden	2569 women (72 twin pairs) who reported use of FA out of a total of 442,906 deliveries between 1995 and 1999.	0.4mg FA tablets available, recommended dose 0.4mg/day Ascertainment: Birth certificate Preconception FA use: 0.6%	Years of involuntary childlessness as surrogate for ART	Excluding women reporting unwanted childlessness: OR for FA and twins = 1.45 (1.06-1.98) Significant RR data presented on DZ twins but unable to determine where this comes from. ART not accounted for satisfactorily.	This is a weak study and results are not clearly presented. Lack of accountability may have resulted in the over interpretation of findings. Findings of this study must be presented together with its limitations.

3.1.3 ART and other infertility treatment

Two studies accounted for ART in convincing ways. [11, 27] The Norwegian study [27] assessed the underreporting rate of IVF in the birth records they were using by contacting fertility clinics in neighbouring countries, from whom they directly established the number of women who had had treatment outside of Norway. It turned out that at least 13% of IVF pregnancies were not reported in Norway and they then adjusted for this in their analysis. In the Chinese study [11] there was no opportunity for women to have infertility treatment, so this was not an issue. Reported involuntary childlessness was used as a proxy in the Swedish studies [23, 26] and in Hungary [25] clomiphene use was recorded and found not to be associated with twinning nor had it increased in use in the time frame. The validity of these latter two efforts at accounting for infertility treatment is questionable. The Swedish data have independently been critiqued following careful examination of vital records, showing that misclassification of IVF has been a serious problem in these studies. [29]

3.1.4 Inclusion of fetal deaths

The only study to include fetal deaths was the study in China [11]. Two studies [23, 26] do not specify the birth status of the populations studied and the other two only examined livebirths. [25, 27] An effect of FA on ‘rescuing’ twin pregnancies would be minimised in studies of livebirths only – twins that had been fetal deaths, recognised as such in prenatal screening programs would not be included anywhere and those that had been ‘rescued’ as a result of FA supplementation would be included as livebirths, but those miscarried or stillborn prior to supplementation would not.

3.1.5 Zygoty

Same sex and like sex twin data were used to estimate MZ rates in one study [27] and found a differential, but non-significant effect with ORs of 0.7 for MZ, and 1.26 for DZ. Risk ratios for same sex and opposite sex twins in the Chinese study were both < 1 . [11] One study [26] also singled out unlike sex twins for analysis and the final figure relates to DZ twins only. The other two studies did not examine zygoty. [23, 25]

3.1.6 Summary of supplementation results

Three of these five studies [23, 25, 26] were of marginal use because of major limitations in methodology and results (see Appendix B). The most convincing study [27] had an adjusted odds ratio (OR) for association between all twins and preconceptional use of FA of 1.02 (0.85-1.24), i.e. a 2% non-significant increased likelihood of twinning having taken a FA

supplement. Much higher ORs were found in the two Swedish studies, [23, 26] and a non-significant high OR (1.6) was found in the Hungarian study, [25] but because of the study limitations, these ORs are not useful.

A relative risk was the point estimate in the Chinese study [11] and this was 0.91 (0.82-1.00). The only reason to question the generalisability of this study is related to the relatively low rate of DZ twinning in the Chinese population, but the authors argue that, with a lower baseline rate of twinning, an increase may be more easily detected. The other possibility is that the high rate of the MTHFR C677T polymorphism in the Chinese population, which plays an integral role in folate metabolism, and was thought to be inversely related to twinning, was somehow reducing the FA effect on twinning. However, a recent rigorous study of the genetic influence on twinning did not find an association between this polymorphism and twinning. [12] Therefore, this polymorphism in the Chinese population is unlikely to be contributing to the non-effect of FA on twinning.

3.2 Fortification studies

Five retrospective cohort studies (Table 2, see also Appendix C) analysed the twinning rates before and after FA fortification – all emanate from the United States where mandatory fortification has existed since 1998. [24, 30-33] Prior to this date there was an optional fortification period from 1996. Data on twinning from 40-50 other countries (eg Canada and a large number of countries in South America) that have mandatory fortification are not yet available in the literature. The published US studies are of varying quality, some on close inspection having considerable limitations in methodology, statistical analysis and presentation of results.

3.2.1 Exposure - intake of FA from food fortification

It is difficult to define the exposure adequately in population studies of food fortification. This is evident in the largely statewide cohorts and in the one instance, a study involving data from the entire US vital statistics system. Inconsistencies appear in the definition of the exposed cohorts in relation to the optional fortification period, 1996-1998. Table 2 shows that two studies included the optional fortification period in their unexposed cohort [30, 33] one study defined it as exposed to FA, [31] one study excluded it [24] and one study only looked at this optional period. [32] In addition to the timing issue, none of the studies were able to determine whether women who delivered twins had the same amount of fortified food as the women who delivered singletons.

Table 2 Retrospective registry-based cohort studies examining twinning rates (TR) following fortification of food with folic acid (FF) in the United States (US FDA mandated folic acid fortification of enriched cereal grain products at 140µg of folic acid per 100g of grain as of 1996. In 1998, full fortification was mandatory)

Authors, (Ref), Year and location	Study population	Time frame	Relevant findings	Comments
Signore et al, 2005 [31] US National Vital Statistics System	Nulliparous women aged 16-19 years (ie ART not relevant), with a live birth or fetal death Singletons: 3,362,245 Twin pairs: 25,065	Unexposed: January 1 1990 – November 30 1996 Exposed: December 1 1996- December 31 2000) (Optional fortification period included)	Constant TR before FF followed by increase of 2.4% per year after FF (= 2 pairs of twin /10,000 confinements). No zygosity data	High quality study accounting for ART confounder, but may not be able to extrapolate to all maternal ages.
Lawrence et al, 2004 [30] Sth California	Women with a live birth in the Kaiser Permanente Health Plan – able to examine ovulation induction medications. Singletons: 215,820 Twin pairs: 3,035	Unexposed: January 1 1994 – September 30 1998 (Optional fortification period included) Exposed: October 1 1998- December 31 2000)	TR was 13.8/1000 before FF and 14.5/1000 after FF but use of ovulation-inducing (OI) drugs was 6.6% in 1994 and 14.9% in 2000 and ART use did not change. After excluding women using OI, TR was 12.7/1000 in both periods No zygosity data	Important study, but statistical analysis very basic.
Kucik et al, 2004 [24] Atlanta	Women with a live birth Singletons: 495,666 Twin pairs: 7,167	Unexposed: January 1 1990 – December 31 1995 (Optional fortification period excluded) Exposed: January 1 1998- December 31 2001	TR was 13.3/1000 before FF and 15.7/1000 after FF Comparison of two periods (excluding intervening years) showed unchanged TR in women less than 30 years in both time periods. Annual increase in TR after FF was 2% in women 30-34 and 0.1% in 35+ No zygosity or ART data	Weak study with issues around methodology and inability to interpret results.
Waller et al 2003 [32] Texas	Women with a live birth resulting from a conception in the time period defined Singletons: 990,520 Twin pairs: 12,687	No pre-post analysis – trend data only: January 1 1996 – December 31 1998 (Optional fortification period included)	After adjustment for season, age, ethnicity, parity, education, the annual increase in TR was 2.4% for 1996-1997 and 4.6% for 1997-1998. Zygosity modelled- some increase in MZ (7%), but not DZ. No ART data	Carefully designed study producing useful trend data on TR, but study period not long enough.
Shaw et al, 2003 [34] California	Women with a live birth or fetal death Singletons: 2,601,175 Twin pairs: 29,665	Unexposed: January 1 1990 – September 30 1998 (Optional fortification period included) Exposed: October 1 1998- December 31 1999	After adjustment for year, age, ethnicity, parity, sex of twin pr (surrogate for zygosity), no increase in TR (not shown) associated with FF. Relative risk ratio for twinning associated with fortification = 1.00 (0.95-1.04) No ART data	Valuable 11 yr trend data on TR, but only last year plus two months to look at rates after FF

3.2.2 Maternal age

Stratification for maternal age found no effect in one study [33]; another study [30] found no change in maternal age distribution across the study years and did not present any maternal age-related results; two other studies adjusted for maternal age in regression analyses [24, 32] and one only looked at teenage mothers. [31] This focus on the young age group (to ensure the ART issue was accounted for – see below), who have lower twinning rates than older women, may limit the generalisability of the results to all ages.

3.2.3 ART and other infertility treatment

The three earliest studies [24, 32, 33] did not take ART into account in their analysis, but this did not really seem necessary, as they recorded no marked increase in the twinning rate over the study period. Two of these three were published in 2003 [32, 33] and the study period following fortification may have been too short to detect any sustained or significantly increased change in twinning rate before and after fortification. The two most recent studies, [30, 31] both of which accounted for ART and ovulation induction, provided quite convincing evidence that there had not been an increase in twinning in the US anywhere near the magnitude as that suggested (40%) in the 2001 review. [1]

3.2.4 Inclusion of fetal deaths

Two studies included fetal deaths [31, 33] and reported either a small increase or no increase in twinning in the study period. If the assumption that FA ‘rescues’ twins from spontaneous fetal death holds, the three studies [24, 30, 32] that only looked at livebirths may have missed some twins fetal deaths (or terminations) in the unexposed period and included an equivalent number of livebirths in the exposed period, which would exaggerate any effect of FA in the population. The effects they showed were minimal, and do not support any major increase in livebirth twinning rates. One could argue that it is only livebirths that are a public health problem, assuming that a miscarriage or perhaps pregnancy termination do not require factoring into the cost-benefit calculations. Many would not agree with this.

3.2.5 Zygosity

Only one study modelled zygosity and showed a marginally significant 7% annual increase in MZ twins, but no increase in DZ twins. [32]

3.2.6 Summary of fortification results

These fortification studies were easier to critique than the heterogeneous mix of supplementation studies. Trend data from the fortification ecological studies allowed for calculation of annual percent increase after the exposure period. This was provided in all but one study [33], which concluded that there was no increase in twinning rate. A weaker study [24] found a low percent annual increase for older women (2%), but no increase for younger women. The other publications [30-32] indicate that there is, at most, a 2.4% to 4.6% annual increase in twinning rates across all ages. If the background rate of twinning is about 15 pregnancies per 1000 and the increase is at most 5%, this would mean an additional 0.75 twin pairs (5% of 15) per 1000 pregnancies or 7.5 per 10,000 extra twin pairs each year.

4. Summary

Weighing up the balance of a beneficial reduction in prevalence of NTDs versus a potentially harmful increase in the twinning rate is important, but only if an increase in the twinning rate is evident. This report has reviewed the latest literature in an attempt to address whether this association does in fact exist. On balance it appears that, if there is an association, it is extremely weak and nowhere near the magnitude suggested in the Cochrane review. [1] The Cochrane review results were heavily weighted by the Hungarian trial which used a relatively high dose of FA (0.8mg) that was part of a multivitamin tablet.

There is no new evidence from intervention studies supporting an association with FA supplementation as the data reviewed in this report have all come from observational studies. Results of the two observational studies that account for infertility treatment adequately, and refer to a supplement containing 0.4mg of FA, do not provide substantial evidence for a relationship between FA and increased twinning. [11, 27]

Other observational studies reviewed in this report have examined twinning in the US since food fortification was introduced in 1996, and mandated in 1998. The highest annual percent increase in twinning rates found in these studies was 4.6%, [32] reflecting, at most, an additional 7.5 twin pairs per 10,000 pregnancies. It could be argued that the dose obtained from fortification alone may be lower than that needed to see an effect on twinning, but the exact daily dose a woman has cannot be determined, being wholly dependent on what she eats (0.14mg per 100g of grain) and whether she also has supplements. Between 1995 and 2003, approximately 25%-30% of non pregnant women of childbearing age in the US were taking

multivitamin supplements including FA, and in 2004 this rose to 40%. [35, 36] Whether the FA had come from fortification alone or from supplementation as well, the amount of FA in the US population in 1999-2000 was enough to see a 27% decline in NTDs [37] and a CDC report showed that the median red blood cell (RBC) level of FA in the blood (a surrogate measure for dose), had by the year 2000, reached the target level of 220ng/ml among women of childbearing age in the US. [38]

FA RBC levels appear to plateau fairly soon after fortification [30, 39], so it is unlikely that there would be any further change in twinning rates related to fortification. It remains to be seen if a further decline in NTDs in the US will occur only if more women also take FA supplements and whether this in turn raises blood levels of FA and increases twinning rates. It will be important to re-examine twinning and NTD data in this context.

Overall, using the 2003 WHO classification for assessing evidence, there is possible evidence for a relationship between periconceptional FA intake and increased twinning. In two acceptable quality studies [30-32], the highest annual increase in twinning rates was 5%, but there was no significant increase seen in three other acceptable studies. [11, 27] A plausible biological relationship exists in terms of the nutrient needs of the developing fetus, and it may be that other micronutrients are just as, or more important than FA. More long term follow up studies are needed, focusing on dose response, by monitoring of FA blood levels, and obtaining accurate data on infertility treatments and all livebirths and fetal deaths of twins. These would help provide more convincing evidence that there is indeed a causative relationship and a negative public health impact, which on the face of the studies reviewed here, at the moment appears to be minimal.

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Appendix A Search strategy

Research questions

1. Does periconceptional supplementation with folic acid increase the risk of multiple pregnancies?
2. Does food fortification with folic acid increase the risk of multiple pregnancies?

Resources Searched

We searched the following databases:

The Cochrane Library, Issue 2, 2005, Medline (OVID), Medline in process (OVID), PubMed National Library of Medicine, CINAHL (OVID), EMBASE (OVID)

Refinements, Searching & Reporting Constraints

Our electronic searching was performed on April 20 2005 and the search was limited to publications dated from 2001- current. MEDLINE in process was last checked on May 4, 2005.

Search strategy

MEDLINE and CINAHL:

1	Folic acid/
2	Folate.tw
3	Folic acid.tw
4	Or/1-3
5	Exp pregnancy, multiple/
6	Exp twins/
7	Twin\$.tw
8	Multiple.tw
9	Or/5-8
10	4 and 9
11	Limit 10 to yr=2001-2005

MEDLINE in Process and EMBASE: (folic or folate) and (twin\$ or multiple)

PubMed and Cochrane Library: (folic OR folate) AND (twin*)

Appendix B Evidence summaries, Folic acid supplementation studies

<p>Evidence Summary</p> <p>Aetiology Observational study</p>	<p>Vollset SE, Gjessing HK, Tandberg A et al (2005). Folate supplement and twin gestation rates. Epidemiology 16:201-205.</p>
<p>STUDY DESIGN Levels of evidence</p>	<p>Retrospective population-based cohort study IIb</p>
<p>DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria</p>	<p>Patients (subjects): 176,042 women giving birth from December 1998 to the end of 2001 as reported to the Medical Birth Registry of Norway. Outcomes: Primary outcome was periconceptual folate intake for singleton or twin pregnancies. Secondary outcome was zygosity. Inclusion Criteria: Not further specified. Exclusion Criteria: Not specified.</p>
<p>VALIDITY: Methodology, rigour, selection</p>	<p>Ascertainment of exposure: Folic acid intake as ascertained from the birth notification form. Health professionals complete 5 items relating to mother's dietary supplements before and during pregnancy. Description of the groups: Overall, 6% of women reported to have used preconceptional folate supplements. There were 3154 twins among 176,042 pregnancies (1.79%). The number of dizygotic twins was 1094 (0.62%). Baseline characteristics of maternal age distribution and parity are not presented. Distribution of prognostic factors: Use of IVF was reported for 2620 pregnancies (1.5%). Preconceptional use of folate was reported 4 times more frequently for IVF pregnancies (24%) and the proportion of twins after IVF was 27%. Confounding factors: The main confounder considered was pregnancies conceived by IVF. Authors account for reporting methods and validity in detail and stratify twinning rates for IVF in the description of their cohort. Maternal age and parity were also taken into account in the multivariate analysis. In addition, authors present a modelled analysis taking into account a misclassification adjustment for underreporting of 12.7% of IVF conceptions and 45% of folate users being misclassified as non-users. Data analysis: Outcome was modelled by Logistic regression and generated odds ratios with 95% confidence intervals. The analysis did not model twinning rates as outcome of folate, rather it worked backwards in the fashion of a case-control study, using singleton pregnancies as the control group .</p>
<p>RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate</p>	<p>Folate use among twin pregnancies: After adjusting for age and parity, folate use before pregnancy was associated with multiple births (OR 1.59, 95% CI 1.41, 1.78). After taking out IVF pregnancies this risk was reduced to 1.13 (0.97, 1.33) (NS) for all twins, and for dizygotic twins, the association was stronger with an OR of 1.43 (1.12,1.83). Folate use during pregnancy was not significantly associated with dizygotic twins. There was no significant association between folate use before or during pregnancy and monozygotic twins. After adjusting for misclassification of IVF and folate intake these associations were further attenuated resulting in no significant associations between folate intake before or during pregnancy and the risk of monozygotic or dizygotic twins (OR 1.02 (0.85-1.24)). In fact, there was a non-significant negative association between preconceptional folate use and risk of monozygotic twins. An association between use of multivitamins during pregnancy only and risk of dizygotic twins was observed even after adjusting for misclassification (OR 1.54 (1.22, 1.96).</p>
<p>AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research</p>	<p>“Confounding by IVF and misclassification of IVF, strongly bias the estimate of the effect of preconceptional folate use on twinning in Norway.” “An association between reported use of multivitamins during pregnancy and twinning remained after adjustment. If causal, this association could indicate decreased risk of spontaneous abortion in women taking vitamins in pregnancy, thus increasing the likelihood of birth of both twins.” Authors discuss their findings in light of previously published studies. Epidemiologic data from other study remains inconclusive and IVF confounding is given as the main reason for those studies that found increased twinning risks. A negative association between folate and twinning rates was found in China, where there are higher proportions of monozygotic twins. 2 studies found no association between folic acid food fortification and the risk of twins. Authors also address the issue of a possible dose-response. In light of folic acid doses used in published studies and their conflicting results, authors conclude that this may explain the heterogeneous results.</p>
<p>OUR COMMENTS: Opportunity for bias, weakness and strength</p>	<p>Opportunity for bias: It is unclear how a birth is defined (eg 20 weeks or later) or if still births and terminations are included in the cohort. There is a higher rate of mortality and preterm birth associated with twin pregnancies and if not all births at 20 weeks or later regardless of their status were included, this may have affected the outcome. All same-sex twins were classified as monozygotic. This may have resulted in an incorrect estimate of the association between zygosity and folate intake. Weakness/es: <i>Validity:</i> The accuracy of folate intake as recorded on birth notification forms has not been formally assessed. Authors estimated a 45% underreporting of use. Strengths: The cohort was large as is required for rare outcomes, and included around 3000 twin pregnancies. Accounting for IVF is thorough and interpretation is easy to follow. The conclusions are justified and within the limitations of the study</p>

Evidence Summary	Czeizel AE and Vargha P (2004). Periconceptional folic acid/multivitamin supplementation and twin pregnancy. American Journal of Obstetrics and Gynecology 191:790-794.
Aetiology Observational study	
STUDY DESIGN Levels of evidence	Retrospective cohort study IIb
DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria	Patients (subjects): 38,151 women who had given birth to a child without congenital abnormalities between 1980 and 1996, as recorded in the Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA) database. Outcomes: Twin births. Determination of outcome is not specified. Inclusion Criteria: Not further specified. Exclusion Criteria: Not specified.
VALIDITY: Methodology, rigour, selection	Ascertainment of exposure: Pre- and postconceptional folic acid and/or multivitamin intake as ascertained from two sources: self-reported information on questionnaire sent to women and antenatal care log book plus medical records including data on twin pregnancies. The latter was asked to be sent by the mother to the study coordinators. Description of the groups: 38,151 women had their information evaluated. No data is provided on the number of questionnaires and/or medical record requests that were sent out and the corresponding response rate. There were 395 women in the cohort who had given birth to twins (1.04%). Of the twin births there were 127 in the no supplement group, 224 in the folic acid group, 20 in the multivitamin group and 24 in the group that reported to have taken both (total of 4 groups in analysis). Distribution of prognostic factors: The mean maternal age was similar across all groups. Mothers of twins were more likely to be of higher parity in all groups than mothers of singletons. Medication use was slightly higher in mothers of twins. Use of clomiphene was not increased in mothers of twins. Confounding factors: Analysis was adjusted for maternal age parity, marital status, employment status and other medicine uses. Data analysis: Outcome was modelled by Logistic regression and generated odds ratios with 95% confidence intervals. Comparison of pre- and postconceptional supplementation was made with chi square tests.
RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate	Likelihood of twin births among folate users: preconceptional: 20 twin pairs, NS, postconceptional: 204 twin pairs, weak association Likelihood of twin births among multivitamin users: preconceptional: 7 twin pairs, OR 2.98 (1.35-6.58), postconceptional: 13 twin pairs, OR 2.08 (1.16, 3.72) p value for the difference between the two: 0.02 Likelihood of twin births among users of both: preconceptional: 1 twin pair, NS, postconceptional: 23 twin pairs, OR 2.59 (1.64, 4.19) p value for the difference between the two: 0.05 Likelihood of twin births among supplement users: preconceptional: 28 twin pairs, OR 1.80 (1.14, 2.85), postconceptional: 240 twin pairs, OR 1.50 (1.15, 1.97)
AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research	Authors discuss possible mechanisms by which folate or multivitamins (eg Vitamin A) may result in multiple pregnancies or protect from neural tube defects, including a putative role for a particular genotype of the MTHFR gene in different populations. Authors also put forward that their findings may be an artefact of early detection of twin pregnancies and improved antenatal care resulting in a reduced perinatal mortality of twins. This improvement in perinatal outcomes of twins is also put forward relating to a potential public health impact of increased twinning rates. In addition, data from an unpublished survey is presented which shows a changing attitude of Hungarian women to twin pregnancies. Women were predominantly happy to accept a twin pregnancy. These two latter findings along with the findings of this study led authors to conclude: “The periconceptional folic acid/multivitamin use is a breakthrough in the primary prevention of neural-tube defects and some other structural births defects. The possible association of periconceptional folic/acid multivitamin supplementation and twin pregnancies needs further studies, but recently we have had a good chance to reduce the previously higher perinatal mortality rate of twins. Thus, the benefits of supplementation obviously outweigh this possible risk.”
OUR COMMENTS: Opportunity for bias, weakness and strength	Opportunity for bias: It is unclear how birth was defined (eg 20 weeks or later) or if still births were included in the cohort. There is a higher rate of mortality and preterm birth associated with twin pregnancies and if not all births at 20 weeks or later regardless of their status were included, this may have affected the outcome. Mothers with better birth outcomes may have been more likely to respond to the questionnaire mail-out. Response rate was not specified and no attempt was made to characterise non-responders. Authors report to have analysed 38,000 plus questionnaires. There may be recall bias in the self reported folic acid intake. The timeframe between birth and questionnaire was not specified. No intake dose could be established. IVF pregnancies were not adjusted for and use of the ovulation-inducing drug clomiphene may not have been an adequate substitute. Zygosity was not taken into account. Weakness/es: <i>Validity:</i> the lack of information on the cohort of responders and non-responders make it difficult to assess the validity of the data evaluated. Multiple adjustments and stratifications were made in the regression analysis where groups contained small numbers. This is apparent in the large CIs returned by the analysis. For example, the authors' main finding that supplementation of folic acid and/or multivitamins before conception increased the rate of twin pregnancies was based on multiple adjusted data from 28 twin pairs. The limitations of the sample size ask for caution in the interpretation of the results. Strengths: Authors interpret their finding of an association between folate/multivitamin intake and twinning carefully and do not overstate their impact.

Evidence Summary	Kallen B (2004). Use of folic acid supplementation and risk for dizygotic twinning Early Human Development 80:143-151.
Aetiology Observational study	
STUDY DESIGN Levels of evidence	Retrospective cohort study IIb
DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria	Patients (subjects): Women recorded in the Swedish Medical Birth Registry from July 1, 1995 to December 31, 2001. Outcomes: Primary outcome was unlike-sex twinning as surrogate outcome for dizygotic twinning. Inclusion Criteria: Not further specified. Exclusion Criteria: "During the analysis, findings resulted in exclusions of certain groups of women from the analysis". Not further specified. Later authors state that women of non-Swedish nationality, women who reported involuntary childlessness or the use of ovulation-inducing drugs or gestagens were excluded from the analysis.
VALIDITY: Methodology, rigour, selection	Ascertainment of exposure: Use of folic acid in early pregnancy (and before) as reported on a standardised antenatal care form. This information is routinely collected by a midwife before the end of the first trimester. Comparisons were made to all women in the register. Our comment: It is not clear how the two groups were independent from each other. Description of the groups: 6953 exposed women and 8676 women who had unlike-sex twins of whom 232 reported folic acid use. Our comment: These groups do not reflect the original groups as identified as exposed/unexposed. 0.03% of women with unlike-sex twins reported folic acid use, it is difficult to see how this can be incorporated in a meaningful analysis. Distribution of prognostic factors: Not specified. Confounding factors: Confounders considered were year of birth, maternal age, parity, smoking and years of involuntary childlessness. Further to these adjustments ORs were presented for 20 different drugs a woman reported taking during pregnancy, BMI and women born outside of Sweden. Later the analysis was also stratified for the years of 2000-2001. Number of previous spontaneous abortions was mentioned in the discussion as having an opposite effect on the risk of twinning, but the results' section never mentioned this. In the final analysis, women with reported involuntary childlessness were not included, but authors did not provide actual numbers of women left in the final regression. Our Comment: Actual numbers for all adjustments and strata would have been quite small. Data analysis: Mantel-Haenzel technique with Odds Ratios and 95% Confidence Intervals as determined by a test-based method. "2 ORs obtained in this way were compared using 2-tailed z tests based on the same variances as those used to determine the 95% CIs."
RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate	Comparison of risk factors for use and reporting of folate use and for dizygotic twinning: Results are presented in 7 tables and some additional results are discussed in the body of the text. However, it is difficult to interpret these outputs. In addition, in light of our comments above regarding the selection and description of the groups and the multitude of adjustments and stratifications it has become unclear how these results can be meaningful in any way.
AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research	"The odds ratio for dizygotic twinning after folic acid supplementation was (then) 1.71 (95% CI 1.21, 2.42) and for the years 2000-2001 even 2.09 (1.39, 3.12)."
OUR COMMENTS: Opportunity for bias, weakness and strength	Opportunity for bias: Involuntary childlessness as a surrogate for infertility was not further defined. Weakness/es: Validity: Comparisons as described did not match actual comparisons made. Exposure status and unexposed comparator not clear. <i>Results:</i> Incomprehensible presentation of results. This study is based on inconsistent methodology, obscure statistical comparisons and flawed interpretation of results.

Evidence Summary Aetiology Observational study	Li Z, Gindler J, Wang H et al (2003). Folic acid supplements during early pregnancy and likelihood of multiple births: a population based cohort study Lancet 361:380-384.
STUDY DESIGN Levels of evidence	Prospective cohort study IIb
DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria	<p>Patients (subjects): Women registered in the folic acid community intervention program in China. These consisted of pregnant women and women who were preparing for marriage and had registered with a pregnancy monitoring system (prenatal care) between October 1993 and September 1995 and who delivered before December 31, 1996.</p> <p>Outcomes: Primary outcome was singleton or multiple births. Secondary outcome was zygosity as estimated by the Weinberg method (twice the number of unlike-sex twins = dizygotic).</p> <p>Inclusion Criteria: All births, stillbirths at 20 weeks gestation or later.</p> <p>Exclusion Criteria: Miscarriages and elective terminations before 20 weeks gestation. Deliveries of a baby with a birth defect or terminations for a birth defect.</p>
VALIDITY: Methodology, rigour, selection	<p>Ascertainment of exposure: Women in the intervention trial were asked to take 400µg of folic acid per day, starting at the time of registration and continuing until, the end of the first trimester. Monthly reports on the number of pills taken and the dates that folic acid taking began and stopped were recorded by health workers.</p> <p>Description of the groups: There were a total of 247,831 live and stillbirths during the study period. 2.3% were excluded because of a birth defect. 52.5% of included women had taken folic acid at some time during pregnancy, 47.5% had not. 1496 were multiple births (0.62%) and 240,519 were singletons. The estimated rate of DZ twins was 2.5/1000 and for MZ twins it was 3.6/1000 births.</p> <p>Distribution of prognostic factors: Women who took folic acid were on average 16 months younger and were more likely to have their first baby than women who did not take the supplement. 98.6% were of the HAN ethnicity, the largest group in China. Most women were farmers or factory workers in both groups and of similar educational level. ART or over the counter vitamins were not available to the women. Women who did not take folic acid were more likely to be entering the second semester when they registered and were having their second child.</p> <p>Confounding factors: Data was stratified for maternal age, parity education, occupation, BMI and plurality.</p> <p>Data analysis: Rates of twinning with folic acid intake when compared to no folic acid intake were calculated as rate ratios with 95% Mantel Haenzel confidence intervals. Data was stratified for potential confounders and three time intervals (intake began before conception, at fertilisation and after conception). Multivariate logistic regression was undertaken but data not shown.</p>
RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate	<p>Overall use of folic acid during pregnancy and risk of multiple births: Overall the rate of multiple births was 0.56% among women who took folic acid and 0.65% among women who did not (RR 0.91 (0.82, 1.00)). 99.5% of multiple births were twins.</p> <p>Use of folic acid before ovulation and risk of multiple births: RR 0.91 (0.82, 1.01)</p> <p>Use of folic acid during fertility and risk of multiple births: RR 0.90 (0.81, 1.00)</p> <p>Use of folic acid after conception and risk of multiple births: RR 0.91 (0.82, 1.00)</p> <p>These results were unchanged when adjusted for age, education and occupation. Rates of same-sex or unlike-sex twins did not differ among women who did or did not take folic acid.</p>
AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research	<p>"Our findings suggest that consumption of 400µg of folic acid alone per day, before and during early pregnancy, does not increase a woman's likelihood of having a multiple birth, whether taken before the estimated date of ovulation, around the estimated time of fertilisation or after conception."</p> <p>Authors put forward that rate of twinning might be expected to increase with increasing dose of folic acid and discuss that this pattern has not been observed.</p> <p>Authors discuss findings of previous studies and present reasons why 4 studies that reported an association between folic acid and twinning, may have been due to chance or confounding.</p> <p>Authors also discuss limitations and strengths of their own study. the major weakness is that Asian populations have a high rate of MZ twinning and that folic acid might be expected to influence the rate of DZ twinning. However, authors state that the mechanism for an association between folate and twinning is unknown.</p>
OUR COMMENTS: Opportunity for bias, weakness and strength	<p>Opportunity for bias: Study participants were not randomised. However, the study population was relatively homogeneous and a number of known confounders were not applicable (ART, multivitamins and ethnicity).</p> <p>The majority of births in other populations occur in the older age group and older women's twinning rates may respond differently to folic acid supplementation. Also there may be an association between parity, folic acid and twinning (most women in this study were primiparous).</p> <p>As discussed by authors, the high rate of MZ twinning in the study population may limit the applicability of this study to other populations.</p> <p>Weakness/es: These mainly relate to the generalisation to other populations.</p> <p>Strengths: This is a carefully designed and executed study. The major confounder in most other studies is use of ART, to which this population had no access. The study of Li et al is the only recent study which was able to provide precise data on folate exposure.</p>

Evidence Summary Aetiology Observational study	Ericson A, Kallen B and Aberg A (2001). Use of multivitamins and folic acid in early pregnancy and multiple births in Sweden Twin Research 80:63-66.
STUDY DESIGN Levels of evidence	Retrospective cohort study IIb
DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria	Patients (subjects): Women recorded in the Swedish Medical Birth Registry from 1995 to 1999. Outcomes: Primary outcome was twinning. Secondary outcome was zygosity as estimated by the Weinberg method (MZ = difference between the number of same-sex and unlike sex twins). Inclusion Criteria: Not further specified. Exclusion Criteria: Not specified.
VALIDITY: Methodology, rigour, selection	Ascertainment of exposure: Use of folic acid in early pregnancy (and before) as reported on a standardised antenatal care form. This information is routinely collected by a midwife before the end of the first trimester. Comparisons were made to all women in the register. Our comment: It is not clear how the two groups were independent from each other. Description of the groups: there were 442,906 deliveries during the study period. 2569 women reported use of folic acid (0.6%) and 1971 women reported using multivitamins (0.4%) There were 72 twin pairs (2.8%) among folate users and 37 (1.9%) among multivitamin users. The twinning rate of the total population was 1.5%. 38 twin pairs were same sex, 31 unlike sex and 3 unknown. Distribution of prognostic factors: Not specified. Confounding factors: No adjustment was made for parity or maternal education. Authors state that these variables had no impact on the twinning rate. Our Comment: It is unclear how this was determined and data was not shown. Most other studies show that parity is an important predictor of twinning. Data were stratified for age, years of involuntary childlessness and year of birth. Our Comment: The legend of Table 1 also states that adjustment for maternal smoking was made, although authors do not mention this again. Our Comment: No actual numbers are provided for any strata. Data analysis: Mantel-Haenzel technique with Odds Ratios and 95% Confidence Intervals as determined by a test-based method. Authors also compared the observed number of DZ and MZ twins (as estimated by the Weinberg method) in the folic acid group with the expected number based on all deliveries. In this comparison no adjustments were made.
RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate	Our Comment: Authors present a number of combinations of data and results, with their main finding cited below. The reference categories used to calculate ORs presented in 2 tables are not clear. In light of the considerable opportunity for bias and lack of accountability in describing the cohort we have not presented these in detail.
AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research	“Women (n=2569) who in early pregnancy reported use of folic acid had an increased rate of twin deliveries after consideration of maternal age and length of involuntary childlessness, both variables being significant confounders. The effect was also seen in women who did not report involuntary childlessness.” “The increased risk seems to be limited to dizygotic twinning (relative risk=2.13, 95% CI 1.64-2.74). If this association is causal, wide-spread supplementation with folic acid may represent a hazard larger than the postulated beneficial effect on neural tube defects, at least in low-risk areas”. In their introduction, authors state that the evidence for any benefits of folic acid supplementation and neural tube defects mainly comes from “interviews in case-control studies and on small RCTs” and fail to cite the major contributors to the body of evidence by 2001.
OUR COMMENTS: Opportunity for bias, weakness and strength	Opportunity for bias and weakness/es: <i>Validity:</i> 0.6% of women in this study reported to have used folic acid at some time before or during pregnancy. In a related study, also presented in this paper, a survey of pregnant women of the same population revealed that 8% had used folate. The accuracy of reporting on the birth registration form has not been tested and appears to be highly unreliable. It is possible that a large number of women in the “unexposed” cohort and who gave birth to singletons had also taken folic acid during pregnancy. Given the issues around the validity of reporting of use of supplementation, it is not clear how accurate the reporting of the twin variable was. ART is most likely to be a strong confounder in the findings of this study. The proportion of women with “involuntary childlessness” as reported on the form was not provided as it is not possible to make any attempt at assessing its accuracy. It is also not clear how involuntary childlessness may be an adequate surrogate for ART. Authors compare women who reported this variable with women who did not and found that ORs of twinning were approximately the same in both groups. Authors use this finding to show that there is most likely no confounding related to ART. However, a more likely explanation is that involuntary childlessness is not a reliable estimate of ART use, resulting in a substantial overestimation of the association between folic acid use and twinning. Further to this, women who conceived a pregnancy by ART may also be more likely to have commenced folic acid supplementation before pregnancy. <i>Results:</i> Results are poorly presented and not easy to interpret. Data needed to follow authors’ arguments are not presented. Authors do not present their findings in light of their study’s limitations and in an unbiased way. There is a high degree of opportunity for bias and lack of accountability in this study, which may have resulted in the over interpretation of findings. Findings of this study must be presented together with its limitations.

Appendix C Evidence summaries, Folic acid fortification studies

<p>Evidence Summary</p> <p>Aetiology Observational study</p>	<p>Signore C, Mills JL, Trumble AC (2005). Effects of Folic Acid Fortification on Twin Gestation Rates. Obstetrics & Gynecology 105:757-762.</p>
<p>STUDY DESIGN Levels of evidence</p>	<p>Retrospective population-based cohort study IIb</p>
<p>DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria</p>	<p>Patients (subjects): Nulliparous non-Hispanic white, non-Hispanic black and Hispanic women aged 16-19 years as recorded by the United States National Vital statistics system for the years of 1990-2000. A total number of 3,387,310 pregnancies of which 25,065 were twin pregnancies Outcomes: Primary outcome was singleton or twin births after 20 weeks gestation, including fetal deaths and still births. Inclusion Criteria: Women aged 16-19 with no previous recorded birth, to minimise influence of ART. Authors estimate that < 0.2% of teenagers receive fertility treatment. Exclusion Criteria: Spontaneous or elective abortion, higher order twins, women of Asian origin (due to low numbers)</p>
<p>VALIDITY: Methodology, rigour, selection</p>	<p>Ascertainment of exposure: US FDA mandated folic acid fortification of enriched cereal grain products at 140µg of folic acid per 100g of grain as of 1996. In 1998, full fortification was mandatory. Exposure was defined as infants born in December 1996 or after were likely to be conceived on or after March 1996, when fortification was introduced. (Dec 1996-December 2000, 4 years and 1 month) Unexposed cohort was defined as infants born before December 1996 (January 1990 –November 1996, 6 years and 11 months) Description of the groups: Baseline characteristics of the cohort are compared to all births for the US during 1990-2000. The crude twin rate for the cohort was 7.4 per 1000 and 13.1 per 1000 for the US population. There were 3,362,245 singleton births and 25,065 twin births in the cohort. Distribution of prognostic factors: There was a temporal trend in both groups, with twin rates in the cohort increasing from 7.2 to 8.2 per 100 (a 13.9% increase) and twin rates for the entire US population increasing from 11.2 to 15.5 per 1000 (a 38.4% increase). Other characteristics described are gestational age at birth and birth weights, both of which were similar in both groups. Women in the cohort were less likely to be married and had lower education attainment. Confounding factors: Maternal age and race were taken into account in the multivariate analysis. Factors not accounted for are parity, voluntary folate supplementation, (although authors argue that awareness of folic acid among teenagers continues to be low), that twinning rates may vary for different age groups and that introduction of fortification may have varied amongst States (authors argue that using national data eliminated this potential confounder. The contribution of fertility treatments to the twinning rates is probably satisfactorily eliminated by choosing the maternal ages of 16-19. Data analysis: Rate of twinning was modelled by Poisson regression and generated rate ratios with 95% confidence intervals. Time trends in the regression model were split to before and after December 1996.</p>
<p>RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate</p>	<p>Twin gestation rates: In the unexposed group (from January 1990 to November 1996) twin rates were essentially constant (slope=0.0052, SE 0.0034, p=0.13). After fortification (from December 1996 to December 2000) there was a small but statistically significant trend for an increasing twinning rate (difference in slope=0.024, SE 0.009, p=0.006). In terms of actual rates, there has been an increase of 2.4% (95%CI 0.1, 4.2%) per annum in the twinning rate in nulliparous non-Hispanic white, non-Hispanic black and Hispanic women aged 16 to 19 years in the US, since the addition of folic acid to the food supply. This equates to an additional 2 sets of twins per 10,000 confinements/ year.</p>
<p>AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research</p>	<p>“Our data show that in the entire US, twin gestation rates in young women who were unexposed to fertility treatments have been slowly increasing since the introduction of folic acid fortification of grain.” Authors discuss their findings in light of previously published studies. Epidemiologic data remains inconsistent and the findings of this study show a much lower increase in twinning rates than previously reported by some studies. Authors also address the issue of biologic plausibility of a continued increase in twinning rates in relation to folic acid. Some time after fortification, serum and red blood cell folate levels should be expected to have reached a plateau and a corresponding plateau in twinning rates should have been observed. (Comment by us: the study by Lawrence et al in 2004 showed that serum folate levels of pregnant women in a large US maternity hospital continued to rise beyond 1998 and 1999, showing in effect that no plateau was reached in the first 2 years following mandatory fortification. Levels have possibly plateaued in 2000). In light of the small increase of 2 twin pairs per 10,000 confinements per annum and the expected plateau of folate stores authors concluding remarks were that “fortification with folic acid does not appear to be causing a serious public health problem related to twinning”.</p>
<p>OUR COMMENTS: Opportunity for bias, weakness and strength</p>	<p>Opportunity for bias: Twinning rates among women aged less than 20 are known to be low. The majority of births occur in the older age group and older women’s twinning rates may respond differently to folic acid fortification. Also there may be an association between parity, folic acid and twinning. Dietary intake of fortified foods may also be different in older women when compared to teenage mothers. By the same token, older mothers may be more likely to take supplements and the dose-response relationship between folate and twinning is not clear. Weakness/es: <i>Validity:</i> There may have been increasing accuracy of reporting to the register regarding plurality over the 11 year study period, resulting in an artificial increase in twinning rates. Authors acknowledge 3 reports on improved reporting accuracy of the US vital statistics system. <i>Results:</i> Zygosity was not taken into account. Strengths: The cohort was large as is required for rare outcomes. Authors’ discussion raises most limitations and strengths and the conclusions directly relate to the study findings.</p>

Evidence Summary	Lawrence JM, Watkins ML, Chiu V et al (2004). Food Fortification with Folic Acid and Rate of Multiple Births, 1994 - 2000. Birth Defects Research (Part A) 70:948-952.
Aetiology Observational study	
STUDY DESIGN Levels of evidence	Retrospective cohort study IIb
DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria	Patients (subjects): Mothers of 215820 live births (also defined as deliveries in some instances, terminology not clear) at Kaiser Foundation Health Plan Hospitals in Southern California between January 1, 1994 and December 31, 2000, as recorded in the perinatal services database. Outcomes: Primary outcome was multiple births/deliveries. Inclusion Criteria: Not further specified. Exclusion Criteria: Not specified.
VALIDITY: Methodology, rigour, selection	Ascertainment of exposure: US FDA mandated folic acid fortification of enriched cereal grain products at 140µg of folic acid per 100g of grain as of 1996. In 1998, full fortification was mandatory. Infants born between October 1, 1998 and December 31, 2000 were defined as exposed to fortification. Births between January 1, 1994 and September 30, 1998 were defined as unexposed. Description of the groups: The overall ethnic distribution was 45% Hispanic, 30% White, 12% Black, 3% Asian/Pacific Islander and 3% unknown. The number of births/deliveries increased slightly every year from 28,077 in 1994 to 32,562 in 2000. Over the entire study period, there were 3035 twin births/deliveries averaging to an actual rate of 14.1 multiple births/deliveries per 1000 live births/deliveries, but peaking in 2000 at 14.8/1000. The maternal age distribution was constant over the 7 year study period. The proportion of women using ART as determined by a random sample of women who had not filled a prescription for ovulation-inducing drugs was 7.9% with no significant changes over the years (chi square 4.04, p=0.67) Distribution of prognostic factors: The rate of multiple births/deliveries increased with maternal age from 8.6/1000 in women aged 19 or less to 19.6/1000 in women aged 35 years and over. Confounding factors: Confounders considered were pregnancies conceived after ovulation-inducing drugs, by IVF and women's ethnic background. Data analysis: Not specified.
RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate	Multiple birth/delivery rates: The rate of multiple births/deliveries in the exposed group was 14.5/1000 compared with 13.8/1000 in the unexposed group. If stratified by ethnicity a similar difference in rates was observed in Whites, Blacks and unknowns, but not in Hispanics and Asians/Pis. Among women with multiple births, 9.6% had filled a prescription for an ovulation-inducing drug (6.6% in 1994 to 14.9% in 2000). After excluding these, the rates for the 2 time periods remained constant at 12.7/1000. All the results presented are of descriptive nature only.
AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research	"While there has been a concern about the effect of fortification of cereal grains with (folic acid) on the multiple birth rates, (our) findings suggest that there has been no change in the multiple birth rates after controlling for the increase in the use of ovulation-inducing drugs."
OUR COMMENTS: Opportunity for bias, weakness and strength	Opportunity for bias: While authors state that one of their sources to identify multiple births was the ICD-9 CM coding system and even list the codes for multiple gestations with fetal losses, only live births were included in the analysis. There is a higher rate of mortality associated with twin pregnancies and all births, regardless of their status, should be included. No information on parity or zygoty was provided in this study. Exposure could not be ascertained reliably. Conclusions are drawn from point estimates that are presented without confidence intervals. No multivariate analysis is presented adjusting for ethnicity, ovulation-inducing drugs or ART. Weakness/es: Validity: The validity of this study is somewhat affected by an overall lack of accountability in the selection of subjects, data analysis and interpretation of results. Results: Authors clearly state that 215,820 live births were included in the study and that there were 14.1 multiple births per 1000 births. This would indicate 7 twin pairs per 1000 births. However in the tables, these numbers are referred to as 215,820 deliveries and 14.1 multiple deliveries per 1000 deliveries, indicating 14 twin pairs. Interpretation of findings is difficult if terminology is inconsistent. Strengths: A simple trend analysis performed by us on the crude yearly multiple delivery rates reveals a chi square for linear trend of 0.669 (p=0.41). It may therefore still be reasonable to assume that the rate of multiple deliveries was unchanged in the study group between 1994 and 2000.

Evidence Summary	Kucik J and Correa A (2004). Trends in twinning rates in Metropolitan Atlanta before and after folic acid fortification. Journal of Reproductive Medicine 49:707-712.
Aetiology Observational study	
STUDY DESIGN Levels of evidence	Retrospective cohort study IIb
DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria	Patients (subjects): Pregnancies resulting in livebirths between 1990 and 2001 in Atlanta, as recorded in the Georgia State Vital records. Outcomes: Primary outcome was twin pregnancies estimated as the number of twin births divided by 2. Inclusion Criteria: Not further specified. Exclusion Criteria: Higher order twins.
VALIDITY: Methodology, rigour, selection	Ascertainment of exposure: US FDA mandated folic acid fortification of enriched cereal grain products at 140µg of folic acid per 100g of grain as of 1996. In 1998, full fortification was mandatory. Infants born between 1998 and 2001 (4 years) were defined as exposed to fortification. Births between 1990 and 1995 (6 years) were defined as unexposed. Description of the groups: There were a total of 510,000 live births of which there were 7167 twin pairs (1.43%). Distribution of prognostic factors: The unadjusted rate of twinning increased with increasing maternal age (RR 2.08 (1.79, 2.42 for 35+ compared to <20 in the unexposed cohort and RR 3.50 (2.82, 4.35 for 35+ compared to <20 in the exposed cohort). There was an interaction between age and ethnicity for both periods. Blacks had a higher rate of twinning than Whites but in women aged 35+ the rate of twinning was higher in Whites. Women who had at least 1 previous child were 2.75 times more likely to have twins than primiparous women in both time periods. Because of this interaction, regression analysis was stratified for age and adjusted for parity and ethnicity. Confounding factors: Confounders considered were maternal age (in 5 categories), parity (0, 1+) and ethnicity (White or Black). Data analysis: Multivariate analysis was performed using Poisson regression (RR and 95% confidence intervals), comparing pre and post fortification time periods. In addition, Mantel Haenzel chi square tests for trend were performed for annual rates of twinning during pre fortification (1990-1996).
RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate	Twinning rates: After adjusting for parity and ethnicity, rates of twinning did not significantly increase in the post fortification period or the transitional period (1996-1997) in women aged 29 years or less. RR for women aged 30-34 was 1.23 (1.10, 1.38) and for women aged 35+ it was 1.49 (1.30, 1.72). The overall rate of twinning increased from 13.3 per1000 births during pre fortification to 15.7 per 1000 during the post fortification period (RR1.18 (1.12, 1.24)). Significant increases in the rate ratios for twinning occurred during the post fortification period for women aged 30-34 (RR 1.23 (1.10, 1.38)) and women aged 35+ (RR 1.49 (1.30, 1.72)). No differences in twinning rates were observed in the younger women. Our Comment: The rate ratios referred to in the text do not match those in the tables. Annual rates by maternal age group showed significant increasing trends for women aged 30-34 and 35+ (p<0.01) even during the pre-fortification time period. Authors report an average annual increase of twinning rates for women aged 30-34 years of 5.5% pre and 2.0% post and for women aged 35+ there was an annual increase in twinning of 5.0% pre and 0.1% post. However, the annual increase derived from 4 point estimates between 1998 and 2001 followed no linear trend (Figure 2) and to combine these into an average figure is misleading. No increased trend in twinning was observed in the younger women. Our Comment: Authors' first finding of significant increases in the older women in rate ratios between pre and post fortification periods (23%) does not make sense in the context of this second finding in which the increases appear to be limited to the prefortification period. The proportion of twins among live births was higher post fortification but the main annual increases occurred during the optional fortification period of 1996 and 1997.
AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research	“Although twinning rates have risen in metropolitan Atlanta during the past decade, the increases have occurred primarily among older women and appear to be limited to the pre fortification period. Twinning rates in other age groups have shown no increases during the pre or post fortification period.” “Increasing trends of twinning were observed only in women older than 30 years, but these trends began prior to folic acid fortification and reached a plateau in recent years.” It is not clear from the presented data and analysis how authors can justify these conclusions.
OUR COMMENTS: Opportunity for bias, weakness and strength	Opportunity for bias: Only live births were included in the analysis. There is a higher rate of mortality and prematurity associated with twin pregnancies and all births regardless of their status should be included. No information on parity or zygosity was provided in this study. Exposure could not be ascertained reliably. ART could not be accounted for. Weakness/es: Validity: The validity of this study is greatly affected by an overall lack of accountability in their data analysis and interpretation of results. Rate ratios quoted in the text do not necessarily match those in the tables, comparisons discussed in the text are not always those presented in the tables and the abstract presents odds ratios in their results section, that are not in the body of the paper. Results: The results section begins with a nice presentation of the cohort and describes an interaction, which is then accounted for appropriately. The multivariate poisson regression reveals age stratified rate ratios adjusted for parity and ethnicity and compares the pre and post fortification periods. However, any analysis following this including an annual trend analysis of twinning rates do not make sense, including their interpretations. The findings of this study are greatly affected by its limitations in accountability and must be presented together with its limitations.

Evidence Summary	Waller DK, Tita ATN, Annegers JF (2003). Rates of twinning before and after fortification of foods in the US with folic acid Paediatric and Perinatal Epidemiology 17:378-383.
Aetiology Observational study	
STUDY DESIGN Levels of evidence	Retrospective population- based cohort study IIb
DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria	Patients (subjects): De-identified birth data from 1996 to 1998 as registered on computersied Texas birth certificates. Outcomes: Primary outcome was singleton or multiple births. Secondary outcome was zygosity as estimated by the Weinberg method (twice the number of unlike-sex twins = dizygotic). Inclusion Criteria: All births resulting from a conception between January 1, 1996 and December 31, 1998. Exclusion Criteria: Twins that could not be linked to a co-twin.
VALIDITY: Methodology, rigour, selection	Ascertainment of exposure: US FDA mandated folic acid fortification of enriched cereal grain products at 140µg of folic acid per 100g of grain as of 1996. In 1998, full fortification was mandatory. Exposure was not defined further. Twinning rate ratios were analysed in nine 4-month groups based on data of conception. Description of the groups: There were a total of 1,003,207 conceptions resulting in birth during the study period. Of these, 12,687 were twin pairs and 990,520 were singletons. 8008 of the twin pairs were characterised as DZ and 4679 as MZ. Distribution of prognostic factors: There was a slightly increased rate ratio of twinning over time. Rate ratios for twinning increased with increasing maternal age, parity and maternal education. Rate ratios of twins were increased in Blacks and decreased in Hispanics and Asians when compared to Whites. Confounding factors: Maternal age, parity, ethnicity, maternal education and season of conception (Jan-Aug and Sep-Dec) were adjusted for in the multivariate analysis. Data analysis: Rates of twinning were modelled by Poisson regression and generated rate ratios with 95% confidence intervals. The analysis included 2 time categories (1997 compared to 1996 and 1998 compared to 1997).
RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate	There was no association between time periods and twinning in the fully adjusted model. RR was 1.024 (0.98, 1.07) for 1997 compared to 1996. This equates to a non-significant 2.4% increase in the rate of twinning. For 1998 when compared to 1997, the RR was 1.046 (1.00, 1.06), which is a non-significant increase of 4.6%. Risk ratios for DZ twinning: In the fully adjusted model, increased risk ratios for twinning were observed with increasing maternal age (RR 4.47 (3.95, 5.06) for mothers aged 35 years and over). No increased risk for DZ twinning was observed with parity or mother's educational level. There was a weak association between season of conception and DZ twinning (Sep-Dec), RR 1.09 (1.04, 1.14). When compared to Whites, Blacks had a 42% increased risk for DZ twins, Hispanics were 35% less likely and Asians were 51% less likely to have DZ twins. Risk ratios for MZ twinning: In the fully adjusted model, increased risk ratios for twinning were observed with increasing maternal age (RR 1.46 (1.26, 1.69) for mothers aged 35 years and over). No increased risk for MZ twinning was observed with season of conception. There was a weak association between increasing parity and mother's educational level and MZ twinning. When compared to Whites, Blacks had a 15% increased risk for MZ twins, Asians 27% and Hispanics were 11% less likely to have MZ twins. Risk ratios for all twinning: In the fully adjusted model, increased risk ratios for twinning were observed with increasing maternal age (RR 2.89 (2.64, 3.17) for mothers aged 35 years and over). Increasing parity slightly reduced the risk of having twins, RR 0.94 (0.90, 0.97). There was a weak association between season of conception (Sep-Dec) and mother's educational level. There was a weak association between increasing parity and mother's educational level and MZ twinning. When compared to Whites, Blacks had a 34% increased risk for twins and Asians and Hispanics were 26% less likely to have twins.
AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research	"The modest yearly increases (in twinning) in this study are most consistent with the ongoing increase in twinning of 1-4% per year observed in the US between 1991 and 1995. This increase has been attributed to the increased use of ovulation-inducing drugs and secondarily to other assisted reproductive technologies." Authors estimate that if folic acid fortification of foods increased the risk of twinning by 40% (as suggested by other studies) but affected only those women who were not taking multivitamins, a 25% total increase in twinning should be observed after fortification.
OUR COMMENTS: Opportunity for bias, weakness and strength	Opportunity for bias: Exposure could not be ascertained reliably. Only livebirths were included. There is a higher rate of mortality and preterm births associated with twin pregnancies and if not all births at 20 weeks or later, regardless of their status, were included, this may have affected the outcome. Rates of ART could not be accounted for. Authors quote results from an Italian study estimating that 5-10% of twins are due to ART. The applicability of these results to the population of Texas is not clear. However, pregnancies conceived in this way are expected to raise the rate of twinning, which has not happened over the study period. Weakness/es: <i>Validity:</i> The validity of the birth certificate reporting has not been described. There may be under reporting of the twin variable on birth certificates for individual children and this in turn may be inconsistent across time and place. <i>Results:</i> The study time was relatively short (3 years) and may not have been long enough to examine trends in twinning following fortification, which only became mandatory in 1998, the last year of the study. The study by Lawrence et al in 2004 showed that serum folate levels of pregnant women in a large US maternity hospital continued to rise beyond 1998 and 1999. Strengths: This study was carefully designed and conducted and the results are clearly presented. The study provides valuable trend data for 1996-1998 on rates of twinning in Texas, taking into account a number of maternal variables. The increases in twinning rates observed were small and may have been due to chance. It would be advisable for authors to choose more careful terminology in their abstract, as their representation of results may be misleading.

Evidence Summary	Shaw GM, Carmichael SL, Nelsen V et al (2003). Food Fortification with folic Acid and Twinning among California Infants American Journal of Medical Genetics 119A:137-140.
Aetiology Observational study	
STUDY DESIGN Levels of evidence	Retrospective population-based cohort study IIb
DESCRIPTION: Patients (subjects), Outcomes, Inclusion & Exclusion Criteria	Patients (subjects): All livebirths and fetal deaths at 20 weeks gestation or more in "selected" California counties between January 1990 and December 1999 as recorded on standard birth and death certificates. Outcomes: Primary outcome was singleton or twin births. Inclusion Criteria: Not further specified. Exclusion Criteria: Not specified.
VALIDITY: Methodology, rigour, selection	Ascertainment of exposure: US FDA mandated folic acid fortification of enriched cereal grain products at 140µg of folic acid per 100g of grain as of 1996. In 1998, full fortification was mandatory. Exposure was not defined further. Twinning rate ratios were analysed by year. Description of the groups: There were a total of 2,663,723 live and stillbirths during the study period. Of these 60,216 were twin births and 2,601,175 were singletons. Distribution of prognostic factors: There was an increased rate of twinning over time, with increasing maternal age and with parity. Rate ratios of twins increased for Non-Hispanic Whites and "Others" but not for Hispanic-Whites. This was presented as proportion of twins of all births and no confidence intervals were presented Confounding factors: Year, maternal age, ethnicity and parity were adjusted for in the multivariate analysis. Data analysis: Rate of twinning was modelled by Poisson regression and generated rate ratios with 95% confidence intervals. Comparisons were also made between January 1, 1990 through September 30, 1998 and October 1, 1998 through December 31, 1999 (fortification period).
RESULTS: Generally favourable or unfavourable, specific outcomes of interest, estimate of experimental effect and precision if appropriate	Twinning associated with fortification: Analysis was simultaneously adjusted for age, parity, ethnicity, sexes of twin pair (as surrogate for zygosity), year of birth and fortification period. This resulted in a relative risk ratio for twinning associated with fortification of 1.00 (0.95, 1.04). Examination of a possible interaction between maternal age and fortification period returned no significant results.
AUTHOR(S) CONCLUSIONS: Limitations, implications for practice and research	"Our results do not suggest an elevated twinning prevalence associated with folic acid fortification of the US food supply among women delivering in California." Authors discuss the study's limitations relating to their inability to assess dietary intake, exact time of fortification and use of fertility drugs. However, authors state they did not observe an effect of fortification on twinning rates in 20-24 year old mothers who would have been unlikely to use ART.
OUR COMMENTS: Opportunity for bias, weakness and strength	Opportunity for bias: Rates of IVF pregnancies and/or use of ovulation-inducing drugs could not be accounted for. However, this was not expected to affect an already negative result. It was not possible to assess the exposure adequately. This may have biased the finding towards the null. Multivariate analysis was adjusted for 2 highly correlated variables, namely year of birth and a derivative (before and after fortification). This may have resulted in unreliable risk ratios and confidence intervals. Weakness/es: <i>Validity:</i> The validity of the birth and death certificate reporting has not been described. There may be under reporting of the twin variable on birth certificates for individual children and this in turn may be inconsistent across time and place. The time period analysed (1990-1999) may not have been appropriate to examine rates of twinning and draw conclusions about an association between folic acid fortification and twinning. Fortification was introduced in 1996 but was not mandatory until 1998. Over the 11 year study period, only the two last years looked at data following fortification. <i>Results:</i> The total number of twins as reported were 60,216. However, table 1 presents twins as pairs which individually add up to 59,330. Authors do not account for this discrepancy of almost 900 twins. Strengths: This was a large cohort as is appropriate for rare outcomes and important trend data in twinning rates for California are presented. These data will be able to be used in future studies examining trends in twinning following food fortification with folic acid.