# Folic acid and risk of twinning: a systematic review of the recent literature, July 1994 to July 2006

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## ABSTRACT

**Objective:** To assess the evidence of an association between periconceptional folic acid (FA) supplementation or fortification of foods with FA and the risk of twinning, using the Food Standards Australia New Zealand (FSANZ) framework for assessing evidence when substantiating nutrition, health and related claims on foods.

**Data sources:** The Cochrane Library Database, MEDLINE, MEDLINE in Process, EMBASE, PubMed National Library of Medicine, and CINAHL were searched to identify systematic reviews and primary intervention and observational studies published from 1 July 1994 to 7 July 2006.

**Study selection:** One prospective and five retrospective cohort studies that assessed the rate of twinning in populations exposed to FA through supplementation, and six retrospective registry-based cohort studies examining twinning rates after fortification of foods with FA.

**Data extraction:** Two reviewers appraised eligible studies and evaluated data independently.

**Data synthesis:** The best maximal risk estimates of twinning after FA supplementation were an adjusted odds ratio (adjOR) of 1.26 (95% CI, 0.91–1.73) for preconceptional supplementation and dizygotic twinning and an adjOR of 1.02 (95% CI, 0.85–1.24) for overall twinning. Data from four FA fortification studies in the United States that allowed for calculation of an annual percentage increase showed a maximal annual increase in twinning rates of 4.6%.

**Conclusions:** Overall, under the FSANZ framework, there is possible evidence for a relationship between periconceptional FA intake and increased twinning. To support this tentative relationship, more well designed, long-term follow-up studies are needed in places where fortification with FA has been introduced, focusing on dose–response and obtaining accurate data on infertility treatments.


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Food Standards Australia New Zealand (FSANZ), a binational independent statutory authority, is currently considering mandatory fortification of food with folic acid (FA) to reduce the risk of neural tube defects (NTDs) in pregnancy. The primary capability of FA to prevent NTDs is well recognised, and is documented in a Cochrane review.[1] However, since a 1994 report of a positive association between multivitamins containing FA (0.8 mg) and an increase in the rate of twinning,[2] there has been ongoing investigation of this subject.

There are obvious public health implications of an increasing prevalence of twins, related to both infant and maternal mortality and morbidity.[3,4] Local, routinely collected data on all twins show preterm delivery in 53% in 2001 and 2002,[5] and a systematic review reported a similar figure of 46%.[6] In 2000, low birthweight (less than 2500 g) was recorded in about 50% of all twins, compared with 5% in singletons.[7] As well as the neurological sequelae in children, which are the consequences of prematurity and low birthweight, there are psychological and economic effects on families, all of which have the potential to affect public health in the long term.[8]

Rising rates of twinning have been observed all over the Western world, with the prevalence in Australia rising from 9.0/1000 in 1977 to 15.1/1000 in 2000.[7] The rate of twinning is particularly high in women aged 35–39, and a quarter to a third of the overall increase is attributed to increasing maternal age.[9] The remaining increase has been attributed to ovarian stimulation and assisted reproductive technologies (ART).

Twin pregnancies have extra requirements for micronutrients and vitamins, including FA, to facilitate epigenetic modifications (eg, DNA methylation) necessary for cell differentiation,[10] and to prevent DNA strand breakage.[11] Twins are known to miscarry early in pregnancy,[8] and low FA levels could reduce their chances of survival. FA and other micronutrients may "rescue" twins about to abort by altering the methylation expression profile to enhance cell differentiation and DNA repair. Further, high folate levels have recently been found to increase the likelihood of a twin birth after multiple embryo transfer.[12]

The Cochrane review, published in 2001,[1] presented a meta-analysis of the 1994 study and two earlier randomised trials,[13,14] both of which investigated the frequency of recurrent NTDs in a high-risk population. The meta-analysis found a non-significant but persistent association between the intake of FA and multivitamins, and twinning (adjusted odds ratio [adjOR], 1.40; 95% CI, 0.93–2.11).[1] Our article is a systematic descriptive review of all primary studies published since that time that specifically examine periconceptional FA supplementation, or fortification of foods with FA, and the association with twinning.

## METHODS

We sought answers to two research questions:

- Does periconceptional supplementation with FA increase the risk of twinning? and
- Has fortification of food with FA increased the risk of twinning?

Details of the search strategy, inclusion criteria and categorisation strategy are shown in Box 1.

## RESULTS

### Supplementation studies

Six observational studies assessed the rate of twinning in populations where supplementation with FA was the exposure (Box 2).[16-21] Two of these studies used data from the Swedish Medical Birth Registry, one examining the years from 1995 to 1999,[21] and the other, a more updated sample from 1995 to 2001.[19] We have included both studies in this review for completeness of reporting.

***Intake of FA from supplementation:*** There was variation in the dose of FA that women were exposed to, ranging from a recommended dose of 0.4 mg/day to a reported 9 mg/day (Box 2). One

**1 Search and categorisation strategy**

| | |
|---|---|
| Databases searched | The Cochrane Library, Issue 4, 2005; MEDLINE (OVID); MEDLINE in Process (OVID); PubMed National Library of Medicine; CINAHL (OVID); EMBASE (OVID). |
| Search key words | Broad searches were performed including MeSH headings where relevant: (folic acid/ OR exp pregnancy OR multiple/ OR exp twins/ OR twin$.tw OR multiple.tw) AND (folic.tw or folate.tw). |
| Search dates | 20 April 2005 (including an update on 7 July 2006), limited to publications dated from 1 July 1994. Studies published prior to this date were included in a Cochrane review. MEDLINE in Process was last checked on 7 July 2006. |
| Other information sources checked | Reference lists of included studies were searched to identify additional sources of information. |
| Method of determining if references should be reviewed | Primary observational or experimental studies directly related to the research questions and involving humans were included.<br>Titles and abstracts were provisionally classified and full text articles obtained for critical appraisal. |
| Results for inclusion | 953 records were initially retrieved and 12 studies met the inclusion criteria (one of which was published in English and later also in Hungarian. We only included the English language publication). |
| Categories of studies retrieved | Six observational studies where supplementation with folic acid (FA) was the exposure.<br>Six retrospective cohort studies where FA fortification was the exposure. |
| Method for assessing and interpreting evidence | Each study was critically evaluated by two independent reviewers for subject inclusion criteria, measured outcomes, study validity and conclusions, and opportunity for bias, weakness and strength.<br>Particular attention was also given to maternal age, use of assisted reproduction and other treatments for infertility, and whether any measure of zygosity had been made. |
| Method for classifying totality of evidence | The Food Standards Australia New Zealand framework for assessing evidence when substantiating nutrition, health and related claims on foods was used.[15] Evidence is assessed as: convincing; probable; possible; or insufficient. ◆ |

study examined the rate of twin pregnancies in patients with sickle cell anaemia who took 1 mg of FA daily for therapeutic reasons, and additional FA-containing multivitamins perinatally (although this term was not further defined).[16] This was a report from the United States, where mandatory fortification of flour with FA existed for part of the study period. Four studies used the recommended dose of 0.4 mg/day. There was also variation in the background timing and use of FA in the different populations studied. The two studies from Sweden reported extremely low periconceptional FA use — 0.6% in the study examining births during 1995–1999,[21] and 1.2% in the later study which included births in 2000 and 2001.[19] The Hungarian study was on births from 1995 to 1996 and data presented indicated 5% preconceptional and 56% periconceptional use.[18] Preconceptional use was 6% and periconceptional use was 21% in the Norwegian study,[17] noting that the authors estimated underreporting of FA use of 45%. The prospective design of the Chinese study[20] resulted in periconceptional use of FA of 53%.[25] Low periconceptional use of FA impacted on statistical power, especially in the study from Hungary where there were only 20 twin pairs whose mothers had taken FA before pregnancy.[18]

The method of determining the proportion of women who took supplements in four of the retrospective studies included asking them to remember what they had taken (with a memory aid),[18] and examination of medical records and birth registry forms.[17,19,21] It was shown in the most recent study that recording of FA supplementation was extremely inaccurate (45% misclassification).[17] This has implications for the interpretation of results in studies where there was no compliance check. The prospective design of the Chinese study[20] allowed for accurate reporting of FA use.

***Maternal age, ART and zygosity:*** Five of the six supplementation studies either stratified or adjusted for maternal age in their analyses, and did not target particular age groups. The study by Ballas et al did not report on maternal age.[16]

Two studies accounted for ART in convincing ways. The Norwegian study assessed the underreporting rate of in-vitro fertilisation (IVF) in the birth records by contacting fertility clinics in neighbouring countries, from which they directly established the number of women who had had treatment outside of Norway.[17] It found that at least 13% of IVF pregnancies were not reported in Norway, and presented an adjusted analysis. In the Chinese study,[20] there was no opportunity for women to have infertility treatment.

Number of years of self-reported involuntary childlessness was used as a proxy in the Swedish studies,[19,21] and the Hungarian study recorded use of the ovulation-inducing drug clomiphene.[18] The validity of these methods of accounting for infertility treatment has been questioned,[22,23] and an independent critique of the Swedish data, after careful examination of vital records, showed that underreporting of ART has been a serious cause of misclassification bias.[24] Use of ART was not reported in the study of patients with sickle cell anaemia.[16]

Data on whether twins were the same sex or unlike sex were used to estimate zygosity in two studies.[17,20] One study singled out unlike-sex twins for analysis and the final figure relates to dizygotic (DZ) twins only.[19] Two studies did not examine zygosity.[18,21] The US study reported that all six twin pairs were DZ, but did not present an explanation of how this was determined.[16]

***Summary of supplementation results:*** Three of the six observational studies examining an association between FA supplementation had major limitations in study design and interpretation of results.[18,19,21] One study reported a high unadjusted rate of twinning associated with FA (5.4%) when compared with a population rate of 0.34%–1.1%, but the report was brief and had small numbers of participants (6 twin pairs in 112 pregnancies).[16]

**2 Cohort studies examining rates of twinning after periconceptional folic acid supplementation**

| Authors, year, place | Study population | Dose, ascertainment and preconceptional use of folic acid | Specific issues addressed | Relevant findings | Comments |
|---|---|---|---|---|---|
| Ballas et al[16]<br>2006<br>United States | 112 pregnant African American women with sickle cell anaemia, 1981–2002. Terminations and miscarriages included.<br>6 twin pregnancies (5.4%), all DZ. | 1 mg of FA daily to assist with erythropoiesis, plus "perinatal" multivitamins containing 0.4 mg FA.<br>Mandatory flour fortification in force for some of the study period. | Beta-globin haplotypes in twin pregnancies. | The only reported finding is the rate of DZ twin pregnancies (5.4%). Authors offer a published population background rate of 0.34%–1.1% for Black and Caucasian pregnant women in the US as comparison.<br>Beta-globin haplotypes showed that women with twin pregnancies did not come from a particular ethnic group. | Very basic study on a small number of pregnancies.<br>High doses of FA.<br>No information on ART.<br>Effect of perinatal multivitamins not described.<br>No statistical analysis offered. |
| Vollset et al[17]<br>2005<br>Norway | 176 042 women who gave birth from 1 December 1998 to 31 December 2001.<br>3154 twin pregnancies (1.79%). | 0.2 or 0.4 mg tablets available; recommended dose 0.4 mg/day.<br>*Ascertainment:* Birth certificate.<br>Preconceptional FA use: 6%.<br>Periconceptional FA use: 21%. | Provided estimates of underreporting:<br>45% misclassification of FA intake;<br>12.7% misclassification of IVF use. | After adjusting for age, parity and underreporting of FA and IVF use, there were no significant associations between FA intake and risk of overall twinning (adjOR, 1.02; 95% CI, 0.85–1.24).<br>Non-significant differential effect seen for same-sex (classified as MZ) twins (adjOR, 0.70; 95% CI, 0.35–1.40) and unlike-sex (classified as DZ) twins (adjOR, 1.26; 95% CI, 0.91–1.73). | Well executed study with a number of adjustments highlighting the importance of confounding by ART.<br>Independent effect of multivitamins also studied. |
| Czeizel et al[18]<br>2004<br>Hungary | 38 151 women who gave birth to a child without congenital abnormalities between 1980 and 1996.<br>395 twin pregnancies (1.04%). | Estimated dose of 3, 6 or 9 mg daily.<br>*Ascertainment:* Birth certificate.<br>Reported overall preconceptional use said to be 32%, but data presented indicate only 5% use.<br>Periconceptional FA use: 56%. | Differentiation between preconceptional and postconceptional use of FA.<br>Clomiphene use. | Use of clomiphene was not increased in mothers of twins.<br>Adjusted likelihood of a twin birth with preconceptional use of FA was not significantly increased (adjOR, 1.60; 95% CI, 0.95–2.69) and with postconceptional use, there was a weak association (adjOR, 1.38; 95% CI,1.04–1.82). | ART not accounted for satisfactorily. See also Berry.[22]<br>Based on small numbers of twins.<br>Very high doses of FA.<br>No data on zygosity.<br>Independent effect of multivitamins also studied. |
| Kallen[19]<br>2004<br>Sweden | 1 July 1995 to 31 December 2001.<br>6953 women who reported use of FA and 8676 women who had unlike-sex twins, of whom 232 reported use of FA, compared with 576 873 women who gave birth. | 0.4 mg FA tablets available; recommended dose, 0.4 mg/day<br>*Ascertainment:* Birth certificate.<br>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. These women and those who reported use of ovulation induction or were marked by midwife as having had ART were excluded. | Adjusted for age, parity, smoking.<br>adjOR for DZ twinning (unlike sex), 1.71 (95% CI, 1.21–2.42) in 2001–2002. Exact data used to obtain this result not shown in report. | There was considerable lack of accountability and potential for misclassification of ART. See also Berry and Kihlberg[23] and Berry et al.[24] |
| Li et al[20]<br>2003<br>China | A cohort of women with 240 519 singletons and 1496 multiple births (0.62%) sourced from the FA community intervention program between October 1993 and September 1995, who delivered before 31 December 1996. | Women were asked to take 0.4 mg of FA/day, starting at time of registration (even if not pregnant) until end of first trimester. Intake was recorded monthly.<br>52.5% of women took FA and 47.5% did not. | No access to ART or over-the-counter multivitamins.<br>Young population with a mean age of around 25 years.<br>Three 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 and the risk of a multiple pregnancy (overall RR, 0.91; 95% CI, 0.82–1.00). | Well executed study. Women had no access to ART, a major confounder.<br>Good data on FA exposure.<br>A limitation is that Asian populations have low rates of twinning, especially DZ. |
| Ericson et al[21]<br>2001<br>Sweden | 2569 women (including 72 twin pairs) who reported use of FA out of a total of 442 906 deliveries between 1995 and 1999. | 0.4 mg FA tablets available; recommended dose, 0.4 mg daily.<br>*Ascertainment:* Birth certificate.<br>Preconceptional FA use: 0.6%. | Years of involuntary childlessness as surrogate for ART. | With women reporting unwanted childlessness excluded, odds ratio for FA and twins, 1.45 (95% CI, 1.06–1.98).<br>Significant RR data also presented for an effect on DZ twinning, but unable to determine how these were derived. | There was considerable lack of accountability and potential for misclassification of ART. See also Berry et al.[24] |

adjOR = adjusted odds ratio. ART = assisted reproductive technologies. DZ = dizygous. FA = folic acid. IVF = in-vitro fertilisation. MZ = monozygous. RR = rate ratio. ◆

After consideration of major confounders and potential for misclassification, the best risk estimate for preconceptional use of FA and DZ twinning[17] had an adjOR of 1.26 (95% CI, 0.91–1.73). Results from this study showed differential effects for monozygotic (MZ) and DZ twins, with an adjusted odds ratio of 0.70 (95% CI, 0.35–1.40) for MZ twins and 1.02 (95% CI, 0.85–1.24) for all twins.

The point estimate in the Chinese study was a relative risk of 0.91 (95% CI, 0.82–1.00).[20] A limitation to the generalisability of this study is 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.[20]

### Fortification studies

Six retrospective cohort studies analysed twinning rates before and after FA fortification (Box 3) — five were from the US, where fortification of enriched cereal grain products has been mandatory since 1998.[27-31] Before 1998, there was an optional fortification period from 1996. The sixth study originated from Chile, where fortification of flour became mandatory in 2000.[26]

***Intake of FA from food fortification:*** Defining exposure to FA adequately in population studies of food fortification is difficult. There are inconsistencies in the definition of the exposed US cohorts in relation to the optional fortification period, 1996–1998. Box 3 shows that two studies included the optional fortification period in their unexposed cohort,[28,31] one study defined it as "exposed to FA",[27] one study excluded it,[29] and one study looked only at this optional period.[30] Further to the variation in timing, there was some overlap in the study populations, with one study examining the whole of the US National Vital Statistics System, but only selecting nulliparous women aged 16–19 years;[27] three reports examining data on women of all ages, but from single states;[29-31] and one selecting women who gave birth within a particular health care plan.[28] None of the studies were able to determine whether women who delivered twins had been exposed to the same amount of fortified food as the women who delivered singletons, or if they were more likely to have taken FA supplements.

***Maternal age, ART and zygosity:*** Stratification for maternal age found no effect in one study.[31] Another study found no change in maternal age distribution across the study years, and did not present any maternal-age-related results;[28] two other studies adjusted for maternal age in regression analyses;[29,30] one only looked at teenage mothers;[27] and one made no mention of the maternal age distribution.[26]

The three earliest studies did not take ART into account in their analyses, but they recorded no marked increase in twinning over the study period.[29-31] Two of these were published in 2003,[30,31] and the study period after fortification may have been too short to detect any sustained or significantly increased change in twinning after fortification. The two most recent studies from the US both accounted for ART and ovulation induction.[27,28] The Chilean study did not account for ART.[26]

Only one study accounted for a measure of zygosity.[30]

***Summary of fortification results:*** Trend data from the studies on FA fortification of foods allowed for calculation of annual percentage increase after the exposure period. This was provided in all but two studies, one of which had concluded that there was no increase in the twinning rate (relative risk, 1.00; 95% CI, 0.95–1.04).[31] The Chilean study analysed data from 3 years and 15 years before fortification and 3 years after fortification, but the study's comparisons had methodological concerns.[26] One US study found a low maximal percentage annual increase for women aged 30 years or over (2%), but no increase for younger women.[29] The other reports indicate that there is, at most, a 2.4% to 4.6% annual increase in twinning rates across all ages.[27,28,30]

## DISCUSSION

Overall, under the FSANZ framework for assessing evidence when substantiating nutrition, health and related claims on foods,[15] there is possible evidence, rather than probable or convincing evidence, for a relationship between periconceptional FA intake and increased twinning. The results of the two observational studies that account for infertility treatment adequately and refer to a supplement containing 0.4 mg of FA do not provide any conclusive evidence for a relationship between FA and increased rates of either overall or DZ twinning.[17,20]

It is important to weigh up the balance of a beneficial reduction in prevalence of NTDs versus a potentially harmful increase in the twinning rate before implementing a public health policy to fortify foods with FA. None of the studies we reviewed identified an increase as great as that proposed by the Cochrane review (40%), which modelled an extra 60 twin pairs per 10 000 pregnancies,[32] around half of whom will require neonatal intensive care. However, there is a possibility that perinatal outcomes for twins "rescued" by FA may be different from ART twins. In this case, the data above[32] may not have resulted in an appropriate estimate of a public health impact.

The well executed large study from Norway, considered the most valid supplementation study, found a non-significant increase in twinning of 26% (adjOR, 1.26; 95% CI, 0.91–1.73),[17] while none of the ecological studies showed a greater than 4.6% annual increase in twinning.[30]

It could be argued that the dose of FA obtained from fortification alone may be lower than that needed to see an effect on twinning, but a woman's exact daily dose of FA cannot be determined, as it depends on what she eats (0.14 mg per 100 g of grain, as well as natural sources in foods) and whether she also takes supplements. Between 1995 and 2003, about 25%–30% of non-pregnant women of childbearing age in the US were taking multivitamin supplements containing FA, and in 2004 this rose to 40%.[33,34] Whether the FA came from fortification alone, or from supplementation as well, the result in 1999–2000 was enough to see a 27% decline in NTDs[35] and a Centers for Disease Control and Prevention report showed that, by the year 2000, the median red blood cell (RBC) level of FA (a surrogate measure for dose) had reached the target level of 220 ng/mL among women of childbearing age in the US.[36]

RBC levels of FA appear to plateau fairly soon after fortification,[28,37] 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 RBC levels of FA and increases twinning rates.

The data reviewed here have all come from observational studies of varying quality and one must apply caution when drawing inferences from their results. However, our findings are supported by a recent review of FA and selected birth outcomes in their discussion on twinning, which cited five of the 12 studies reviewed here.[38] Further, there is no new evidence from interven-

**3 Retrospective registry-based cohort studies examining twinning rates following fortification of food with folic acid**

| Authors, year and place | Study population | Time frame* | Relevant findings | Comments |
|---|---|---|---|---|
| Nazer et al[26]<br>2006<br>Chile | All live and still births as recorded in the maternity database of the University of Chile Hospital.<br>Singletons: 63 700.<br>Twin pairs or triplets: 619 (0.98%). | ***Unexposed:*** 1983–1997 (49 115 singletons, 451 twin pairs) and 1998–2000 (7360 singletons, 73 twin pairs).<br>***Exposed:*** 2001–2004 (7225 singletons, 95 twin pairs). | Twinning was not presented as pairs, but calculation of TR was possible based on raw numbers (data on triplets were only available for the post FF period):<br>TR was 9.2/1000 for 1983–1997, 9.9/1000 for 1998–2000 and 13.1/1000 for 2001–2004 (after FF).<br>Relative risk ratio for twinning in the 3 years after FF compared with the 3 years before FF was 1.32 (95% CI, 0.98–1.79; $P = 0.07$). | Useful data on before and after FF.<br>Analysis based on number of twins per births rather than the number of twin pairs per pregnancies. This resulted in the over-interpretation of results.<br>Increase in TR after FF was only significant if the large number of births in the 15 years between 1983 and 1997 were included in the comparison (as authors had done). |
| Signore et al[27]<br>2005<br>US National Vital Statistics System | Nulliparous women aged 16–19 years with a live birth or fetal death.<br>Singletons: 3 362 245.<br>Twin pairs: 25 065 (0.74%). | ***Unexposed:*** 1 January 1990 to 30 November 1996.<br>***Exposed:*** 1 December 1996 to 31 December 2000. (optional fortification period included). | Constant TR before FF followed by increase of 2.4% per year after FF.<br>No zygosity data. | Well executed study accounting for ART confounder.<br>May not be able to extrapolate to all maternal ages. |
| Lawrence et al[28]<br>2004<br>Southern California | Women with a live birth within the Kaiser Permanente Health Plan.<br>Singletons: 215 820.<br>Twin pairs: 3035 (1.39%). | ***Unexposed:*** 1 January 1994 to 30 September 1998 (optional fortification period included).<br>***Exposed:*** 1 October 1998 to 31 December 2000. | TR was 13.8/1000 before FF and 14.5/1000 after FF, but use of ovulation-inducing drugs was 6.6% in 1994 and 14.9% in 2000, and ART use did not change. After excluding women using ovulation induction, TR was 12.7/1000 in both periods.<br>No zygosity data. | Well executed study, but only basic statistical analysis.<br>Able to examine ovulation-inducing medications. |
| Kucik et al[29]<br>2004<br>Atlanta, Georgia | Women with a live birth.<br>Singletons: 495 666.<br>Twin pairs: 7167 (1.43%). | ***Unexposed:*** 1 January 1990 to 31 December 1995 (optional fortification period excluded).<br>***Exposed:*** 1 January 1998 to 31 December 2001. | TR was 13.3/1000 before FF and 15.7/1000 after FF.<br>Comparison of two periods (excluding intervening years) showed unchanged TR in women aged less than 30 years in both time periods.<br>Annual increase in TR after FF was 2% in women aged 30–34 years and 0.1% in those aged 35+.<br>No zygosity or ART data. | Lack of accountability around methods and interpretation of results. |
| Waller et al[30]<br>2003<br>Texas | Women with a live birth resulting from a conception in the defined time period.<br>Singletons: 990 520.<br>Twin pairs: 12 687 (1.26%). | No pre–post analysis — trend data only: 1 January 1996 to 31 December 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.<br>Zygosity modelled — some increase in MZ (7%), but not DZ twins.<br>No ART data. | Useful trend data on TR, but study period not long enough. Conceptions for deliveries before October 1998 occurred before FF; only 3 months of post FF data. |
| Shaw et al[31]<br>2003<br>California | Women with a live birth or fetal death.<br>Singletons: 2 601 175.<br>Twin pairs: 29 665 (1.13%). | ***Unexposed:*** 1 January 1990 to 30 September 1998 (optional fortification period included).<br>***Exposed:*** 1 October 1998 to 31 December 1999. | After adjustment for year, age, ethnicity, parity, sex of twin (surrogate for zygosity), no increase in TR (not shown) associated with FF.<br>Relative risk ratio for twinning associated with fortification was 1.00 (95% CI, 0.95–1.04).<br>No ART data. | Valuable 11-year trend data on TR, but given that there was an annual increase in TR, the single FF time point may not have been enough to detect a sustained and significant increase in TR caused by FF. |

* The United States Food and Drug Administration mandated folic acid fortification of enriched cereal grain products at 140 μg of folic acid per 100 g of grain as of 1996 (designated "optional fortification period" above). In 1998, full fortification was mandatory. Chile mandated folic acid fortification of flour with 200 μg of folic acid per 100 g of flour as of 2000.

ART = assisted reproductive technologies. DZ = dizygous. FF = folic acid fortification. MZ = monozygous. TR = twinning rate. ◆

tion studies supporting an association between FA supplementation and twinning.

There is a plausible biological relationship between FA and the nutrient needs of the developing fetus, and it may be that other micronutrients are equally important or more important than FA, as indicated by the two studies that also examined an effect on twinning of multivitamins (including FA) taken during pregnancy.[17,18] These showed odds ratios of 1.30 (95% CI, 1.14–1.49)[17] and 2.08 (95% CI, 1.16–3.72)[18] for an increase in the likelihood of overall twinning.

This raises 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 its overall odds ratio — was based on a supplement that had a dose of FA of 0.8 mg as part of a multivitamin tablet. In an analysis of five US datasets where data on multivitamin supplementation were collected retrospectively, four showed a non-significant pattern of 30%–60% greater prevalence of periconceptional supplementation in mothers of twins.[39] Further, a randomised trial in Nepal of the effect on infant health and survival of taking vitamin A or beta carotene before concep-

tion and during pregnancy also found an increase in the rate of twins of 30%–44%.[40]

More well designed, long-term follow-up studies are needed in places where fortification with FA has been introduced, focusing on dose–response, by monitoring of FA blood levels, and obtaining accurate data on infertility treatments. These would help provide more convincing evidence that there is indeed a causal effect on twinning and a negative public health impact. On the face of the studies reviewed here, these effects are of a smaller magnitude than previously proposed.

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## COMPETING INTERESTS

Jane Halliday has been a member of the Flour Fortification Initiative Australia and New Zealand.

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