

Papers

Impact of misclassification of in vitro fertilisation in studies of folic acid and twinning: modelling using population based Swedish vital records

R J Berry, Reinhold Kihlberg, Owen Devine

Abstract

Objective To determine whether failure to adequately adjust for a reported 40% misclassification of use of in vitro fertilisation (IVF) as reported in a Swedish study could have led to a false finding that folic acid increases dizygotic twinning.

Design Modelling with population based data.

Setting Swedish vital records for 1995-9.

Main outcome measures Rates of twinning calculated according to whether women used IVF to become pregnant. Estimated unadjusted and adjusted odds ratios of the association between use of folic acid and twinning by use of IVF.

Results In 1995-9, Swedish women who used IVF had an almost 20 times the chance of having twins than women who did not use IVF (rate ratio 19.7, 95% confidence interval 18.7 to 20.6). In the absence of a true effect of folic acid, the use of a 40% misclassified surrogate variable to adjust for use of IVF would have resulted in a false finding that folic acid was associated with a more than twofold increase in twinning.

Conclusion Use of IVF is a strong confounder because it is associated with both use of folic acid and twinning. Even when misclassification of IVF was reduced to 5%, this bias persisted in the adjusted model. Using a 40% misclassified surrogate to adjust for IVF, as reported in the Swedish study, probably led to a false finding that folic acid increased dizygotic twinning.

Introduction

Folic acid is recommended throughout most of the world for women of childbearing age to prevent neural tube defects.¹ No European country, however, has implemented mandatory fortification of food, partly because of concerns that use of folic acid is associated with an increased risk of twinning.²⁻³ A Swedish study found that use of folic acid was associated with a more than twofold increase in dizygotic twinning,² but the analysis included 40% of the women who had used in vitro fertilisation (IVF) but were not identified as having done so in the Swedish medical birth registry.⁴ The strong association of IVF with both twinning⁵ and the use of vitamins make it a potentially strong confounder. Adjustment for a confounder using a misclassified surrogate variable can lead to substantial bias.⁶ The authors of the Swedish paper claimed they had adjusted for all confounding but did not provide numbers and rates of twinning for women who used IVF by their use of folic acid, which we have previously discussed.⁷⁻⁸ Recently available reports on Swedish vital records have enabled us to calculate background rates of twinning by women's use of

IVF.⁹⁻¹⁰ We used these rates along with data and assumptions based on findings reported in the original Swedish study to model the conditions in which that study was conducted. We used probabilistic simulations to assess bias caused by misclassification of the use of IVF.

Method

Definitions—IVF does not include use of ovarian-stimulating drugs alone, but does include all other assisted reproductive technologies as defined in Swedish reports.⁹⁻¹¹

Rates from database—We used numbers of births and twin pregnancies for the years 1995-9 from Swedish vital records⁹⁻¹⁰ to calculate rates of twinning for women who did and did not use IVF. We defined twinning as the number of pregnancies resulting in a twin birth. The Swedish study reported that 8% of women used folic acid during pregnancy; however, only 0.6% of the women in their analysis actually reported using folic acid.²

Assumptions—In all models we assumed that folic acid does not cause twinning. Our assumption that 50% of women who used IVF took folic acid was supported by a statement in the original study: "folic acid supplementation is often given, notably at IVF."²

Unadjusted analysis—We used rates of use of folic acid to estimate the number of women who did and did not use folic acid and used the total numbers to calculate the unadjusted rate ratio and 95% confidence intervals for the observed association between folic acid and twinning. We then used different estimates of use of folic acid in Sweden to assess how the unadjusted rate ratio would change.

Adjusted analysis—We used a probabilistic simulation model to examine the effect on the Mantel-Haenszel odds ratio of adjustment with a misclassified surrogate variable for use of IVF and an under-reported measure of the use of folic acid (see details of analysis on bmj.com).

Results

During the five year period 1995-9, the rate of twinning in Sweden was 1.5% (6960/450 697). During this period 1.8% (7958/450 697) of all deliveries and 26.1% (1814/6960) of all twin deliveries occurred among women who used IVF. The rates of twinning were 22.8% (1814/7958) among women who used IVF and 1.2% (5146/442 739) among women who did not.



Further details of the analysis, a figure, and an extra table are on bmj.com.

Table 1 Numbers of singleton and twin births by use of in vitro fertilisation (IVF) and unadjusted rate ratio and 95% confidence intervals for observed association between estimated use of folic acid and twinning (based on Swedish vital records 1995-9*)

Used folic acid during pregnancy	Singleton births			Twin births			Unadjusted rate ratio (95% CI)
	Yes (n=6110)	No (n=437 489)	Total (n=443 599)	Yes (n=1814)	No (n=5146)	Total (n=6960)	
Yes	3055†	34 999‡	38 054	907†	412‡	1319	2.44 (2.30 to 2.59), P<0.0001
No	3055	402 490	405 545	907	4734	5641	Reference

*Excludes 138 triplets and higher order births.
†50% use of folic acid among women who used IVF.
‡8% use of folic acid among women who did not use IVF.

Women who used IVF were almost 20 times more likely than those who did not use IVF to have a twin pregnancy (rate ratio 19.7, 95% confidence interval 18.7 to 20.6). We have excluded triplets and higher order births from all tables (n = 138).

In the absence of a true effect of folic acid, a 50% use of folic acid among women who used IVF and an 8% use among those who did not use IVF produces a unadjusted rate ratio of 2.44 (P<0.0001) for the false association between folic acid and increased twinning (table 1). Table 2 shows how varying the rates of use of folic acid among women who used IVF from 25% to 90% and among women who did not use IVF from 4% to 12% produced highly significant unadjusted rate ratios that varied from 1.31 to 6.15.

In the absence of a true effect of folic acid, the use of a 40% misclassified surrogate variable to adjust for use of IVF in the adjusted analysis would have resulted in a finding that folic acid was associated with a more than twofold increase in twinning (see table A on [bmj.com](#)).

Discussion

This modelling of Swedish vital records provides strong evidence that the use of a 40% misclassified surrogate variable to adjust for IVF in the original Swedish study probably led to a substantial upward bias in the estimation of the effect of folic acid on twinning. In 1995-9, almost 2% of Swedish mothers used IVF,^{9 10} which, combined with the differential patterns of use of vitamins between groups of women who did or did not use IVF, made IVF a potentially strong confounder. Even 5% misclassification of use of IVF in the adjusted model produced a twofold false association between folic acid and twinning (see table A on [bmj.com](#)). The problem of using a misclassified surrogate variable to adjust for confounding is well known.⁶

The magnitude of the potential effect and its implication for public health policy, however, are not fully appreciated, which is illustrated by two new articles that reported that folic acid and vitamins were associated with twinning. The new paper from Sweden adds two years' data to the original study, but it is unclear whether the author adequately adjusted for confounding.¹² The

paper from Hungary included women who used ovarian stimulation, and it did not seem to adjust for their increased rate of twinning.¹³ In Sweden the rate of twinning among women using ovarian stimulation was 5.9% (rate ratio 4.3).¹⁴ While use of these drugs does not result in twin pregnancies as commonly as does IVF, misclassification of women who use ovarian stimulation is likely to further increase the bias for the observed association between use of folic acid and dizygotic twinning.

All numbers and rates for twinning and misclassification used in modelling were based on population based vital records from Sweden^{9 11} and were consistent with other published Swedish studies.^{5 14 15}

If the true rates of use of folic acid in Sweden were different from those we used, then our estimate of the false association between folic acid use and twinning would change, but the association would not disappear unless the rates were equal. Given the large numbers of women in the Swedish study, use of almost any set of different rates of use of folic acid in our model produced a biased odds ratio that was highly significant. The false association we found is strengthened if the rate of use of folic acid was lower than 8% among women not using IVF or was higher than 50% among women using IVF (table 2). Another study from Sweden during 1995-9 reported that the use of folic acid during pregnancy was less than 5%.¹⁶ We believe that in the original study the true rates of use of folic acid during pregnancy were probably closer to 4% among women who did not use IVF and 75% among women who used IVF, which would have produced an observed unadjusted rate ratio of 5.24 for the false association between folic acid and twinning (table 2).

If the use of folic acid substantially increased twinning, then the increased adverse pregnancy outcomes associated with twinning^{5 15 17} could outweigh the known benefits of using folic acid to prevent neural tube defects.¹⁸ A study in China,⁷ where none of the women used IVF, provides strong evidence that no association exists between use of folic acid and increased twinning. More recently, three studies of secular trends of twinning rates in the United States before and after 1998, when mandatory folic acid fortification began, found no evidence that fortification has increased twinning.¹⁹⁻²¹ The studies that have found that folic acid or vitamins are associated with an increase in twinning have been conducted in populations where IVF and ovarian stimulation are often used.^{2 3 12 13} Our modelling suggests that the findings from Sweden are biased and raises questions about whether such studies can be conducted in areas where use of treatments for subfertility is common. Use of IVF is so strongly related to the occurrence of twins that, unless the use of IVF and ovarian-stimulating drugs is known for virtually all women and included in the analysis, a false association between twinning and any drug or vitamin used in combination with IVF will probably be observed. This result should reassure women planning pregnancies, their healthcare providers, and the wider health community that the evidence of an association between folic acid and an increase in twinning is probably false. This knowledge should aid government agencies and other organisations in

Table 2 Estimated unadjusted rate ratios for observed association between estimated use of folic acid during pregnancy and twinning according to estimated use of folic acid among women who did and did not use in vitro fertilisation (IVF) (based on Swedish vital records, 1995-9*†)

Estimated use of folic acid among women who used IVF	Estimated use of folic acid among women who did not use IVF		
	4%	8%	12%
25%	2.29	1.57	1.31
50%	3.77	2.44	1.93
75%	5.24	3.38	2.63
90%	6.15	3.99	3.10

*All rate ratios are significant P<0.0001.
†Estimated rate ratios calculated from total columns in table 1. Proportion of both singleton and twin births in columns in table 1 are changed by applying different rates of use of folic acid by use of IVF.

What is already known on this topic

Almost a quarter of Swedish women who used IVF in 1995-9 had a twin pregnancy, most of which were dizygotic

Women who use IVF often take vitamins, including folic acid

What this study adds

Use of IVF is such a powerful confounder in studies of folic acid and twinning that even a 5% misclassification of its use leads to substantial bias

The finding from a recent Swedish study that folic acid was associated with an increase in dizygotic twinning is probable incorrect because of the reported 40% misclassification of the use of IVF

evaluating the available evidence when they consider implementing folic acid fortification of food or other interventions to increase consumption of folic acid during pregnancy to prevent neural tube defects.

Contributors: RJB and RK conceived the study; RJB designed the study; RJB and OD supervised epidemiological and statistical analyses; and RK collected and interpreted of Swedish reports. All authors critically reviewed and contributed to the final draft of the paper. RJB is guarantor.

Funding: US federal government.

Competing interests: None declared.

Ethical approval: Not required.

- 1 Cornell MC, Erickson JD. Comparison of national policies on periconceptional use of folic acid to prevent spina bifida and anencephaly. *Teratology* 1997;55:134-7.
- 2 Ericson A, Källén B, Åberg A. Use of multivitamins and folic acid in early pregnancy and multiple births in Sweden. *Twin Res* 2001;4:63-6.
- 3 Czeizel AE, Metneki J, Dudas I. The higher rate of multiple births after periconceptional multivitamin supplementation: an analysis of causes. *Acta Genet Med Gemellol (Roma)* 1994;43:175-84.
- 4 EpC Research Report. *The Swedish medical birth registry—a summary of content and quality*. Stockholm, Sweden: National Board of Health and Welfare, 2003 (article 2003-112-3).
- 5 Bergh T, Ericson A, Hillensjö T, Nygren K-G, Wennerholm U-B. Deliveries and children born after in-vitro fertilization in Sweden 1982-95: a retrospective cohort study. *Lancet* 1999;354:1579-85.

- 6 Marshall J. Methodologic and statistical considerations regarding use of biomarkers of nutritional exposure in epidemiology. *J Nutr* 2003;133:881-7S.
- 7 Li Z, Gindler J, Wang H, Berry RJ, Li S, Correa A, et al. Folic acid supplements during early pregnancy and likelihood of multiple births: a population based cohort study. *Lancet* 2003;361:380-84.
- 8 Kihlberg R, Bui TH, Nielsen T, Söderhjelm L. Studien visar inte att folsyra ökar risk för tvillingfödsel. [The study does not show that folic acid increases the risk of twinning]. *Dagens Medicin* 2002;41:38.
- 9 EpC Report. *Assisterad befruktning 2001*. [Assisted reproduction—results of treatment 2001]. Stockholm, Sweden: National Board of Health and Welfare, 2004 (article 2004-42-4).
- 10 EpC Report. *Fakta om mammor, förlossningar och nyfödda barn—Medicinska födelserregistret 1973 till 2000*. [Facts concerning mothers, pregnancies, and newborn children—Swedish Medical Birth Registry—1973 to 2000.] Stockholm, Sweden: National Board of Health and Welfare; 2002 (article 2002-125-12). (Tables updated to 2002 on 9 August 2004.)
- 11 EpC Report. *Barn födda i Sverige efter provrörsbefruktning 1982-1997*. [Children born in Sweden after in-vitro fertilization 1982-1997.] Stockholm, Sweden: National Board of Health and Welfare, 2000 (article 2000-18-001).
- 12 Källén B. Use of folic acid supplements and risk for dizygotic twinning. *Early Hum Dev* 2004;80:143-51.
- 13 Czeizel AE, Vargha P. Periconceptional folic acid/multivitamin supplementation and twin pregnancy. *Am J Obstet Gynecol* 2004;191:790-4.
- 14 Källén B, Olausson P-O, Nygren K-G. Neonatal outcome in pregnancies from ovarian stimulation. *Obstet Gynecol* 2002;100:414-9.
- 15 Ericson A, Nygren K-G, Olausson PO, Källén B. Hospital care utilization of infants born after IVF. *Hum Reprod* 2002;17:929-32.
- 16 George L, Mills JL, Johansson ALV, Nordmark A, Olander B, Granath F, et al. Plasma folate levels and risk of spontaneous abortion. *JAMA* 2002;288:1867-73.
- 17 Kinzler WL, Ananth CV, Vintzileos AM. Medical and economic effects of twin gestations. *J Soc Gynecol Investig* 2000;7:321-7.
- 18 Lumley J, Watson L, Watson M, Bower C. Modelling the potential impact of population-wide periconceptional folate/multivitamin supplementation on multiple births. *BJOG* 2001;108:937-42.
- 19 Waller DK, Tita ATN, Annegers JF. Rates of twinning before and after fortification of foods in the US with folic acid, Texas, 1996 to 1998. *Paediatr Perinat Epidemiol* 2003;17:378-83.
- 20 Shaw GM, Carmichael SL, Nelson V, Selvin S, Schaffer DM. Food fortification with folic acid and twinning among California infants. *Am J Med Genet* 2003;119A:137-40.
- 21 Kucik J, Correa A. Trends in twinning rates in metropolitan Atlanta before and after folic acid fortification. *J Reprod Med* 2004;49:707-12.

(Accepted 10 January 2005)

doi 10.1136/bmj.38369.437789.82

National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Department of Health and Human Services, 1600 Clifton Road, MS E-86, Atlanta, GA 30333, USA

R J Berry medical epidemiologist

O Devine senior statistician

Riddarvägen 37B, 184 51, Österskär, Stockholm, Sweden

R Kihlberg lecturer

Correspondence to: R J Berry RJBerry@cdc.gov

[Further details of analysis](#)

[Figure](#)

[Table](#)

Probabilistic simulation of the Swedish study of folic acid and twinning using Swedish vital records

Method

All the definitions and rates used in the unadjusted modeling are unchanged in the probabilistic simulation.

Variables in the model – source and calculation.

Twinning and use of IVF

Swedish vital records 1995 through 1999: ^{1, 2}

Women who used IVF (1.8%)

Twinning rate when woman used IVF (23%)

Twinning rate when woman did not use IVF (1.2%)

Involuntary childlessness

An evaluation of the Medical Birth Registry found that 6.4% of women reported any period of involuntary childlessness, which was used as a surrogate variable to adjust for use of IVF in the original analysis.³

However, this evaluation showed that among women who used IVF, 40% did not report a period of involuntary childlessness and among those who did not use IVF, 5% reported a period of involuntary childlessness. This evaluation of the content and quality of the Medical Birth Registry showed that only 1% to 2 % of records were missing and that twins were accurately counted.

Misclassification of the surrogate variable used to adjust for IVF

Women who used IVF but did not report a period of involuntary childlessness (40%)

Women who did not use IVF but did report a period of involuntary childlessness (5%)

Use of folic acid

An unpublished survey cited in the Swedish study reported that 8% of women used folic acid during pregnancy; however, only 0.6% of women in the original Swedish article reported using folic acid to the Medical Birth Registry.⁴ Therefore, only about 7% (0.6%/8%) of women who were estimated to have used folic acid reported such use and were included in the analysis. Therefore use of folic acid was approximately 93% underreported in the original study. We used a more conservative estimate of 75% underreporting.

Women who used folic acid during pregnancy (8%)

Women who reported use of folic acid during pregnancy (0.6%)

Underreporting of use of folic acid in original study (75%)

Assumptions:

No association between folic acid and twinning – true odds ratio = 1

Rate of folic acid use among women who used IVF (50%)

Adjusted analysis

We used probabilistic simulation to model conditions that existed during the Swedish study. In all models we held data-based rates of twinning and use of folic acid constant. In scenario 1, we used the true status for use of folic acid and IVF to adjust for these variables. In scenario 2, we added a misclassified surrogate to adjust for use of IVF. In scenario 3, we added a 75% underreporting of the use of folic acid.

For each scenario we created a hypothetical population of 100 000 women; for each subject we used Swedish rates to generate Bernoulli random variables having values of either 1 (yes) or 0 (no). We then analyzed 5000 simulated studies using this population. For each of the 5000 studies we calculated the unadjusted and Mantel-Haenszel odds ratios and 95% confidence intervals for the association between use of folic acid and twinning. We summarized the 5000 studies in each scenario by calculating the mean unadjusted and Mantel-Haenszel odds ratios and plotting the distribution of the 5000 Mantel-Haenszel odds ratios.

Results

Table A shows that with no misclassification or underreporting in the model the mean Mantel-Haenszel odds ratio equaled 1.0, the null value. When we added a 40% misclassified surrogate variable to adjust for use of IVF (scenario 2), the mean Mantel-Haenszel odds ratio was 2.28, with 100% of the simulated 95% confidence intervals excluding the null value of 1. Even when we lowered the level of the misclassification to 5%, the mean Mantel-Haenszel odds ratio was still biased (2.02) and highly significant. When we added a 75% underreporting for use of folic acid (scenario 3), the mean Mantel-Haenszel odds ratio (2.07) remained high. In the figure, the distributions of the Mantel-Haenszel odds ratios for each of the 3 scenarios show the marked shift away from the null produced by using a misclassified surrogate to adjust for IVF (scenario 2) and the shift back toward the null when adding a underreported measure of the use of folic acid (scenario 3).

Discussion

Our probabilistic simulations allowed us to examine how well the Mantel-Haenszel procedure would be able to adjust for confounding using a misclassified surrogate variable for use of IVF and an underreported measure of the use of folic acid (table A). With no misclassification of IVF the Mantel-Haenszel procedure worked as expected, correcting the mean unadjusted odds ratio of 2.48 to 1.0 (table A). However, the Mantel-Haenszel procedure failed when a 40% misclassified surrogate was used to adjust for IVF. The mean Mantel-Haenszel odds ratio only corrected to 2.28, which was highly significant. This biased estimate was lowered to 2.07 by the addition of 75% underreporting of use of folic acid, but this should not be viewed as a correction of a biased estimate, but as combination of competing biases of unknown magnitudes.

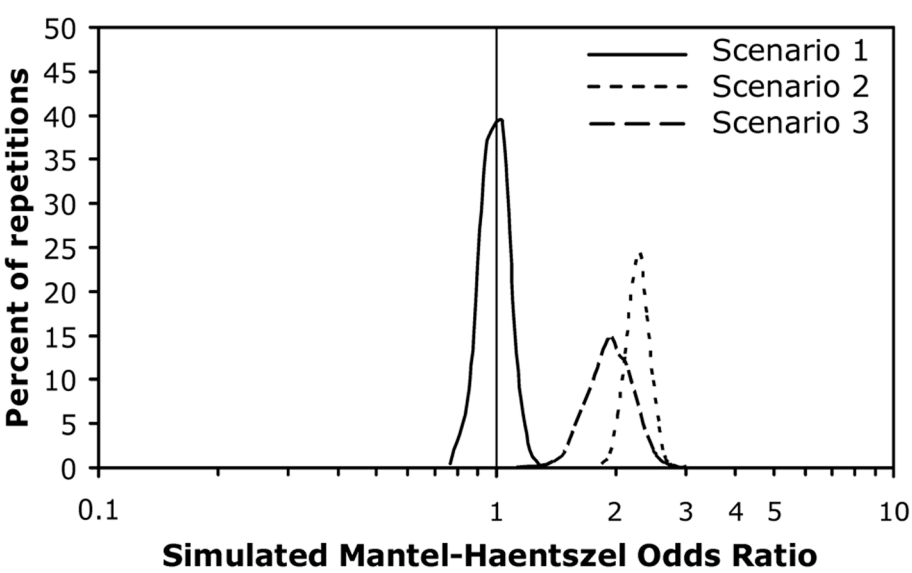
1 EpC Report. *Assisterad befruktning 2001*. [Assisted reproduction—results of treatment 2001]. Stockholm, Sweden: National Board of Health and Welfare, 2004.

2 EpC Report. *Fakta om mammor, förlossningar och nyfödda barn—Medicinska födelseregistret 1973 till 2000*. [Facts concerning mothers, pregnancies, and newborn children—Swedish Medical Birth Registry—1973 to 2000]. Stockholm, Sweden: National Board of Health and Welfare; 2002. [Tables updated to 2002 on 9 August 2004].

3 EpC Research Report. *The Swedish medical birth registry—a summary of content and quality*. Stockholm, Sweden: National Board of Health and Welfare, 2003 (article No: 2003-112-3).

4 Ericson A, Källén B, Åberg A. Use of multivitamins and folic acid in early pregnancy and multiple births in Sweden. *Twin Research* 2001;4:63-6.

Figure [posted as supplied by author] Distribution of Mantel-Haenszel odds ratios from three scenarios in Table A and the observed association between use of folic acid and twinning according to misclassification of use of in vitro fertilisation (IVF) and underreporting of use of folic acid – (based on Swedish vital records 1995–9)



Footnotes to legend

Adjustment in scenario 1 uses the true status for use of folic acid and use of IVF (unadjusted odds ratio = 2.48).

Adjustment in scenario 2 uses the true status for use of folic acid and a 40% misclassified surrogate to adjust for use of IVF (unadjusted odds ratio = 2.48).

Adjustment in scenario 3 uses a 40% misclassified surrogate to adjust for use of IVF and a 75% underreported measure to adjust for use of the use of folic acid (unadjusted odds ratio = 2.27).

[Posted as supplied by author] **Table A** Probabilistic simulation of unadjusted and Mantel-Haenszel odds ratios for the observed association between use of folic acid and twinning according to misclassification of use of in vitro fertilisation (IVF) and underreporting of use of folic acid – (based on Swedish vital records 1995–9)

Scenario	Misclassification of use of IVF	Underreporting of use of folic acid	Unadjusted odds ratio		Mantel-Haenszel odds ratio	
			Mean *	Percent of 95% confidence intervals that excluded the null *	Mean *	Percent of 95% confidence intervals that excluded the null *
1	0%	0%	2.48	100%	1.00	5%
2	40%	0%	2.48	100%	2.28	100%
	10%	0%			2.05	100%
	5%	0%			2.02	100%
3	40%	75%	2.27	100%	2.07	100%

* Each scenario is based on 5000 simulated studies with the following rates held constant:

Rates based on data from Swedish vital records reports:

Women who used IVF (1.8%),

Twinning rate when woman used IVF (23%),

Twinning rate when woman did not use IVF (1.2%),

Women who reported involuntary childlessness but who did not use IVF (5%),

Rates based on survey reported in original article:

Rate of folic acid use among women, who did not use IVF (8%)

Rates based on assumptions:

No association between folic acid and twinning, and Rate of folic acid use among women who used IVF (50%).