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.


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. 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. A Swedish study found that use of folic acid was associated with a more than twofold increase in dizygotic twinning, 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. The strong association of IVF with both twinning 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. 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. Recently available reports on Swedish vital records have enabled us to calculate background rates of twinning by women’s use of IVF. 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.

Rates from database—We used numbers of births and twin pregnancies for the years 1995-9 from Swedish vital records 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 0.8% of the women in their analysis actually reported using folic acid.

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.”

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.
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 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).

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 studies that have included 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. 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.

Table 2

<table>
<thead>
<tr>
<th>Estimated use of folic acid among women who did or did not use IVF</th>
<th>Estimated use of folic acid among women who did not use IVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>4%</td>
<td>2.29</td>
</tr>
<tr>
<td>8%</td>
<td>1.57</td>
</tr>
<tr>
<td>12%</td>
<td>1.31</td>
</tr>
<tr>
<td>25%</td>
<td>3.77</td>
</tr>
<tr>
<td>50%</td>
<td>2.44</td>
</tr>
<tr>
<td>75%</td>
<td>1.93</td>
</tr>
<tr>
<td>90%</td>
<td>5.24</td>
</tr>
</tbody>
</table>

*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 column 1 are changed by applying different rates of use of folic acid by use of IVF.

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Table 1

<table>
<thead>
<tr>
<th>Used folic acid during pregnancy</th>
<th>Singleton births</th>
<th>Twin births</th>
<th>Unadjusted rate ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>3551</td>
<td>34895</td>
<td>20.49</td>
</tr>
<tr>
<td>No</td>
<td>402</td>
<td>264</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*Excludes 138 triplets and higher order births.
†5% use of folic acid among women who did not use IVF.
‡15% use of folic acid among women who did use IVF.
§20% 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 50% 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).
elevating 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.

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(Accepted 10 January 2005)

doi: 10.1136/bmj.38369.437789.82

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