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

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### 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 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

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



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**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.

tube defects.<sup>1</sup> 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.<sup>2-3</sup> A Swedish study found that use of folic acid was associated with a more than twofold increase in dizygotic twinning,<sup>2</sup> 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.<sup>4</sup> The strong association of IVF with both twinning<sup>5</sup> 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.<sup>6</sup> 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.<sup>7-8</sup> Recently available reports on Swedish vital records have enabled us to calculate background rates of twinning by women's use of IVF.<sup>9-10</sup> 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.<sup>9-11</sup>

**Rates from database**—We used numbers of births and twin pregnancies for the years 1995-9 from Swedish vital records<sup>9-10</sup> 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.<sup>2</sup>

**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."<sup>2</sup>

**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](http://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. 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](http://bmj.com)).

**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.

## 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,<sup>9 10</sup> 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.<sup>6</sup>

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.<sup>12</sup> The paper from Hungary included women who used ovarian stimulation, and it did not seem to adjust for their increased rate of twinning.<sup>13</sup> In Sweden the rate of twinning among women using ovarian stimulation was 5.9% (rate ratio 4.3).<sup>14</sup> 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<sup>9 11</sup> and were consistent with other published Swedish studies.<sup>5 14 15</sup>

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%.<sup>16</sup> 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<sup>5 15 17</sup> could outweigh the known benefits of using folic acid to prevent neural tube defects.<sup>18</sup> A study in China,<sup>7</sup> 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

### 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 probably incorrect because of the reported 40% misclassification of the use of IVF

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.<sup>19-21</sup> 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.<sup>2 3 12 13</sup> 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 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.

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## Utility of testing for monoclonal bands in serum of patients with suspected osteoporosis: retrospective, cross sectional study

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### Abstract

**Objective** To determine whether measuring monoclonal bands (M component) in serum should be part of the investigation of patients referred to osteoporosis clinics.

**Design** Retrospective, cross sectional, observational study.

**Setting** Referral centre for osteoporosis in a university hospital, Denmark.

**Participants** 799 people (685 women) aged 19 to 94 years newly referred with suspected osteoporosis.

**Main outcome measures** Proportion of patients fulfilling the Nordic Myeloma Study Group definition for target condition and proportion of patients with other important haematological conditions.

**Results** 4.9% (18 of 366) of patients with osteoporosis and 2.2% (9 of 408) of patients without osteoporosis had M components in serum ( $\chi^2 = 3.66$ ,  $P = 0.04$ ). Multiple myeloma was diagnosed in three patients with osteoporosis (absolute risk 0.8%, 95% confidence interval 0.11% to 1.7%). The relative risk of multiple myeloma in patients presenting with osteoporosis was 75 (10 to 160). As a diagnostic test for multiple myeloma in patients with osteoporosis, M component in serum had a specificity of 95.0% and a positive predictive value of 17.6%. 122 blood electrophoreses were carried out for each case of multiple myeloma diagnosed. All patients with multiple myeloma had a history of fragility fractures. If lymphoma was included as a target condition, the specificity increased to 95.3% and the positive predictive value increased to 23.5%. Monoclonal gammopathy of undetermined significance was diagnosed in 13

(3.6%) participants with osteoporosis and in eight (2.0%) participants with normal bone mineral density or osteopenia.

**Conclusions** Patients presenting with osteoporosis should be tested for M component in serum, as 1 in 20 patients with newly diagnosed osteoporosis had multiple myeloma or monoclonal gammopathy of undetermined significance.

### Introduction

Compared with osteoporosis, multiple myeloma is a rare disease, yet vertebral fractures and pain are common to both. Multiple myeloma would be expected to be seen more often in osteoporosis clinics than in most other areas of medicine dealing with the care of elderly people. Moreover, monoclonal gammopathy of undetermined significance is a common disorder, with a similar age distribution to that of osteoporosis.

National guidelines in Denmark do not advocate the routine measurement of monoclonal bands (M component) in the serum or urine of patients presenting with osteoporosis. We determined the prevalence of multiple myeloma and monoclonal gammopathy of undetermined significance in unselected patients newly referred with osteoporosis to assess whether measurement of M component should form part of the investigation of patients with suspected osteoporosis.



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