In utero exposure to tobacco smoke and subsequent reduced fertility in females

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Submitted on March 18, 2010; resubmitted on June 18, 2010; accepted on July 29, 2010

BACKGROUND: Animal studies have shown that in utero exposure to chemicals in tobacco smoke reduces female fertility, but epidemiological findings have been inconsistent.

METHODS: We examined the association between in utero exposure to tobacco smoke and female fertility among women in the Norwegian Mother and Child Cohort Study, enrolled from 1999 to 2007. Around the 17th week of pregnancy, participants reported how long they took to conceive (time to pregnancy), and whether their mother smoked while pregnant with the participant. This analysis included 48 319 planned pregnancies among women aged 15–44 years. We estimated fecundability odds ratios (FORs) using a discrete-time survival analysis, adjusting for age, education and adult tobacco smoking.

RESULTS: The adjusted FOR for in utero exposure to tobacco smoke among all subjects was 0.96 [95% confidence interval (CI): 0.93, 0.98], among subjects reporting no adult tobacco smoking or passive exposure it was 0.96 (95% CI: 0.93, 0.99) and among subjects reporting adult tobacco smoking or passive exposure it was 0.95 (95% CI: 0.91, 0.99). We performed a probabilistic sensitivity analysis to estimate the effect of exposure and outcome misclassification on the results, and, as expected, the association became more pronounced after taking misclassification into account.

CONCLUSIONS: This large cohort study supports a small-to-modest association between in utero exposure to tobacco smoke and reduced fertility.

Key words: tobacco smoking / in utero exposure / fertility

Introduction

Smoking is a well-established risk factor for many human health conditions, including impaired reproduction (CDC, 2004a). Women who smoke have an increased risk of subfertility, infertility, pregnancy loss, preterm delivery and of giving birth to an infant who is small-for-gestational age (Augood et al., 1998; Kharrazi et al., 2004; Wilks and Hay, 2004; Tchi and Hossain, 2007). About 10–30% of women in western countries smoke during pregnancy (CDC, 2004b; Egebjergh Jensen et al., 2008). The effects of passive smoking on reproductive outcomes are less clear (CDC, 2006). Animal studies, however, have shown that in utero exposure to smoking-related chemicals reduces female fertility. For example, mice exposed in utero to polycyclic aromatic hydrocarbons had fewer and smaller litters (MacKenzie and Angevine, 1981). In rats, in utero exposure to nicotine caused decreased ovarian function and increased time to pregnancy (TTP) (Holloway et al., 2007). Epidemiological studies on the association between a mother’s smoking during pregnancy and her daughter’s fertility have, however, yielded inconsistent findings, which may in part be related to limited sample size (Baird and Wilcox, 1986; Weinberg et al., 1989; Jensen et al., 1998, 2006; Joffe and Barnes, 2000). To examine the association between a mother’s smoking during pregnancy and a daughter’s fertility in a large study, we analyzed data from a pregnancy cohort in Norway.
Materials and Methods

The Norwegian Mother and Child Cohort Study

This study is based on the Norwegian Mother and Child Cohort Study (MoBa), conducted by the Norwegian Institute of Public Health (Magnus et al., 2006). Enrollment was from 1999 to 2008 and about 107 000 pregnancies among 90 000 women were included. The present study is based on the 90 190 pregnancies recruited from 1999 to 2007 whose data appeared in the MoBa version 4.201 data set. The majority of all pregnant women in Norway were invited to participate, and the response rate was about 44%. During weeks 17–18 of gestation, participants were asked to complete a questionnaire about demographic characteristics, reproductive health, disease and medication history, lifestyle and socioeconomic status. The Regional Committee for Medical Research and the Norwegian Data Inspectorate approved the study, and informed consent was obtained from each participant.

In utero exposure to tobacco smoking

Women were asked: 'Did your mother smoke when she was pregnant with you?' Women could answer 'yes', 'no' or 'don’t know'. Those who answered 'yes' to this question were classified as having been exposed to tobacco smoke while in utero; those who responded 'no' were considered unexposed.

Time to pregnancy

TTP is a measure of a couple’s ability to conceive with regular intercourse and no use of birth control (Baird et al., 1986). To ascertain TTP, women were first asked: 'Was this pregnancy planned?' Those who planned their pregnancy were further asked: 'How many months did you have regular intercourse without contraception before you became pregnant?' Women could choose one of three response options: 'less than 1 month', '1–2 months' and '3 months or more'. Women choosing the '3 months or more' option were further asked to report the actual number of months. Women were also asked if they had received any infertility treatment in connection with this pregnancy.

Exclusions

As noted above, data for 90 190 pregnancies were available. The selection of the analysis sample with the exclusion criteria is shown in Fig. 1. We restricted our analysis to the woman’s first MoBa pregnancy. Women who met at least one of the following criteria were excluded from the primary analysis: (i) age <15 or 44 years old; (ii) reported an unplanned pregnancy (including those who reported contraceptive failures) or did not report this information; (iii) did not report TTP or reported TTP inconsistently (TTP was considered as inconsistent if, for example, a woman reported that she became pregnant within ‘less than 1 month’ or ‘1–2 months’ but then answered ≥3 to the next question [‘number of month if more than 3’]); and (iv) did not report her mother’s smoking when pregnant (question not answered or answered ‘don’t know’) or reported inconsistent information. The reported information on the mother’s smoking was considered inconsistent if a woman was enrolled for more than one pregnancy and gave a different answer on two questionnaires. Women with missing values for covariates (n = 2231) were excluded from the final data analysis as described below. Overall, 48 319 women met the criteria for inclusion in the primary analysis.

Data analysis

To analyze the association between in utero exposure to tobacco smoke and female fertility among women in the Norwegian Mother and Child Cohort Study (MoBa), enrolled from 1999 to 2007. Characteristics of these subjects are shown in Table I. **Characteristics of these women are shown in Table II.

Figure 1 Flowchart for subject selection in the study of the association between in utero exposure to tobacco smoke and female fertility among women in the Norwegian Mother and Child Cohort Study (MoBa), enrolled from 1999 to 2007. Characteristics of these subjects are shown in Table I. **Characteristics of these women are shown in Table II.
In utero tobacco exposure and fecundability

In utero tobacco exposure and fecundability

Table I Characteristics of pregnancy planners and non-planners in the study of the association between in utero exposure to tobacco smoke and female fertility among women in the Norwegian Mother and Child Cohort Study, enrolled from 1999 to 2007.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Planners (n = 59 917)</th>
<th>Non-planners (n = 17 167)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother smoked when she was pregnant with the subject (%) (^b)</td>
<td>No 64.5</td>
<td>59.7</td>
</tr>
<tr>
<td></td>
<td>Yes 24.0</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>Don’t know 10.0</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>Missing 1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Subject’s age at enrollment [mean (SD), years]</td>
<td>29.8 (4.3)</td>
<td>28.4 (5.6)</td>
</tr>
<tr>
<td>Subject’s age at enrollment (%)</td>
<td>&lt; 20 0.6</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>20–24 10.0</td>
<td>21.3</td>
</tr>
<tr>
<td></td>
<td>25–29 37.2</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>30–34 38.1</td>
<td>27.4</td>
</tr>
<tr>
<td></td>
<td>35–39 12.9</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>≥40 1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Subject’s completed education (%)</td>
<td>&lt; High school 1.8</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>High school 26.7</td>
<td>38.9</td>
</tr>
<tr>
<td></td>
<td>College or university 62.1</td>
<td>41.1</td>
</tr>
<tr>
<td></td>
<td>Other education 6.4</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>Missing 3.1</td>
<td>6.2</td>
</tr>
<tr>
<td>Subject’s adult tobacco smoking before pregnancy (%)</td>
<td>None 64.7</td>
<td>48.9</td>
</tr>
<tr>
<td></td>
<td>Active smoking only 22.4</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>Passive smoking only 5.0</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Both 5.3</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Missing 2.7</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*All differences between pregnancy planners and non-planners shown in the table were statistically significant (\(\chi^2\) or \(t\)-test) at the \(P < 0.001\) level. \(^b\)663 women who reported inconsistent information on this item were excluded for this comparison.

We conducted sensitivity analyses that have been recommended to assess the potential effect of biases that can influence the results of retrospective studies of TTP (Weinberg et al., 1994; Olsen et al., 1998; Joffe et al., 2005). We fit a logistic model to evaluate whether contraceptive failure was associated with in utero exposure to tobacco smoke (Joffe et al., 2005). We repeated the primary analysis after (i) including non-planners with imputed TTPs of 1, 2, 3 or 4; (ii) excluding all subjects with a TTP of 1 or 2; (iii) censoring TTP at different thresholds (6, 9, 12 and 14 months); (iv) recalculating TTP in terms of menstrual cycles instead of months (restricted to those reporting regular cycles); and (v) after assigning those who were missing data or who reported ‘do not know’ for in utero smoking (13% of the study population) as either exposed or unexposed.

In other sensitivity analyses, we repeated the primary analysis after (i) adjusting for the use of hormonal contraceptives in the past year (in the subset of women with a TTP < 13 months); (ii) adjusting for parity; (iii) adjusting for amount smoked by the mother before pregnancy (non-smoker, <10, 10–20, 20+ cigarettes/day); and (iv) restricting the analysis to the first MoBa pregnancy that was also the woman’s first pregnancy.

Finally, beginning with the 59 917 women with planned pregnancies (Fig. 1), we used multiple imputation with chained equations to impute values that were missing for TTP, in utero smoking, education and prepregnancy exposure to tobacco (Raghunathan et al., 2007). Adjusted FORs for in utero exposure to tobacco smoke based on five imputed data sets were calculated, and the entire procedure was repeated to assess its reliability.
Results

Compared with planners, a higher proportion of non-planners reported in utero exposure to tobacco smoke (Table I). Planners tended to be older than those who had not planned their pregnancies. Sixty-two percent of planners had completed college or university, versus 41% among non-planners. A lower proportion of planners (27%) were exposed to active or passive smoking before the pregnancy than non-planners (36%). The two groups did not differ substantially on other variables (data not shown).

Among planners, the proportion with TTP > 12 months was higher among women with in utero exposure to tobacco smoke (12%) than in those without (11%) (Table II). The average age in the two groups was similar. Compared with unexposed subjects, women exposed prenatally to tobacco smoke were less likely to have completed college or university, and a higher proportion of them were exposed to active or passive smoking.

The unadjusted FOR for exposure to in utero tobacco smoke among all subjects was 0.93 (95% confidence interval (CI): 0.91, 0.96), and the adjusted FOR was 0.96 (95% CI: 0.93, 0.98) (Table III). We saw no effect modification between a subject’s adult tobacco smoking history before pregnancy and in utero tobacco smoke exposure (likelihood ratio test, P = 0.65, 3 degrees of freedom). Regardless of the non-significance of the effect modification test, we stratified the analysis by the subject’s adult tobacco smoking before pregnancy (two categories: no adult tobacco exposure, with adult tobacco exposure including passive smoking) because subjects exposed in utero had higher proportions of exposure to adult tobacco smoking. The association among those exposed in utero but without any adult exposure would be less likely to be biased by residual confounding by adult smoking. The adjusted FORs for those who had no adult tobacco smoking exposure was 0.96 (95% CI: 0.93, 0.99) and for those who had adult tobacco smoking exposure it was 0.95 (95% CI: 0.91, 0.99).

After taking into account exposure misclassification, the FOR for all subjects was 0.92 (95% CI: 0.88, 0.95), for subjects without adult tobacco smoking exposure it was 0.94 (95% CI: 0.89, 0.98) and for subjects who had a history of such exposure it was 0.91 (95% CI: 0.86, 0.95) (Table III). The association also became more pronounced after taking into account outcome (TTP) misclassification.

The odds of contraceptive failure were unrelated to in utero exposure to tobacco smoke (data not shown). The association between TTP and in utero exposure to tobacco smoking was slightly attenuated when we included non-planners in the analysis [e.g. adjusted FOR = 0.97 (95% CI: 0.95, 0.99), after imputation of non-planners’ TTP as 1 month]. The other sensitivity analyses also indicated that the results were robust. For example, the estimated association was virtually the same when we restricted the analyses to first pregnancies (not shown), and when we assigned the 7506 women who provided an uncertain answer about the analyses to first pregnancies (not shown), and when we assigned the 7506 women who provided an uncertain answer about the analyses to first pregnancies (not shown). Using different censoring thresholds or adjusting for additional variables did not change the association (not shown).

Discussion

In the present study, we found a small-to-modest association between in utero exposure to tobacco smoke and reduced fertility. As noted earlier, the findings of previous epidemiological studies on the association between in utero exposure to tobacco smoke and fertility have been inconsistent (Table IV) (Baird and Wilcox, 1986; Weinberg et al., 1989, 1998; Jensen et al., 1998, 2006; Joffe and Barnes, 2000). The reason for the inconsistency remains unclear, although in both of the prospective studies a reduced fecundability was found (Weinberg et al., 1989; Jensen et al., 1998). Although we also found reduced fecundability, the magnitude of the association was small enough that residual confounding could account for it. Once the effect of misclassification was considered, however, the possibility of a true effect was more evident.

As noted earlier, animal studies provide some evidence for mechanisms through which in utero exposure to tobacco smoke could cause reduced fertility in females. Prenatal exposure to cigarette smoke or polycyclic aromatic hydrocarbons (important toxicants in cigarette smoke) induced fetal ovarian germ cell loss, resulting in a significant loss of primordial follicles in mice (Vahakangas et al., 1985;
Prenatal exposure to nicotine resulted in subsequent ovarian dysfunction and increased TTP in adult female rats offspring (Holloway et al., 2006). Furthermore, other toxic components in cigarette smoke may have similar effects (Miller et al., 2004; Rogers, 2008). In utero tobacco smoke exposure has recently been associated with earlier age at menopause (Strohsnitter et al., 2008). This finding provides additional support for an adverse effect of in utero smoke exposure on female reproduction.

A daughter’s report about her mother’s smoking status during pregnancy has been previously shown to be reasonably reliable and valid (Sandler and Shore, 1986; Coultas et al., 1994; Joffe and Barnes, 2000). Retrospective TTP data have also been shown to be fairly accurate (Zielhuis et al., 1998; Simard et al., 2004). The data on the validity of the measures employed suggested that the effect of non-differential misclassification on our results would be modest. The results of the probabilistic sensitivity analysis verified this.

Our additional sensitivity analyses to assess other potential sources of bias (Weinberg et al., 1994; Olsen et al., 1998; Joffe et al., 2005) did not suggest any substantial differences in the associations between in utero exposure to smoke and TTP when planning status, infertility treatment history, TTP cutoffs or unknown reports for in utero exposure were accounted for, suggesting that the effect of these biases was probably minor in the present study.

Nonetheless, our questionnaire-based study had several limitations. A daughter’s reports about her mother’s smoking status during pregnancy have not been validated with biomarkers of the mother’s tobacco exposure. Some evidence from biomarker-based studies suggests underreporting of smoking during pregnancy (Shipton et al., 2009), and this would attenuate observed associations. We did not have data on the number of cigarettes smoked per day by any subject’s mother or information about the subjects’ childhood exposure. Studies have shown that most mothers who smoked during pregnancy continued smoking after pregnancy (Weinberg et al., 1989; Simard et al., 2008), suggesting that adjusting for childhood exposure may lessen the association of in utero smoke exposure with fertility. On the other hand, the production of oocytes occurs only during fetal life and this may be a critical window of susceptibility (Pryor et al., 2000). Although retrospective reports of TTP are good (Zielhuis et al., 1992), they are not perfect. Furthermore, we cannot rule out the possibility that differential misclassification affected our findings. Finally, selection bias may have occurred owing to the exclusion of sterile women and pregnancies ending before participation (about the 17th week of gestation), possibly causing an underestimation of effect (Weinberg and Wilcox, 2008).

In summary, we observed a small-to-modest association between in utero exposure to tobacco smoke and reduced fertility in this large cohort study, and the association was more pronounced after accounting for exposure and outcome misclassification.

### Table IV A summary of epidemiological studies on in utero exposure to tobacco smoke and female fertility.

<table>
<thead>
<tr>
<th>Author</th>
<th>Location</th>
<th>Sample size</th>
<th>Smoking status reporter</th>
<th>TTP data collected</th>
<th>FORs a,b</th>
<th>95% CI b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baird and Wilcox (1986)</td>
<td>USA/Minnesota</td>
<td>663</td>
<td>Daughter</td>
<td>R t, in months</td>
<td>1.0</td>
<td>0.9, 1.2</td>
</tr>
<tr>
<td>Weinberg et al. (1989)</td>
<td>USA/North Carolina</td>
<td>221</td>
<td>Daughter</td>
<td>P t, in cycles</td>
<td>0.5</td>
<td>0.4, 0.8</td>
</tr>
<tr>
<td>Jensen et al. (1998)</td>
<td>Denmark</td>
<td>423</td>
<td>Daughter</td>
<td>P, in cycles</td>
<td>0.64 d</td>
<td>0.47, 0.87 d</td>
</tr>
<tr>
<td>Joffe and Barnes (2000)</td>
<td>UK</td>
<td>2587</td>
<td>Mother (at the time of delivery)</td>
<td>R, in months</td>
<td>1.02</td>
<td>0.92, 1.13</td>
</tr>
<tr>
<td>Jensen et al. (2006)</td>
<td>Denmark</td>
<td>1653*</td>
<td>Daughter (20%) and mother (80%)</td>
<td>R, in months</td>
<td>0.81</td>
<td>0.65, 1.02</td>
</tr>
<tr>
<td>Present study</td>
<td>Norway</td>
<td>48 319</td>
<td>Daughter</td>
<td>R, in months</td>
<td>0.96</td>
<td>0.93 0.98</td>
</tr>
</tbody>
</table>

*For early life smoking exposure: yes versus no. a FORs, fecundability odds ratios; CI, confidence interval. b R, retrospectively; P, prospectively. c Weighted average of stratified FORs (0.70 and 0.53) given in their Table 2. d Twins.

### Supplementary data


### Acknowledgements

We thank Dr Tim Lash from the University of Boston for providing his probabilistic bias analysis SAS program. Government Department: National Institutes of Health, Department of Health and Human Services.

### Funding

This study was supported in part by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences. The Norwegian Mother and Child Cohort Study is supported by the Norwegian Ministry of Health, NIH/NIEHS (grant no. N01-ES-85433), NIH/NINDS (grant no. 1 UO1 NS 047537-01) and the Norwegian Research Council/FUGE (grant no. 151918/S10).
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