Fecundability and Parental Exposure to Ambient Sulfur Dioxide

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Recently it has been observed that birth rates in Teplice, a highly polluted district in Northern Bohemia, have been reduced during periods when sulfur dioxide levels were high. This study, which is based on data from 2,585 parental pairs in the same region, describes an analysis of the impact of SO_2 on fecundability in the first unprotected menstrual cycle (FUMC). We obtained detailed personal data, including time-to-pregnancy information, via maternal questionnaires at delivery. We estimated individual exposures to SO_2 in each of the 4 months before conception on the basis of continual central monitoring. Three concentration intervals were introduced: < 40 µg/m^3 (reference level); 40–80 µg/m^3; and ≥ 80 µg/m^3. We estimated adjusted odds ratios (AORs) of conception in the FUMC using logistic regression models. Many variables were screened for confounding. AORs for conception in the FUMC were consistently reduced only for couples exposed in the second month before conception to SO_2 levels as follows: 40–80 µg/m^3, AOR 0.57 [95% confidence interval (CI), 0.37–0.88; $p < 0.011$]; ≥ 80 µg/m^3, AOR 0.49 (CI, 0.29–0.81; $p < 0.006$). The association was weaker in the second 2 years of the study, probably due to the gradual decrease of SO_2 levels in the region. The relationship between SO_2 and fecundability was greater in couples living close to the central monitoring station (within 3.5 km). The timing of these effects is consistent with the period of sperm maturation. This is in agreement with recent findings; sperm abnormalities originating during spermatid maturation were found in young men from Teplice region who were exposed to the increased levels of ambient SO_2. Alternative explanations of our results are also possible. ***Key words*****: air pollution, environmental exposure, fecundability, human fertility, reproductive effects, SO_2, sperm maturation.** ***Environ Health Perspect*** **108:647–654 (2000). [Online 5 June 2000]**
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Several recently published papers have suggested that air pollution has detrimental effects on reproduction (*1–5*). Reduced birth rates in periods with high sulfur dioxide levels were found in a heavily polluted region of Northern Bohemia during the late 1980s (*6,7*). Šrám and colleagues (*6,7*) hypothesized that SO_2 or some associated copollutants may reduce reproductive success through adverse effects on oocyte fertilization, an effect that had been produced experimentally (*8*). Detailed information on the course and outcome of almost all pregnancies occurring in the same region of the Czech Republic was collected over the last 5 years; relevant pollution data were also obtained. The purpose of the present paper is to verify the impact of SO_2 on fertility in the population.

The probability of conceiving during the menstrual cycle—fecundability—varies considerably, even for healthy and reasonably sexually active pairs in the human population. Some proportion of couples will conceive in the first unprotected menstrual cycle (FUMC), but others need more time to become pregnant. Little is known about the particular causes of these differences (*9*). Obviously, this variation is mostly due to the biologic and social differences among parental pairs (*10*); however, fecundability may be also influenced by environmental factors such as temperature, photoperiodicity, and food availability or quality (*11*). There is even growing evidence that some occupational (*12–14*) and lifestyle factors (*15,16*) and some environmental noxae can adversely affect human fertility (*7,17*).

Exogenous factors may influence the reproductive ability of parental pairs by affecting different functions in various levels and stages of the reproductive process (*17*); biologic as well as behavioral functions of one or both partners may be impaired. Couples exposed to adverse factors take longer time to achieve a clinically recognizable pregnancy. Therefore, the common consequence of such effects—irrespective of the particular mechanism—may be a conception delay. Thus, fecundability can be simply measured as the number of nonprotected menstrual cycles (MCs) required to become pregnant. This approach called time to pregnancy (TTP) was introduced in the late 1980s (*13,18*) and it has been useful in numerous epidemiologic studies (*19–23*).

A conception is usually not recognized before the 5th week of gestation, and only approximately two-thirds of pregnancies reach this stage (*24,25*). Data about TTP obtained from parents can be related only to recognized pregnancies. These data reflect not only the effects of factors that reduce the probability of conception itself (interfering with gametogenesis, transport of gametes, or their fertilization ability); they also include the effects of losses of conceived fetuses (loss during transport of zygotes, failure to successfully implant, and especially subclinical abortion).

The TTP approach does not allow for differentiating among these mechanisms. However, it may be sensitive enough to determine relatively weak effects on human fertility that could be expected for such common toxins as air pollutants. Thus, we applied a modified version of the TTP method in the present study to investigate the impact of SO_2 on human fertility.

Materials and Methods

The present study was designed as a prevalence population study with interviews at delivery. The background sample included all full-term singleton births in the district of Teplice between April 1994 and March 1998. We excluded all couples who admitted that they "did something to prevent the index pregnancy" or they "were treated for fertility disorders." Only spouses of European origin were enrolled to avoid additional variability related to ethnic and cultural differences. The sample was further restricted to the mother's first delivery during the study period. The completion of a written informed consent form was the final condition for enrollment in the study.

Personal and lifestyle data were obtained via questionnaires and medical records. Self-administered maternal questionnaires were completed in the hospital after delivery, with the assistance of a specially trained nurse. These data included occupational and other

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risks, smoking, and consumption of alcohol, health status and medication and detailed information on reproductive history and habits. The main questions concerning TTP from the standard short questionnaire recommended by Baird et al. (*26*) were included:

> 1) Were you (or your partner) doing something to prevent pregnancy at the time you most recently got pregnant? If no: 2) Did you get pregnant during the first menstrual cycle of unprotected intercourse? 3) the second 4) the third 5) other? Please specify which cycle …

Mothers who answered "yes" to question 1 were excluded from the study.

Because the fathers were not interviewed, the women were proxy reporters for their partners' information; more data are therefore missing for paternal characteristics. Hospital staff abstracted medical and health care data on the course and outcome of the index pregnancy from the clinical records.

We estimated the gestational age in weeks using each woman's prenatal history log (her maternity card), which included her reported last menstrual period (LMP), plus data on prenatal visits, ultrasound measurements, etc. We calculated the estimated date of conception (EDC) using the gestational age and correcting from the LMP (decreasing the gestational age by 2 weeks) (*5*).

In the original version of the TTP method, an effect on fecundability is measured by comparing the distribution in exposed and nonexposed couples of the number of menstrual cycles required to become pregnant. In this arrangement, a single exposure estimate is not meaningful for permanently changing exposures such as air pollution. On the other hand, the exposure and its timing can be easily defined for parents who become pregnant in the FUMC. Moreover, according to Baird et al. (*9*), the proportion conceiving in the first cycle gives an unbiased estimate of the mean fecundability in the cohort.

Based on these considerations, we developed a simplified version of the TTP method: only couples achieving a clinically recognized pregnancy in the FUMC were categorized as successfully "conceived"; others were classified as "nonconceived." This approach examines whether the proportion of women who become pregnant in the FUMC may differ according to the exposure of the parents to air pollution before conception. We estimated the exposure of each parental pair using mean 30-day averages of SO_2 levels in each of 4 months before the EDC. We used three empirically chosen concentration intervals for SO_2 according to exposure distribution in population (low, < 40 µg/m^3; medium, 40 to > 80 µg/m^3; and high, ≥ 80 µg/m^3). The cutoffs were near tertiles in the first 2 years of study (*27*); they fell to 55, 25, and 20%, respectively, during the last 2 years as a consequence of the downgrading of SO_2 in ambient air. We sorted parental pairs by exposure to SO_2 levels in the particular month before pregnancy and by conception success in the FUMC. Conception rates in the FUMC were estimated for each concentration interval and each month before pregnancy.

Centralized air pollution monitoring in the Teplice district was organized in cooperation with the U.S. Environmental Protection Agency (U.S. EPA) (*28*). The monitoring station was located in the center of town of Teplice. Concentrations of SO_2 were measured continuously by the pulse fluorescence method using model 43A (Thermo Environmental Instruments, Inc., Franklin, MA). Several other pollutants were also measured, including nitrogen oxide, particulate matter, and polycyclic aromatic hydrocarbons.

Individual exposures to SO_2 were derived from centralized monitoring of ambient air. To reduce inevitable misclassification and improve the exposure estimate, we used some additional variables. A unified system of protection for inhabitants has been in place in the Teplice District since 1993. In periods with extremely high air pollution levels (for example, meteorologic inversion) district authorities broadcast a special signal via local media and establish a special phone service [3-hr average SO_2 > 400 µg/m^3 or the sum of 24-hr average SO_2 + (2 times total suspended particles) > 750 µg/m^3]. Inhabitants are encouraged to reduce outdoor activities. They are also encouraged to delay or reduce airing of their homes. Information about the timing of such signals was obtained from district authorities. A variable signal was defined as "yes" for each 30-day period that included a high-pollution episode, under the assumption that behavior would be altered during those times, resulting in a reduction of personal exposure.

Two other approaches were applied to take into account the influence of nonstandard behavior of inhabitants during inversions: we excluded all parental pairs who were considered exposed to extreme levels of pollution before conception. Two versions of this sample restriction were *a*) the exclusion of couples who were considered to be exposed to monthly mean concentrations > 110 µg/m^3, which were observed only in rare inversion episodes, and *b*) the exclusion of couples who were exposed to inversion situations during the 4 months before conception.

We expected that the authenticity of the exposure estimates would be higher for couples living nearer to the monitor irrespective of the distances of sources or the wind direction. To test this presupposition, we analyzed the relationship between estimated exposure to pollution and fecundability separately for parents living at different distances from the centralized monitor. For this purpose, parental pairs were classified into two groups (in and out) according to the distance of maternal permanent residence from the monitoring station in the year before delivery. Couples living within 3.5 km of the monitoring station were classified as in; all others were classified as out (Figure 1).

Many characteristics are related to human fertility (*17,26*) and to SO_2 exposure. We initially examined the relationships between fecundability in the FUMC and the characteristics of the parents using *t*-test and chi-square analyses. These results were used to construct logistic regression models.

We estimated adjusted odds ratios (AORs) and their confidence intervals (CIs) and Wald's chi-squares using logistic regression procedures (*29*). Conception in the FUMC resulting in a live birth was entered as the outcome measure in logistic regression models. A spectrum of parental characteristics associated with the outcome (fertility) and some parameters associated with the exposure (pollution) were tested for inclusion in the final model. These characteristics were maternal age (< 19, 19–34, ≥ 34 years), maternal and paternal education (basic, high school, university), marital status (currently married, other), parity (first, second or third, higher), spontaneous abortion (< 2, > 1), induced abortion (< 2, > 1), alcohol habits of mother and father (< 1 drinks in a week, other), maternal smoking before conception (0, 1–9, > 9/day) passive smoking (0, 1–9, > 9/day) and paternal smoking (0, 1–9, > 9/day), employment of mother and father (employed yes or no), and occupational risk of mother and father (yes or no). Eight types of occupational risk (as radiation, chemicals, dust, infection, etc.) were screened in the models.

A possible confounding effect due to other pollutants was also taken into account. The findings of several preliminary studies showed that simultaneous inclusion of highly correlated variables in one model gives rather misleading results (*27,30*). The mutual correlations between levels of SO_2 and such pollutants as particulate matter or NO_x were high in both regions studied; therefore, we did not include other pollutants in the models. Although pollutant levels were associated with season, season by itself might also be a surrogate for other changes such as weather patterns and the consumption of fruits and vegetables. We defined summer as the months from April through September and winter as the months from October through March. We also tested other definitions of the seasons using four 3-month annual periods.

Secular and/or seasonal rhythms of conceptions in Czech population were estimated on the basis of 10-year data from official statistics (*31*); a parameter rhythm was introduced, weighing each calendar month by its relative contribution in long-time year totals.

Temperature is an important factor that may influence fertility (*11*). For that reason, we introduced temperature in logistic models as expressed in four variables: avg (monthly average temperature), avg_{max} (monthly average of daily maximum temperatures), max (dummy variable introduced for months that included > 10 days that were warmer than 80% of the days in a year), and avg_{min} (monthly average of daily minimum temperatures). Common respiratory infections such as influenza are more frequent in the winter. The sexual behavior of couples (coital frequency) may change during such periods. We defined a weekly incidence above 1,500/100,000 of acute respiratory diseases in the Teplice District as an epidemic situation. Using a dummy variable epidemic situation, couples were differentiated according to their exposure to this situation during the particular month before conception; we tested the variable for inclusion into logistic models.

Because air pollution levels have changed in Teplice District over time, the potential for secular changes was evaluated by comparing results from the two 2-year periods (period I, from April 1994 to March 1996 and period II, from April 1996 to April 1998).

Each month before pregnancy was analyzed separately, allowing some factors to vary over time (e.g., pollution levels, temperature, and season). Some factors considered for analysis were highly correlated (e.g., mother's active and passive smoking and paternal smoking; temperature variables; season and conception rhythms). Therefore, development of the final model used a stepwise approach to select the most appropriate factors. After this initial stage, we examined other factors for potential confounding by examining AORs of SO_2 exposures to see how much change results from inclusion versus exclusion of the potential confounder. Change by > 15% (> 0.15) for beta was used as a reasonable criterion for inclusion (*29*). We used SAS program 6.12 for statistical analysis (*32*).

Results

There were 3,651 singleton live births to parental pairs between April 1994 and March 1998 in Teplice District. More than one quarter of the parental pairs [964 (26.4%)] admitted they did something to prevent the index pregnancy and were therefore excluded from the study; another 102 were excluded because of previous consultation or treatment for infertility. Of the 2,585 parental pairs included, 587 (22.7%) conceived in the FUMC.

Monthly mean levels of SO_2 during the 4 years of the study are shown in Figure 2. Average concentrations were generally lowest during summer and highest during winter. Annual mean levels of SO_2 decreased consecutively during the 4 years of the study: 1994–1995 = 54.3 μg/m^3, 1995–1996 = 50.5 μg/m^3, 1996–1997 = 48.9 μg/m^3, and 1997–1998 = 38.0 μg/m^3. The overall decrease would have been greater had it not been for an inversion episode (20 days of extreme SO_2 levels from 20 December 1996 through 20 January 1997) (Figure 2). The mean levels of SO_2 in 2-year periods decreased from 53.6 ± 27.6 μg/m^3 in period I to 44.6 ± 35.7 μg/m^3 in period II.

Results of descriptive analysis are presented in Table 1. They tend to suggest that only a few characteristics of parents who conceived in the FUMC differ significantly from those of less successful pairs. Couples who became pregnant in the FUMC were significantly more frequently single (32.2 vs. 26.7%; $p < 0.01$) and conceived more often during the summer months (55.2 vs. 50.2%; $p < 0.05$) compared to the others. Mothers from successful pairs were more frequently unemployed or employed without occupational risk.

Table 2 presents the results of multivariate analysis of the impact of SO_2 exposure in different months before conception on fecundability in the FUMC. AORs for conception in the FUMC were consistently reduced, with higher levels of SO_2 in the second month (30–60 days) before conception. The AOR for the medium SO_2 level was 0.57 (CI, 0.37–0.88; $p < 0.011$); it was 0.49 (CI 0.29–0.81; $p < 0.006$) for the high level. No significant association was observed in any other period before conception (Table 2).

We conducted a similar analysis for those parental pairs delivering in the first and the second 2-year periods (n = 1,527

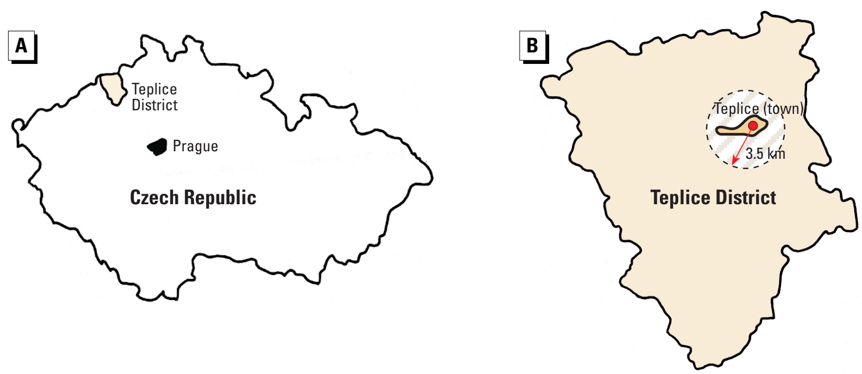

Figure 1. (*A*) The Czech Republic showing Teplice District. (*B*) The Teplice District. The dotted circle indicates the "in" area (up to 3.5 km away from the monitoring station in the center of the city). Approximately one-half of the couples (n = 1,297) were living in this area (according to the permanent residence of the mother).

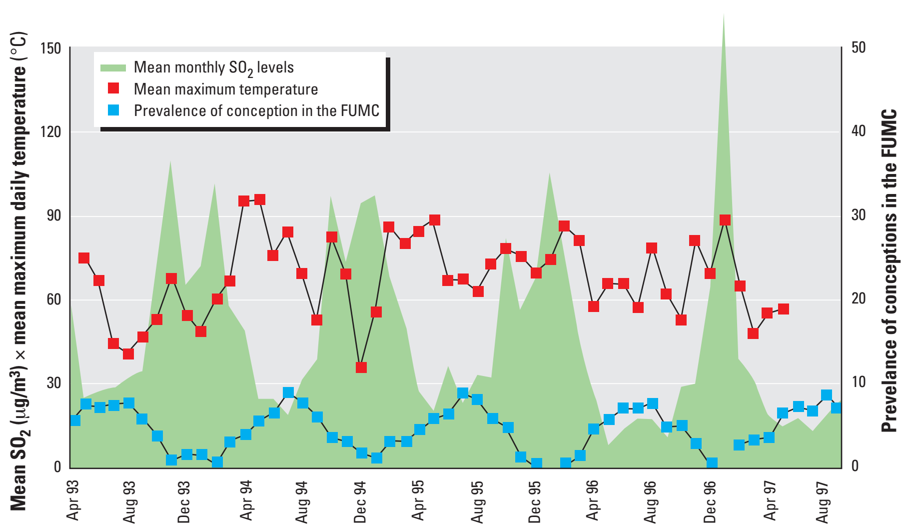

Figure 2. 30-day running averages of SO_2 levels (in micrograms per cubic meter) and 30-day maximal daily temperatures (°C) compared to percent conception in the FUMC by the second month before conception.

and 1,058 couples, respectively). The only clear-cut relationship between fecundability and SO_2 was observed for pairs delivering in period I in the second month before conception: for medium SO_2 levels the AOR = 0.49 (CI, 0.25–0.96; $p < 0.037$) and for high levels the AOR = 0.43 (CI, 0.20–0.93; $p < 0.033$). In period II there was a consistent but nonsignificant tendency (Table 3).

The variable signal was associated with SO_2 exposure. Inclusion of this covariate into logistic models tended to decrease slightly the AORs for the fourth and first-months before conception; we did not see an influence on the AORs for the third and second preconceptional months. Another way to reduce possible distortion of the association between exposure and effect during inversion episodes was to exclude cases exposed to inversion from the sample. After the exclusion of 63 parental pairs exposed to monthly means > 110 µg/m^3 from the whole sample, the AORs for both medium and high SO_2 levels were further reduced (0.51 and 0.42 instead of 0.57 and 0.49), and significance levels were higher (Table 4). This approach was also applied to the analysis of samples for periods I and II. The exclusion of exposures > 110 µg/m^3 did not change the results in period I. In contrast, the results for period II change considerably when high exposures are excluded (Tables 3 and 4): the AOR for medium exposures was 0.54 (CI, 0.28–1.07; $p < 0.08$) and the AOR for high exposures was 0.44 (CI, 0.18–1.09; $p < 0.08$). Thus, after exclusion of extremely high exposures, the results in the second 2 years of the study are similar to those from the first 2 years, though with the marginal significance only. The results of the alternative analysis of the samples after exclusion of the 92 couples exposed to inversions before conception yielded similar results (Table 4). Approximately one-half of the couples (1,297) lived up to 3.5 km away from the central monitor (classified as "in") (Figure 1). Results in Table 5 show that the association between conception success in the FUMC and SO_2 exposure in the second preconception month is greater for the group who lived closer to monitor ("in"). In the "in" group, the AOR of conception in the FUMC was consistently decreased for medium (0.56; CI, 0.31–1.00) and high (0.36; CI, 0.17–0.73) SO_2 exposure. The fecundability/SO_2 association in the "out" group was weaker and nonsignificant (Table 5). Excluding both the "out" group and the inversion cases magnified even more the effect of SO_2 on fecundability in the second month before conception (Table 5).

Table 1. Background characteristics of study groups.

Variable	Conceived in the FUMC (n = 587) Mean ± SD	No. (%)	Conceived later (n = 1,998) Mean ± SD	No. (%)
First 2 years of study (period I)	–	334 (56.9)	–	1,193 (59.7)
Second 2 years of study (period II)	–	253 (44.1)	–	805 (40.31)
Ambient levels[a] of SO_2				
Period I	49.6 ± 27.2	–	54.7 ± 27.7	–
Period II	43.4 ± 34.5	–	48.7 ± 38.9	–
Summer at conception	–	324 (55.2)*	–	1,003 (50.2)
Maternal characteristics				
Age at conception (years)				
< 19	24.6 ± 4.8	38 (6.5)	24.8 ± 4.6	108 (5.4)
20–29	–	469 (79.9)	–	1,606 (80.4)
≥ 30	–	80 (13.6)	–	384 (14.2)
Body mass				
< 22	–	40 (6.8)	–	123 (6.2)
22 to < 27	–	491 (83.6)	–	1,687 (84.3)
≥ 27	–	56 (9.6)	–	188 (9.5)
Parity				
First	1.70 ± 0.97	304 (51.7)	304 (51.7)	1,095 (54.8)
Second–third	–	258 (44.0)	–	833 (41.7)
≥ Fourth	–	25 (4.3)	–	70 (3.5)
Previous abortion				
Spontaneous	0.15 ± 0.43	–	0.16 ± 0.46	–
Induced	0.49 ± 1.00	–	0.38 ± 0.72	–
Employed	–	330 (56.1)	–	1,552 (59.5)
Occupational risk	–	209 (35.5)	–	1,020 (39.1)
Education				
Basic[b]	–	113 (19.3)	–	368 (18.4)
High school[c]	–	446 (76.3)	–	1,547 (77.5)
University	–	26 (4.4)	–	81 (4.1)
Currently married	–	397 (68.0)	–	1,460 (73.3)
Currently single/other	–	187 (32.0)**	–	531 (26.7)
Smoking before conception				
Active	–	226 (38.5)	–	768 (38.4)
Passive	–	318 (54.2)	–	1,110 (55.6)
Cigarettes/day among smokers	11.9 ± 6.1		11.7 ± 7.0	
Alcohol before conception ≥ 1/week	–	106 (20.9)	–	360 (20.4)
Paternal characteristics				
Employed	–	524 (89.1)	–	2,310 (88.6)
Occupational risk	–	302 (51.4)	–	1,354 (51.9)
Education				
Basic[b]	–	76 (13.4)	–	275 (14.2)
High school[c]	–	463 (81.6)	–	1,569 (80.8)
University	–	28 (4.9)	–	97 (5.0)
Smoking	–	312 (55.0)	–	1,081 (56.1)
Cigarettes/day among smokers	14.3 ± 7.3		14.8 ± 9.8	
Alcohol ≥ 1/week	–	251 (55.9)	–	880 (57.4)

[a]Ambient levels of pollutants during the second month before conception. [b]Basic education = approximately 9 years. [c]High school (with maturity exam) = approximately 11–12 years in the Czech Republic during the relevant period. *$p < 0.05$. **$p < 0.01$.

Discussion

Teplice District lies in a highly industrialized mining area of Northern Bohemia with heavy industry and many large power plants using low-energy-quality brown coal with high sulfur content. Pollution reaches its highest levels during meteorologic inversions, which are not infrequent events in this mountainous area. Ambient air monitoring tends to suggest that SO_2 levels were falling during the 4 years of this study. This trend seems mainly to be due to changes in industry profiles, technological improvement of large power plants, and a rapid conversion of local heating systems from coal to gas in the Teplice area.

The longitudinal version of the TTP method presents a problem for evaluating the impact of air pollution on fecundability. All potential parents were continually exposed in various periods before a particular conception and the levels of pollutants are continually changing. Appropriate comparisons for couples conceiving in the FUMC are easier to achieve. Baird et al. (*9*) suggested that data about the proportion conceiving in the first cycle give an unbiased estimate of the mean fecundability in the cohort, providing a rationale for the design of the present study. In this approach, information concerning the distribution of later conceptions is lost. On the other hand, a bivariate outcome measure and yes or no responses make it possible to apply logistic regression (*9*), a powerful device for controlling potentially confounding covariates. It should be emphasized that the approach used is based on data obtained at delivery; therefore, all early losses were included within the nonconceived group; on the other hand, all conceptions that resulted in clinical spontaneous or induced abortion were omitted.

We used the EDC as a reference date for analysis. Stolwijk et al. (*33*) recently showed that this approach might involve substantial bias arising from a seasonal pattern of pregnancy planning. The authors instead recommended using the date of onset of TTP, which is not biased in this manner. This recommendation cannot be followed in the present study, as data about TTP onset cannot be obtained from couples with longer conception delay. Therefore, we included annual conception rhythms observed longitudinally in the Czech population in logistic models; thus a possible influence of seasonality in pregnancy planning was reduced. According to Stolwijk et al. (*33*), a residual effect of this bias can cause an underestimation of the strength of the relation, but not a change in the direction of the effect estimators.

Results of descriptive analysis suggested that parental pairs conceiving in the FUMC did not differ from less successful couples in most of the characteristics listed in Table 1. Mothers conceiving in the FUMC were more frequently single and unemployed. The employed women from this group were less likely to be exposed to occupational risk. After the inclusion of particular occupational risks into logistic models, the AOR of conception in the FUMC was significantly reduced for mothers exposed to any risk (0.75; CI, 0.58–0.97), and to radiation (0.30; CI, 0.69–1.12); the same is true for fathers exposed to dust (0.64; CI, 0.46–0.89). Inclusion of these variables in the models did not influence the final association between SO_2 and fecundability in the FUMC. Success in the FUMC is more frequent during the summer (24.4%) than during the winter (20.9%). This could be due to better opportunities for intercourse during vacation time and to other supporting influences of summer (*10*). Moreover, couples successful in the FUMC were exposed to lower levels of SO_2 during the second month before conception (Table 1). Thus, association between SO_2 and fecundability may also contribute to higher conception success in the FUMC during summer.

We used monthly means of SO_2 to characterize the exposure. We examined shorter as well as longer periods in preliminary investigations to find an optimal measure. Misclassification of exposure estimates may be frequent, and the accuracy of the EDC is limited in the present study. An interval shorter than 30 days is inadequate to ensure the reliability of data. On the other hand, the biologic sensitivity window (e.g., in the case of influence on spermatogenesis) can be relatively narrow, on the order of weeks. To hit this small target using such an inaccurate device requires choosing the optimal interval, such as 1 month, because only analyses based on 30-day periods yielded consistent results.

Ambient SO_2 is only one of many components of a complex mixture. The possible effects of SO_2 and four other noxae, namely NO_x, particulate matter ≤ 10 µm in aerodynamic diameter (PM_{10}), particulate matter ≤ 2.5 µm in aerodynamic diameter, and polycyclic aromatic hydrocarbons, on fecundability were examined in two preliminary studies using the same approach (*27,30*). Levels of all five noxae were mutually highly correlated in a range of 0.55–0.83. Analyzing each pollutant in a separate model, we observed the only consistent relationship to fecundability was that for SO_2. A much weaker association with PM_{10} could be explained by the high correlation between SO_2 and PM_{10} levels ($r = 0.83$; $p < 0.0001$). An analysis of the simultaneous effects of the two pollutants in one model yielded an increased SO_2 effect (AORs for SO_2 40–80 µg/m^3 = 0.53; CI, 0.39–81 and AORs for SO_2 ≥ 80 µg/m^3 = 0.41; CI, 0.25–0.70) and eliminated any suggestion of PM_{10} association (AORs for

Table 2. AORs of the fecundability in the FUMC by exposure to SO_2 before conception.

	Medium[a]			High[a]		
Month	AOR[b]	CI	*p*-Value	AOR[b]	CI	*p*-Value
- 4	1.32	0.90–1.91	0.15	0.93	0.57–1.51	0.78
- 3	0.95	0.63–1.48	0.75	0.90	0.55–1.48	0.68
- 2	0.57	0.37–0.88	0.011	0.49	0.29–0.81	0.006
- 1	1.01	0.68–1.51	0.95	0.96	0.58–1.58	0.86

Four years: April 1994 to March 1998, European births in Teplice (n = 2,585).
[a]Low < 40 µg/m^3 (reference level); medium 40 to < 80 µg/m^3; and high ≥ 80 µg/m^3. [b]Adjusted for maternal age, parity, conception seasonality, currently married, temperature average, temperature maxima, signal, year and season, and epidemic situation.

Table 3. AORs of the fecundability in the FUMC by exposure to SO_2 before conception.

	Medium[a]			High[a]		
Month	AOR[b]	CI	*p*-Value	AOR[b]	CI	*p*-Value
First 2 years of study[c]						
- 4	1.58	0.85–2.74	0.16	1.26	0.58–2.71	0.56
- 3	0.88	0.49–1.57	0.66	0.86	0.41–1.82	0.70
- 2	0.49	0.25–0.96	0.037	0.43	0.20–0.93	0.033
- 1	1.14	0.67–1.97	0.62	1.20	0.58–2.48	0.62
Second 2 years of study[d]						
- 4	0.90	0.51–1.61	0.74	0.88	0.41–1.85	0.73
- 3	0.85	0.45–1.57	0.59	0.96	0.45–2.03	0.91
- 2	0.67	0.36–1.28	0.22	0.59	0.36–1.28	0.20
- 1	1.16	0.59–2.29	0.66	1.15	0.59–3.59	0.31

[a]Low < 40 µg/m^3 (reference level); medium 40 to < 80 µg/m^3; and high ≥ 80 µg/m^3. [b]Adjusted for maternal age, parity, conception seasonality, currently married, temperature average, temperature maxima, signal, year and season, and epidemic situation. [c]April 1994 to March 1996, European births in Teplice (n = 1,527). [d]April 1996 to March 1998, European births in Teplice (n = 1,058).

Table 4. Effect of exclusion of extremely high exposures to SO_2 in the second month before conception.

SO_2 exposure	Medium[a]			High[a]		
in the second month	AOR[b]	CI	*p*-Value	AOR[b]	CI	*p*-Value
All exposed[c]						
I	0.49	0.25–0.96	0.037	0.43	0.20–0.93	0.033
II	0.67	0.36–1.28	0.22	0.59	0.36–1.28	0.20
Total	0.57	0.37–0.88	0.011	0.49	0.29–0.81	0.006
Exposed to < 110 µ/m^3[d]						
I	0.49	0.25–0.96	0.038	0.43	0.20–0.93	0.033
II	0.54	0.28–1.07	0.08	0.44	0.18–1.09	0.08
Total	0.51	0.33–0.80	0.004	0.42	0.25–0.71	0.001
Exposed to inversion situation[e]						
I	0.49	0.25–0.96	0.037	0.43	0.19–0.93	0.033
II	0.54	0.27–1.09	0.08	0.41	0.17–0.99	0.049
Total	0.51	0.33–0.81	0.004	0.42	0.25–0.72	0.002

First and second 2-year periods of study.
[a]Low < 40 µg/m^3 (reference level); medium 40 to < 80 µg/m^3; and high ≥ 80 µg/m^3. [b]Adjusted for maternal age, parity, conception seasonality, currently married, temperature average, temperature maxima, signal, year and season, and epidemic situation. [c]n = 2,585. [d]n = 2,522. All mean month SO_2 exposures > 110 µg/m^3 occurred during the inversion episode from 15 December 1996 through 20 January 1997; thus, only results for the second 2-year period II (April 1996 to March 1998) were influenced. [e]n = 2,493. Results of analysis after exclusion of couples exposed to the inversion situation did not differ from those calculated after exclusion of extremely exposed parents. Either extremely high exposures or altered behavior of people during the inversion episode 15 December 1996 through 20 January 1997 might distort the SO_2/fecundability detected in this study.

Table 5. Influence of the distance from the monitor on AOR of fecundability in the second month before conception.

	Medium[a]			High[a]		
Distance	AOR[b]	CI	*p*-Value	AOR[b]	CI	*p*-Value
< 3.5 km	0.56	0.31–1.00	0.05	0.36	0.17–0.73	0.005
> 3.5 km	0.58	0.31–1.08	0.09	0.70	0.34–1.45	0.34
Total	0.57	0.37–0.88	0.011	0.49	0.29–0.81	0.006
Combined effect[c]	0.51	0.27–0.95	0.034	0.28	0.13–0.61	0.0012

European births in Teplice: < 3.5 km, n = 1,297; > 3.5 km, n = 1,288.
[a]Low < 40 µg/m^3 (reference level); medium 40 to < 80 µg/m^3; and high ≥ 80 µg/m^3. [b]Adjusted for maternal age, parity, conception seasonality, currently married, temperature average, temperature maxima, signal, year and season, and epidemic situation. [c]Combined effect of the limitation of the distance of residence from the monitoring station (< 3.5 km) and the limitation of considered exposure to SO_2 (< 110 µg/m^3).

PM_{10} 40–50 µg/m^3 = 1.18; CI 0.90–1.56 and AORs for PM_{10} ≥ 50 µg/m^3=1.30; CI, 0.92–1.93) (*30*). These results would be misleading in view of the high correlation of both variables. Therefore, the analysis was concentrated on the effect of SO_2 alone in the present communication. The rather complex question of the simultaneous effects of copollutants will be discussed in a separate study.

Multivariate analysis of data from all 4 years showed a relatively strong inverse association between the concentration of SO_2 during the second month before conception and conception success in the FUMC (Table 2). The AOR of conception in the FUMC was reduced to 0.57 for couples exposed to SO_2 levels of 40–80 µg/m^3 in the second month before conception. This value falls to 0.49 for those exposed to levels > 80 µg/m^3 in the same preconceptional stage. A similar relationship was also observed when data were divided into two subsets corresponding to the first (period I) and second (period II) 2-year periods of the study (Table 3). However, the results show unequivocally that the association is much stronger in period I. The particular AOR values were 0.49 and 0.43 for medium and high SO_2 exposures; lower than for the total 4-year sample (Table 2). On the other hand, the association in period II was much weaker; in fact, it was no longer significant. This tendency may be rooted in the permanent decline of SO_2 levels in the region during the past several years.

Misclassification in exposure estimates is a usual weakness in studies based on centralized monitoring. To reduce the deleterious effects of misclassification, we conducted a sensitivity analysis. The variable signal should correct possible exposure-protective behaviors of people to the warning system during inversion episodes. However, an introduction of this variable into logistic models did not affect the results in any preconception period. Another method used to control the influence of possible protective behavior of inhabitants during inversion episodes was the exclusion of inversion-exposed cases from the analysis. This approach seemed to clarify the exposure/outcome associations. An exclusion of parental pairs exposed to monthly means of SO_2 > 110 µg/m^3 (63 couples) had the same effect as the exclusion of 92 couples who were exposed to an inversion episode before pregnancy (Table 4). The reduction of AORs for medium and high exposures in the second month before conception was stronger and more significant. It may be particularly important that the difference between the effect of SO_2 on fecundability in periods I and II was reduced after controlling for the influence of inversion situations.

We derived the mean monthly exposure of couples to SO_2 from daily measurements by a central monitor. Exposure estimates should be more accurate for persons living near the monitoring station than for others, irrespective of the distance of pollution sources or wind direction. If the relationship between SO_2 and fertility is real, the statistical association between estimated levels of SO_2 and fecundability derived from logistic models should be stronger for the "in" sample. The present results show unequivocally that the relationship between SO_2 and fertility for couples living < 3.5 km from the monitor station is much stronger than for other pairs (Table 5). Approximately one-half of the inhabitants of the district were living inside and the other half outside this area (1,297 and 1,288, respectively): this made the cutoff optimal for comparison of both subsamples. This result strengthens the hypothesis that some part of the variation in fecundability may be explained due to the changes in SO_2 levels.

It has been suggested that temperature may affect human fertility (*11*). Temperature may influence hormonal levels (*34*), frequency of intercourse (*11*), and spermatogenesis (*35*). In our study, average as well as maximal temperatures in the second preconceptional month showed significant association with fecundability in the FUMC in models without pollutants (p < 0.003 and p < 0.03); no consistent association of fecundability with temperature during other months was observed. In models containing SO_2, both maximal and average temperatures significantly influenced AORs, especially in the second month before conception. On the other hand, the influence of SO_2 in models without temperature reduced the fecundability significantly although the AORs were lower than in the complete model [medium SO_2 level AOR = 0.67 (CI, 0.44–0.96; p < 0.03); high SO_2 level AOR = 0.60 (CI, 0.38–0.97; p < 0.03)]. Minimal temperatures did not affect the SO_2/fecundability association and were not included into final models. Success in the FUMC was observed significantly more frequently in the summer (from April through September) than in the winter (Table 1). Conception rates in the FUMC were also positively related to the warmest periods in the logistic models used. These observations are not surprising because the conditions for fertilization are generally more favorable during the warmer months in temperate latitudes (*36*). Loose summer clothing enables better scrotal cooling, which optimizes spermatogenesis and sperm quality. Other influences such as higher coital frequency in the summer can also contribute to this seasonal fluctuation (*37*). It seems that seasonal epidemics of respiratory diseases may influence fecundability. The observed fecundability/SO_2 association in the second month before conception decreased slightly after controlling for the influence of epidemic situations. On the other hand, AORs for other periods before conception tended to decline to unity as if those epidemics may explain a part of the variability in fertility. Some changes in sexual behavior during such epidemics can be hypothesized. Thus, fecundability may vary due to many seasonal factors other than pollution.

Currently, few papers have examined possible associations between human fertility and air pollution. Recently, reduced birth rates were observed during periods of high SO_2 concentrations in the Teplice region (*6,7*). It has been suggested that high SO_2 or some associated pollutants may reduce the ability of oocytes to be fertilized. Jagiello (*8*) previously observed this effect experimentally. Šrám et al. (*7*) hypothesized that an effect of other environmental mutagens, inducing mutations in gametes or early embryos, may be potentiated by SO_2 due to the suppression of DNA repair mechanisms.

Whether SO_2 affects fertility through genetic mechanisms remains an open question. There are conflicting data about the genotoxic effects of SO_2 in humans. Schneider and Calkins (*38*), Nordensen et al. (*39*) and Yadav and Kaushik (*40*) observed clastogenic effects in workers exposed to high concentrations of SO_2. Chromosomal aberrations and sister chromatid exchanges (SCEs) in exposed workers were observed by Meng and Zhang (*41*). On the other hand, Sorsa et al. (*42*) observed neither chromosomal aberrations nor SCEs.

Little is known about the background variability in fecundability in the normal human population (*10,17,26*). It is clear that delays in conception may result from a spectrum of pathogenetic processes in one or both sexes (*17*). The present approach cannot differentiate among the processes involved; therefore, we can only speculate about mechanisms of the observed adverse effects of SO_2. However, the timing of the acute effect coincides with some important stages of the reproductive process in men, namely sperm maturation. This finding is interesting in light of the recent results of Selevan et al. (*43*), in which semen quality was repeatedly analyzed in healthy young men from Teplice. Highly significant adverse but transient effects of increased SO_2 levels on sperm morphology and motility during one spermatogenic cycle were observed in multivariate analysis. The results tended to suggest an effect on spermatogenesis rather than an acute influence on epididymal sperm function. These effects should operate during transformation of the round spermatids into differentiated sperm cells (*43*). These results support our findings with regard to the timing of conception: it is noteworthy that in our results a significant reduction of the conception rate in the FUMC was associated with SO_2 levels only in the second month before fertilization. This is the same period described by Selevan et al. (*43*) as the most probable stage of sperm maturation damage associated with SO_2 exposure. In addition, these authors' results contribute to the substantive question of the relationship between SO_2 and fertility: there are indications that the type of sperm damage observed by Selevan et al. (*43*) may actually reduce fertility (*44*).

In spite of this late preconception period, an interference of SO_2 or some copollutant with the respective stages of oogenesis cannot be excluded. Damage to gametes might reduce the efficiency of reproduction, subsequently affecting fertilization, implantation, and early embryogenesis. All of those events could increase the risk of early losses (subclinical abortion). On the other hand, toxic pollutants may also interfere with these processes directly.

We are aware that our results are based on some data of unequal reliability. Some questionnaire data (parental ages, date of delivery, and other personal data) were cross-checked using other information sources. Pollution data were measured using the regularly calibrated equipment and standardized methods developed by the U.S. EPA; these data should be reliable. However, we made an important assumption in the assignment of exposure to different time periods: the measured exposure level at the central monitoring station may not be entirely representative of an individual woman's or man's exposure. Wide variations in exposure may result from individuals living at various distances from the monitor, varying wind conditions, varying personal habits, and differences in daily routine. Exposure estimates were more relevant for couples living near the monitoring station (Table 4). The exposure–effect relationship may be distorted in periods with extremely high levels of pollution (inversions).

Another possible source of error in exposure estimation may be incorrect determination of the EDC. Errors in EDC could blur or wholly dissolve the exposure-period relationship; systematic error may shift the important exposure to another time period. To prevent this, we made the EDC determination using maternal prenatal records obtained in early pregnancy. Thus, systematic errors in the EDC are less likely.

Conclusions

The results of the present study suggest that AORs of conception in the FUMC may be reduced in couples exposed to mean SO_2 levels > 40 $\mu g/m^3$ in the second month before conception. No consistent relationship was observed in any other period during the 4 months before conception. The exposure–effect association tends to be strengthened by the exclusion of couples living larger distances from the monitor station and/or couples exposed to extreme inversion situations, when behavior may alter exposures. The timing of the effect coincides with the sperm maturation period. These results are in agreement with the findings of Selevan et al. (*43*), who observed spermatogenesis damage in the same stage in young healthy men exposed to ambient SO_2; the observed types of damage may reduce fertility.

The impact of preconceptional exposure to other pollutants will be evaluated in the future using the same methods.

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