# Semen Quality and Reproductive Health of Young Czech Men Exposed to Seasonal Air Pollution

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This study of male reproductive health in the Czech Republic resulted from community concern about potential adverse effects of air pollution. We compared young men (18 years of age) living in Teplice, a highly industrialized district with seasonally elevated levels of air pollution, to those from Prachatice, a rural district with relatively clean air. Surveys were scheduled for either late winter, after the season of higher air pollution, or at the end of summer, when pollution was low. Participation included a physical examination, donation of a semen sample, and completion of a questionnaire on health, personal habits, and exposure to solvents and metals through work or hobby. Analysis of data from 408 volunteers showed that the men from Teplice and Prachatice were similar in physical characteristics, personal habits, and work- or hobby-related exposures. Sixty-six percent (272) of these men donated a single semen sample for routine semen analysis, computer-aided sperm motion analysis, and sperm chromatin structure assay. The mean (median) sperm concentration and sperm count were 61.2 (44.0) million/mL semen and 113.3 (81.5) million, respectively, and were not associated with district of residence or period of elevated air pollution. However, periods of elevated air pollution in Teplice were significantly associated with decrements in other semen measures including proportionately fewer motile sperm, proportionately fewer sperm with normal morphology or normal head shape, and proportionately more sperm with abnormal chromatin. These results suggest that young men may experience alterations in sperm quality after exposure to periods of elevated air pollution, without changes in sperm numbers. *Key words*: air pollution, epidemiology, human, semen, sperm chromatin, sperm count, sperm morphology, sperm motility. *Environ Health Perspect* 108:887–894 (2000). [Online 2 August 2000]
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Air pollution in the Czech Republic increased dramatically with the advent of industrialization in the 1950s, primarily the result of increasing use of brown coal (with high sulfur content) for both home heating and industry. Sulfur dioxide emissions in Czechoslovakia amounted to 0.9 million tons in the 1950s and increased to 3.5 million tons by 1985 (*1*). This increase was particularly pronounced in the mountainous region of Northern Bohemia, where coal comes from mammoth open-pit mines and is used to heat homes and generate power for local industry. During the 1980s, ambient $SO_2$ levels associated with high levels of particulate matter (PM) in the Teplice district of Northern Bohemia frequently exceeded U.S. and Czech air pollution standards (*2*,*3*) in winter, when the use of coal increases and thermal inversions favor retention of the air pollution in the valley (*4*).

The Teplice Program, an international research program, was initiated in 1991 in response to concerns over potential health effects of this pollution. This program sponsored cooperative research among the Czech Institute of Hygiene, the Czech Ministry of the Environment, and the U.S. Environmental Protection Agency to compare health status in Teplice district to that in Prachatice district (*5*). We chose Prachatice because of its relatively cleaner air. A critical component of this program was the establishment of air monitoring in both districts to measure aerosol and gas-phase air pollutants [PM, including volatile and semivolatile polycyclic aromatic hydrocarbons (PAHs) and toxic metals] as well as $SO_2$, nitrous oxides ($NO_x$), and carbon monoxide on an ongoing basis. Monitoring confirmed that levels of these air pollutants were considerably higher in Teplice than in Prachatice, and were higher in the winter than during the rest of the year in both districts (*5*,*6*). The Teplice Program includes studies of a number of health outcomes, including respiratory and neurologic effects in children, biomonitoring of mutagens in adults, and reproductive health in pregnant women and young men (*5*).

Reproductive health studies were prompted by reports that rates of conception and incidence of congenital anomalies were affected by seasonal increases in air pollution (*7*). To examine the potential relationship between the season of elevated air pollution and male reproductive health, we surveyed young (18-year-old) men and evaluated their semen quality. Metabolites of the PAHs present in this industrial air pollution can form protein or DNA adducts in body tissues (*8*) and thus have the potential to damage germ cell DNA. PAHs also reportedly alter male reproductive function in test species (*9*,*10*), providing additional rationale for this study. Furthermore, metals such as lead and cadmium that are present in the particulate fraction of air pollution have been associated with decrements in human semen quality (*11*).

## Methods

This project was a collaborative effort between Czech and U.S. scientists. The study protocol was reviewed and approved by the Institutional Review Board of the Regional Institute of Hygiene of Central Bohemia, Prague, Czech Republic.

*Subject recruitment.* All young men turning 18 in the two districts over the 6 months before sampling were sent a letter from their district Hygiene Station with an appointment for a physical examination. Appointments were scheduled within 1 week's time in either early fall (1993) or late winter (1993 and 1994) to allow comparisons between recent exposures to periods of either high (winter) or low (summer) pollution. When each man presented for his physical examination, the reproductive study was explained to him,

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including his right to decline to participate and/or donate a semen sample, and written informed consent was obtained from each participant. No financial incentive was provided for participation. A pilot study was conducted in fall 1992 to estimate participation levels, establish field methods for the laboratory measures, and field test the questionnaire. Because methods for recruiting the participants in the pilot study differed from those used in the main study, data from the pilot study are not included in this report. However, a preliminary summary report of the study findings included the pilot data (*5*).

*Questionnaire, physical examination, and semen sampling.* The Czech study team traveled to the Teplice and Prachatice District Institutes of Hygiene for each sampling cycle (5 days) with the necessary supplies and equipment. Data were collected by questionnaire, physical examination, and semen sampling. A structured questionnaire, customized for use in the Czech Republic, was given by two trained interviewers. The purpose of the questionnaire was to obtain information on health status; other exposures such as metals, solvents, or pesticides encountered through hobbies or work (for those men undertaking apprenticeships); lifestyle data; and reproductive history including the date of last semen emission. Questions on other factors that could affect semen quality (such as fever, medications, exposure to X rays, cigarette use, consumption of alcohol and caffeinated beverages, and use of briefs) covered the previous 3 months. The physical examination included a urogenital evaluation and determination of testis volume based on caliper measures of testis length and width.

A single semen sample was collected on site by masturbation and sperm were videotaped within 1 hr of collection for subsequent motility analysis. Routine semen analysis included semen volume, sperm concentration [determined by hemocytometer according to World Health Organization guidelines; WHO (*12*)], total number of sperm per sample, percentage of motile sperm, percentage of sperm with normal morphology (entire cell considered), and percentage with normal head morphology. For the morphology assessment, we evaluated 300 sperm per sample from air-dried Papanicolaou-stained preparations and classified as either normal or abnormal according to strict criteria (*12*). The remaining semen was aliquoted into small tubes or straws, snap frozen on dry ice, and archived at –70°C.

*Computer-aided sperm analysis (CASA).* Within 1 hr of collection, an aliquot of semen was diluted at least 1:1 with Dulbecco's phosphate buffered saline to obtain a concentration suitable for CASA analysis (*13*), loaded into a 20-micron-deep chamber (Microcell, Fertility Technologies, Inc., Natick, MA), placed on a stage warmer set to 37°C, and videotaped (10× negative phase contrast with green filter). Video images were evaluated using a Hamilton-Thorne Integrated visual optical system (HTM-IVOS) semen analyzer (version 10.6; Hamilton-Thorne Research, Inc., Beverly, MA). We analyzed each field for 30 frames at 60 frames/second with minimum track length set at 20 points and examined enough fields to obtain velocity measures on at least 100 motile sperm. For oligospermic samples with < 100 motile sperm on the tape, mean velocities are included only when ≥ 25 motile sperm were analyzed. We visually determined the percentage of motile sperm from the videotapes after scoring at least 100 sperm per sample. Because of technical difficulties, videotapes were unavailable for 10 men evaluated on 1 day in the Teplice (the late winter 1993 group).

CASA outcomes include indicators of *a*) sperm progression: straight-line velocity (VSL), straightness (STR = VSL/VAP × 100, where VAP is the average velocity along a mathematically smoothed sperm path), and linearity (LIN = VSL/VCL × 100, where VCL is the curvilinear velocity); and *b*) vigor: curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF), as described in detail elsewhere (*14*). Some of these outcomes have been associated with fertility status (*15–18*) and have been affected by occupational exposures (*19,20*). We also calculated two composite outcomes: the total number of motile sperm per sample (sperm count × %motile) and the total number of progressive sperm per sample (total motile × an index of progressive motility defined as percent of motile sperm with VSL 25 microns/second or greater).

*Sperm chromatin structure assay (SCSA).* Archived semen was shipped to South Dakota State University (Brookings, SD) for analysis using the SCSA, a measure of sperm nuclear integrity (*21–23*). Briefly, thawed and diluted semen was incubated for 30 sec in acid (pH 1.2) to potentially denature nuclear DNA, then immediately stained with the metachromatic dye, acridine orange (AO). AO intercalated into native double stranded DNA fluoresces green; AO complexed with single stranded DNA fluoresces red. We used flow cytometry to detect green (515–530 nm band pass filter) and red (630 nm long pass filter) fluorescence in 5,000 individual sperm per sample. The presence of DNA denaturation in each cell was observed as a shift from green to red fluorescence and was quantitated by the expression "$\alpha_t$," defined as the ratio of red/(red + green) fluorescence. We used the "cells outside the main population" (COMP $\alpha_t$) variable, which represents the percentage of cells containing denatured DNA. Normal sperm chromatin is resistant to acid induced DNA denaturation and fluoresces green. Increased red fluorescence indicates abnormal chromatin packaging and/or DNA damage. High COMP $\alpha_t$ values have been associated with spermatogenic disorders and infertility (*21–28*).

*Air pollution data and exposure categories.* Air pollution data were provided by I. Beneš and R. Stevens from the air monitoring program of the Teplice Project (*5,6*). These data include particulate matter < 10 µm in aerodynamic diameter ($PM_{10}$) obtained using the versatile air pollution sampler [VAPS (*5,6*)], PM-total suspended particulates (TSP), $SO_2$, CO, and $NO_x$. Because VAPS data were incomplete for earlier phases of the study, we present both VAPS and TSP data. The correlation between TSP and $PM_{10}$ was very high ($r$ = 0.96, $p$ = < 0.01, $n$ =171) for those days where both were available. $SO_2$ data (an indicator of coal-derived pollution) were more complete than PM data. $PM_{10}$ levels were significantly correlated with $SO_2$ ($r$ = 0.81, $p$ < 0.01, $n$ = 274), with $NO_x$ ($r$ = 0.58, $p$ < 0.01, $n$ = 274), and CO ($r$ = 0.49, $p$ < 0.01, $n$ = 252).

The process of spermatogenesis involves a series of complex steps (stem cell replication, meiosis, and spermiogenesis) over approximately 74 days in humans (*29,30*). Epididymal transit time [estimated at 3–12 days (*31*)] and abstinence interval (controlled in the analysis) can add several weeks to the time before mature sperm are ejaculated. Thus an exposure period of approximately 90 days is generally accepted as being of sufficient duration for detecting effects on any stage of spermatogeneis when using semen measures as the biologic end points. Therefore, for purposes of estimating and categorizing exposures relevant to seasonal changes in air pollution, the air pollution data for the 90-day period preceding sampling were considered relevant. Examination of the mean levels of pollutants (Table 1) shows that they were uniformly low in the 90 days preceding the fall sampling periods in both districts and in the late winter sampling period in Prachatice in 1994, and somewhat higher preceding the late winter sampling in Prachatice in 1993. Because all mean values were well below both U.S. (*3*) and Czech standards (*2*), and individual values rarely, if ever, exceeded air quality standards, we considered these to be periods of low air pollution for the purposes of this study. In contrast, the mean levels of air pollutants were considerably higher in Teplice during the 90 days preceding the late winter samplings (Table 1), with 1993 levels considerably higher than 1994 levels. Furthermore, individual daily

values frequently exceeded air quality standards. For descriptive purposes, we therefore considered winter 1993 in Teplice as a period of high air pollution and the winter of 1994 in Teplice as a period of medium air pollution. Air pollution peaks were episodic; severe episodes lasted a few days to a week (*6*). If such an episode were to affect a particular cell type in the testes, the time between exposure and sampling would be important in detecting that effect. Because the time between severe episodes and sampling was different in the winters of 1993 and 1994, the exposure/effect relationship could also be different. Consequently, a clear cut exposure–response relationship would not necessarily be expected. Therefore, the high and medium exposure periods were analyzed as dummy variables so as not to impose a linear exposure–response relationship. Furthermore, season of semen collection has also been associated with sperm concentration (*32*) and some measures of sperm motility and morphology (*33*), and needs to be considered when interpreting any associations between periods of elevated air pollution and semen outcomes.

*Statistical analyses.* We entered all data into SAS (SAS Institute, Cary, NC) and calculated summary statistics. Initially we used the Wilcoxon and Kruskal-Wallis tests for unadjusted comparisons between the two districts and the three exposures. We conducted multivariable regression in tiers: first, differences for each outcome were examined by district, then by season, and finally by exposure categories. In addition, season was considered a potential confounder, because it is related to exposure in these communities, for those outcomes with *a priori* data suggesting an association with season (*32*,*33*).

Outcome data were analyzed as continuous variables after transformation as needed to correct for nonnormal distribution: Log (count, concentration, number of progressive sperm, and SCSA COMP$\alpha_t$); square root [percent sperm with normal morphology, percent sperm with morphologically normal heads, semen volume, and curvilinear velocity (CASA)].

In all analyses we assessed risk factors known or suspected to be associated with poor semen quality (*34*) for inclusion in the models. Factors considered included sexual abstinence [< 2 days vs. longer (*35*,*36*)], high fever (> 38°C) within the last 3 months, wearing briefs vs. loose-fitting underwear, alcohol consumption (0–25 mL ethanol/week, 25–199 mL/week, or ≥ 200 mL/week), cigarette smoking [none, < one pack/day, or ≥ 1 pack/day (*37*)], caffeine consumption [< 1/2 coffee cup equivalents, 1/2–3 cups, or ≥ 3 cup/day (*38*)], and hobby or work with solvents (≥ 10 hr/week vs. less) or with metals (≥ 10 hr/week vs. less). The volume of ethanol was estimated based on the self-reported numbers of servings of beer, wine, or liquor consumed per week and the serving size (which is regulated precisely in the Czech Republic and determines the amount of alcohol per drink). We considered other factors such as the use of medications or the presence of genital tract conditions found by physical examination or by questionnaire, but the number of men affected was too small to be informative, and all of the men presented semen within the range of normal values as specified by the WHO (*12*).

## Results

*Participation and description of the study group.* Sixty-one percent of those men who were sent appointment notices came to the study center for their physical examination and completed the questionnaire portion of the study (408 of 670). Reasons for missing the appointment were not ascertained but are unlikely to affect response to the request for

**Table 1.** Air pollution levels in the two communities for 90 days preceding collection of semen samples.

| Location, pollutant | Characteristic | Winter 1993 | Summer 1993 | Winter 1994 | Standards U.S. | Standards Czech |
|---|---|---|---|---|---|---|
| Teplice | | | | | | |
| $PM_{10}$ µg/m$^3$ (VAPS) | Average ± SD | 184.7 ± 211.9 | 35.5 ± 19.7 | 61.3 ± 41.9 | 50[a] | 150[c] |
| | Median | 125.3 | 28.5 | 46.0 | 150[b] | |
| | Range | 10.6–832.0 | 13.6–95.6 | 8.0–183.5 | | |
| | No. days w/data | 38 | 22 | 90 | | |
| | No. days > 150 (%days) | 16 (42.1%) | 0 (0%) | 4 (4.4%) | | |
| PM-TSP µg/m$^3$ | Average ± SD | 195.2 ± 241.0 | 41.5 ± 17.2 | – | | |
| | Median | 86.2 | 37.0 | – | | |
| | Range | 8–960 | 15–80 | – | | |
| | No. days w/ data | 44 | 62 | 0 | | |
| | No. days > 150 (%days) | 15 (34.1%) | 0 | – | | |
| $SO_2$ µg/m$^3$ | Average ± SD | 164.0 ± 161.0 | 30.6 ± 14.9 | 79.8 ± 39.9 | 80[a] | 150[b] |
| | Median | 106.9 | 25.1 | 81.4 | 365[b] | |
| | Range | 14.4–697.9 | 10.6–70.0 | 11.2–230.7 | | |
| | No. days w/ data | 90 | 90 | 90 | | |
| | No. days > 150 (%days) | 26 (28.9%) | 0 (0%) | 4 (4.4%) | | |
| $NO_x$ µg/m$^3$ | Average ± SD | 109.12 ± 72.06 | 42.1 ± 20.1 | 77.6 ± 40.9 | 100[a] | 100[b] |
| | Median | 83.9 | 38.6 | 70.3 | | |
| | Range | 7.20–367.20 | 0–103.4 | 12.8–193.3 | | |
| | No. days w/ data | 90 | 90 | 90 | | |
| | No. days > 100 (%days) | 37 (41.1%) | 1 (1.1%) | 24 (26.7%) | | |
| CO mg/m$^3$ | Average ± SD | 1.72 ± 0.95 | 0.19[d] | 2.76 ± 0.86 | 10[e] | 1[a] |
| | Median | 1.60 | 0.19 | 2.66 | | |
| | Range | 0.0–5.50 | | 0–4.62 | | |
| | No. days w/ data | 90 | 1 | 90 | | |
| | No. days > 10 (%days) | 0 (0%) | 0 (0%) | 0 (0%) | | |
| Prachatice | | | | | | |
| $PM_{10}$ µg/m$^3$ (VAPS) | Average ± SD | 65.9 ± 47.6 | 18.2 ± 8.2 | 29.4 ± 20.3 | 50[a] | 150[c] |
| | Median | 49.8 | 14.6 | 23.0 | 150[b] | |
| | Range | 7.5–174.3 | 6.8–31.2 | 3.1–106.5 | | |
| | No. days w/ data | 25 | 13 | 86 | | |
| | No. days > 150 (%days) | 1 (4%) | 0 (0%) | 0 (0%) | | |
| PM-TSP µg/m$^3$ | Average ± SD | 45.5 ± 30.9 | 24.1 ± 9.2 | 28.5 ± 13.4 | | |
| | Median | 32 | 21 | 24 | | |
| | Range | 13–158 | 12–55 | 13–80 | | |
| | No. days w/ data | 90 | 88 | 90 | | |
| | No. days > 150 (%days) | 1 (1.1%) | 0 (0%) | 0 (0%) | | |
| $SO_2$ µg/m$^3$ | Average ± SD | 41.5 ± 35.3 | 6.1 ± 3.2 | 17.4 ± 12.6 | 80[a] | 150[b] |
| | Median | 33.5 | 6 | 13 | 365[b] | |
| | Range | 7–192 | 1–14 | 1–63 | | |
| | No. days w/ data | 90 | 74 | 90 | | |
| | No. days > 150 (%days) | 1 (1.1%) | 0 (0%) | 0 (0%) | | |
| NOx µg/m$^3$ | Average ± SD | 25.1 ± 29.6 | 19.9 ± 14.0 | 18.2 ± 17.9 | 100[a] | 100[b] |
| | Median | 16 | 17 | 13 | | |
| | Range | 0–140 | 3–70 | 0–87 | | |
| | No. days w/ data | 90 | 88 | 90 | | |
| | No. days > 100 (%days) | 5 (5.6%) | 0 (0%) | 0 (0%) | | |
| CO mg/m$^3$ | Average ± SD | 0.75 ± 0.52 | 0.30 ± 0.11 | 0.53 ± 0.25 | 10[e] | 1[a] |
| | Median | 0.6 | 0.27 | 0.49 | | |
| | Range | 0.20–2.60 | 0.12–0.73 | 0–1.32 | | |
| | No. days w/ data | 90 | 88 | 90 | | |
| | No. days > 10 (%days) | 0 (0%) | 0 (0%) | 0 (0%) | | |

[a]Annual arithmetic mean. [b]24-hr average (in the United States; not to be exceeded more than once a year) (*3*). [c]A limit is not officially determined for $PM_{10}$ in the Czech Republic, but 150 is generally accepted (*2*). [d]Only one sample was available. [e]8-hr average not to be exceeded more than once a year.

semen samples because the appointment notice did not specifically mention a reproductive health assessment. Of the men who completed the physical and questionnaire, 67% (273) agreed to provide a semen sample. One specimen container leaked, which left a total of 272 semen samples available for analysis.

Based on physical examination and questionnaire data, men from Teplice and Prachatice were similar with respect to descriptive factors (height, total testicular volume, age at first semen appearance, cigarette, and alcohol and caffeine consumption) (Table 2). The only differences by district were that on average, men in Prachatice weighed more than men in Teplice and were more likely to consume alcohol. None of the men were judged to be sexually immature on the basis of testis size or physical development. No differences were observed in any of these factors between those who were only interviewed and those who provided semen samples (Table 2). Table 3 shows other potential risk factors and confounders examined for inclusion in the regression models by exposure category. Of these, differences in distribution were noted for amount of alcohol and caffeine consumed per day and for the number of men with > 10 hr/week exposure to solvents or metals through work or hobbies. Several potential risk factors were present in the medical histories of only a few men (mumps, one case; injury to testes, one case; hydrocele, one case; and varicocele, six cases in men from Prachatice and two cases in men from Teplice). These cases were reviewed individually and because each had semen measures above the WHO reference values (*39*), none were excluded from the database.

*Semen quality.* Semen volume and sperm numbers. The mean (median) semen volume for the entire group was 1.96 (1.80) mL and did not differ by district (Table 4). Two men were azospermic (0.5%) (one from each district) but neither was sampled after periods of medium or high pollution. There was no *a priori* reason to exclude them from the analysis; however, excluding them did not alter the sample median (data not shown). The mean (median) sperm concentration for all sampled men was 61.2 (44.0) million/mL semen (Table 4). The current WHO reference value for sperm concentration is ≥ 20 million/mL (*39*). In this group of men, 21% had sperm concentrations < 20 million/mL (21% in Teplice and 22% in Prachatice). No significant difference was observed by exposure category (Table 5).

The duration of sexual abstinence in these young men averaged 4.6 days (± 4.4 days SD; range < 1–31 days), however, 18% were abstinent for < 2 days. Secondary analyses of abstinence data revealed that the best model to correct for short abstinence for this data set was < 2 days versus ≥ 2 days (*40*). After controlling for short abstinence, no relationship was found between sperm concentration or total sperm count and exposure to periods of medium or high air pollution (Table 6).

*Sperm motility and motion.* In all samples, the average (mean) percentage of motile sperm was 33.6% (32.9%) and the mean for Prachatice donors was slightly, but significantly, higher than that for Teplice donors (Table 4). These mean values fall below the WHO reference value for percentage of motile sperm which is > 50% (*38*). Percent motile was also different by exposure category (Table 5). After controlling for appropriate lifestyle factors, the multivariable analysis showed a significant relationship between the percentage of motile sperm and a period of medium, but not high, air pollution; this relationship remained after control of confounding by season (Table 6). This decrease in percent motile sperm translates into a significant exposure-related association with the total number of motile sperm per sample and the total number of progressive sperm (VSL ≥ 25 μm/sec) for the medium exposure group (Table 6). However, after control for confounding by season, these associations were no longer significant.

CASA measures the quality of motion of the motile population of sperm. Although data were generated for seven CASA parameters for each sperm track, some of these measures were significantly correlated with each other and therefore are not independent. For example, VAP was highly correlated with VSL ($r$ = 0.94), STR was highly correlated with LIN ($r$ = 0.85), and three vigor terms were highly correlated: ALH and BCF were highly correlated with VCL ($r$ = 0.89 and $r$ = 0.31, respectively). We selected three measures that describe different aspects of sperm motion: VSL (the absolute distance traveled over time), a measure of progression, VCL (the average point to point velocity), a measure of vigor, and LIN (VSL/VCL × 100), a measure of swimming pattern, and considered them individually. These measures are also relatively independent of the type of CASA instrument used; therefore, results for these measures are more readily compared with those in other studies.

There were no associations between district of residence and any of these three measures (Tables 4 and 6). In contrast, there was a

**Table 2.** Characteristics of 18-year-old males in the study (mean ± SD).

| | All men interviewed | | Semen donors | |
|---|---|---|---|---|
| Characteristic | Teplice ($n$ = 215) | Prachatice ($n$ = 193) | Teplice ($n$ = 154) | Prachatice ($n$ = 118) |
| Height (cm) | 177.6 ± 6.8 | 178.7 ± 7.4 | 177.9 ± 6.8 | 178.9 ± 7.3 |
| Weight (kg) | 71.5 ± 13.2 | 73.9 ± 11.5* | 71.0 ± 11.4 | 74.0 ± 11.4* |
| Total testicular volume ($cm^3$) | 44.0 ± 19.4 | 44.6 ± 19.7 | 43.2 ± 16.7 | 43.1 ± 17.2 |
| Age semen appeared (years) | 14.1 ± 1.1 | 14.1 ± 1.1 | 14.0 ± 1.1 | 14.0 ± 1.1 |
| Smoker? (% yes) | 37.7% | 43.0% | 40.9% | 43.2% |
| Cigarettes/day | 11.6 ± 8.4 | 10.3 ± 7.0 | 11.4 ± 8.0 | 10.7 ± 6.9 |
| Drinker? (% yes) | 70.2% | 80.8%* | 67.5% | 78.8%* |
| Alcohol/week (mL) | 102.1 ± 105.9 | 111.7 ± 132.1 | 109.4 ± 117.1 | 134.5 ± 156.2 |
| Coffee equivalent/day[a] | 1.3 ± 1.3 | 1.3 ± 1.5 | 1.3 ± 1.3 | 1.4 ± 1.4 |
| Work/hobbies with solvents > 10 hr/week? (% yes) | 15.8% | 16.2% | 14.9% | 16.1% |
| Work/hobbies with metals > 10 hr/wk? (% yes) | 13.0% | 11.9% | 14.9% | 12.7%* |

[a]Calculated as in Pastore and Savitz (38). *$p$ < 0.05 by Wilcoxon or Chi Square test comparing Teplice to Prachatice; no differences were found in the comparison of all men interviewed to semen donors.

**Table 3.** Distribution of factors (%) by exposure category[a] in men with semen samples.

| Factor | Low ($n$ = 162) | Medium ($n$ = 63) | High ($n$ = 47) |
|---|---|---|---|
| District* | | | |
| Teplice | 27.2 | 100.0 | 100.0 |
| Prachatice | 72.8 | 0.0 | 0.0 |
| Smokes cigarettes | | | |
| None | 59.3 | 50.8 | 63.8 |
| 1–19/day | 32.7 | 31.8 | 31.9 |
| 20+ /day | 8.0 | 17.5 | 4.36 |
| Drinks alcohol* | | | |
| < 25 mL/day | 33.3 | 47.6 | 48.9 |
| 25–199 mL/day | 51.9 | 42.9 | 38.3 |
| 200+ mL/day | 14.8 | 9.5 | 12.8 |
| Caffeine*[b] | | | |
| None (< 0.5) | 34.2 | 39.7 | 10.6 |
| 0.5– < 3 cups/day | 55.9 | 44.4 | 74.5 |
| 3+ cup/day | 9.9 | 15.9 | 14.9 |
| Abstinence | | | |
| 2+ days | 83.1 | 74.6 | 89.4 |
| < 2 days | 16.9 | 25.4 | 10.6 |
| Fever > 38°C | | | |
| No | 88.9 | 88.9 | 87.2 |
| Yes | 11.1 | 11.1 | 12.8 |
| Wears briefs | | | |
| No | 19.9 | 14.3 | 17.4 |
| Yes | 80.1 | 85.7 | 82.6 |
| Work/hobbies with metals* | | | |
| < 10 hr/week | 90.1 | 84.1 | 74.5 |
| 10+ hr/week | 9.9 | 15.9 | 25.5 |
| Work/hobbies with solvents | | | |
| < 10 hr/week | 85.2 | 90.5 | 74.5 |
| 10+ hr/week | 14.8 | 9.5 | 25.5 |

[a]Low: Prachatice, all samples; Teplice, fall 1993; medium: Teplice, winter 1994; high: Teplice, winter 1993 (see Table 1). [b]Calculated as in Pasture and Savitz (*38*). *$p$ < 0.05 by Mantel-Haenszel chi square.

strong positive association between VSL and LIN, but not VCL, and season (Table 6), suggesting that sperm may swim faster and with straighter paths in the winter. Mean VSL was also higher in samples obtained after exposure to the season of high pollution (winter 1993) (Table 5), but the significance of this association disappeared after control for confounding by season (Table 6). Interestingly, VCL was also higher in this group of samples (Table 5), and this association remained significant (though relatively weak) after control for confounding by season (Tables 5 and 6). This apparent increase in sperm vigor (VCL), without an increase in progression (VSL), resulted in lower mean LIN (Table 5) and a significant negative association between exposure and LIN (Table 6), apparently because mean VCL increased relatively more than mean VSL. With increases in both VSL and VCL, the decrease in LIN would not be considered a sign of weakened-sperm motility. In the samples obtained after exposure to periods of medium air pollution (winter 1994), mean VSL was unchanged but mean VCL was decreased (Table 5) compared to the reference group (periods of low air pollution), resulting in an apparently contradictory positive association between exposure and LIN (Tables 5 and 6). These results may be biased by two factors that reduced the sample size for CASA analysis: *a*) 10 of 47 (31%) samples obtained in winter 1993 could not be analyzed by CASA because of technical problems; and *b*) samples with fewer than 25 motile sperm tracks were not included in the analyses because we did not consider means based on < 25 sperm to be representative of the sample. These samples without CASA data were distributed across exposure groups as follows: 21 of 160 or 13% in the low-exposure group, 3 of 63 or 5% in the medium-exposure group, and 6 of 47 or 13% in the high-exposure group.

*Sperm structure.* The mean (± SD) percentage of normal sperm for all samples was 17.8 ± 8.0% (Table 4). The most recent WHO guidance (*39*) does not specify a reference value for this measure because multicenter population-based studies are underway to derive one using standardized strict criteria (as used in this study) for scoring each cell. Nevertheless, the guidance notes that as sperm morphology falls below 15% normal forms (using strict criteria for scoring sperm as normal), the fertilization rate *in vitro* decreases. We observed significant negative relationships between district of residence and/or exposure to periods of medium or high air pollution and the percent of sperm with overall normal morphology (considering head, midpiece, and tail) as well as the percent with normal head morphology (Tables 4–6). As demonstrated in Tables 4 and 5, we observed significant differences by district alone and by air pollution category alone, but these outcomes were unrelated to season. In the multivariable regression analyses, both the percentage of normal sperm and the percentage of normal sperm heads were significantly

**Table 4.** Semen outcomes: summary and by district.

| | Summary | | | | Prachatice | | | | Teplice | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outcome | No. | Mean ± SD | Median | Range | No. | Mean ± SD | Median | Range | No. | Mean ± SD | Median | Range |
| Production of viable sperm | | | | | | | | | | | | |
| Semen volume (mL) | 272 | 1.96 ± 1.06 | 1.80 | 0.5–6.0 | 118 | 2.09 ± 1.09 | 2.00 | 0.5–6.0 | 154 | 1.86 ± 1.03 | 1.70 | 0.5–5.5 |
| Concentration (millions/mL) | 272 | 61.2 ± 60.9 | 44.0 | 0–456 | 118 | 60.6 ± 66.3 | 39.0 | 0–456 | 154 | 61.7 ± 56.6 | 49.5 | 0–421 |
| Total count (millions/sample) | 272 | 113.3 ± 119.2 | 81.5 | 0–780 | 118 | 119.3 ± 137.0 | 79.0 | 0–780 | 154 | 108.6 ± 103.7 | 82.1 | 0–624 |
| Percent motile* | 256 | 33.6 ± 17.2 | 32.9 | 0–84 | 113 | 36.1 ± 17.9 | 36.0 | 0–75 | 143 | 31.6 ± 16.3 | 31.1 | 0–84 |
| Total motile (in millions) | 256 | 44.2 ± 68.4 | 24.3 | 0–579.7 | 113 | 52.5 ± 82.5 | 27.6 | 0–579.7 | 143 | 37.5 ± 54.2 | 22.5 | 0–398.1 |
| Total progressive (in millions)[a,b] | 228 | 33.3 ± 45.2 | 19.8 | 0.6–354.8 | 105 | 38.6 ± 54.2 | 22.2 | 0.6–354.8 | 123 | 28.9 ± 35.4 | 18.0 | 0.6–261.7 |
| Sperm structure | | | | | | | | | | | | |
| Percent normal morphology* | 262 | 17.8 ± 8.0 | 16.7 | 1–53.5 | 111 | 19.3 ± 8.6 | 17.7 | 1.0–53.5 | 151 | 16.6 ± 7.3 | 16.0 | 1.0–36.3 |
| Percent morphologically normal heads* | 262 | 36.5 ± 10.1 | 35.5 | 10.7–76.0 | 111 | 39.3 ± 11.0 | 39.0 | 10.7–76.0 | 151 | 34.4 ± 8.7 | 33.7 | 15.0–60.7 |
| SCSA COMP$\alpha_t$ | 266 | 20.2 ± 14.0 | 15.9 | 2.0–81.0 | 116 | 19.8 ± 12.1 | 15.9 | 2.7–57.6 | 150 | 20.5 ± 15.4 | 15.8 | 2.0–81.0 |
| Quality of sperm motion—CASA[b] | | | | | | | | | | | | |
| VSL | 228 | 44.3 ± 9.6 | 45.0 | 20.1–72.0 | 105 | 44.1 ± 9.6 | 45.2 | 21.5–65.7 | 123 | 44.5 ± 9.6 | 44.0 | 20.1–72.0 |
| VCL | 228 | 91.8 ± 20.8 | 90.9 | 48.6–139.3 | 105 | 93.0 ± 21.9 | 91.9 | 51.7–139.3 | 123 | 90.7 ± 19.9 | 90.1 | 48.6–132.3 |
| Linearity | 228 | 48.6 ± 8.0 | 49.0 | 28.0–69.0 | 105 | 48.0 ± 8.5 | 48.0 | 31.0–68.0 | 123 | 49.2 ± 7.5 | 49.0 | 28.0–69.0 |

[a]Total progressive = Total motile × percent sperm with VSL > 25 µm/sec. [b]Only for samples with at least 25 sperm tracks; VSL and VCL are in µm/sec. *Different by district, $p < 0.05$ by Wilcoxon test.

**Table 5.** Semen outcomes by exposure.

| | Low | | | | Medium | | | | High | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outcome | No. | Mean ± SD | Median | Range | No. | Mean ± SD | Median | Range | No. | Mean ± SD | Median | Range |
| Production of viable sperm | | | | | | | | | | | | |
| Semen volume* (mL) | 162 | 2.00 ± 1.07 | 1.90 | 0.5–6.0 | 63 | 1.65 ± 0.77 | 1.6 | 0.5–4.0 | 47 | 2.24 ± 1.28 | 2.0 | 0.5–5.5 |
| Concentration (millions/mL) | 162 | 59.9 ± 64.3 | 39.5 | 0–456 | 63 | 65.4 ± 61.6 | 56.0 | 0.1–421 | 47 | 60.1 ± 46.7 | 42.0 | 6–210 |
| Total count (millions/sample) | 162 | 113.5 ± 130.7 | 69.9 | 0.0–780 | 63 | 100.9 ± 97.6 | 82.6 | 0.1–560 | 47 | 129.1 ± 103.1 | 106.4 | 4.5–383 |
| Percent motile* | 156 | 36.2 ± 17.1 | 35.2 | 0–76 | 63 | 27.9 ± 18.1 | 25.0 | 0–84 | 37 | 32.5 ± 13.2 | 33.8 | 0–51 |
| Total motile (in millions) | 156 | 50.6 ± 79.6 | 25.0 | 0–580 | 63 | 29.8 ± 46.6 | 18.0 | 0–337 | 37 | 41.6 ± 40.4 | 33.2 | 0–161 |
| Total progressive (in millions)*[a,b] | 139 | 36.6 ± 50.5 | 20.3 | 0.6–354.8 | 58 | 23.5 ± 36.3 | 17.2 | 0.6–61.7 | 31 | 37.2 ± 31.4 | 28.7 | 2.6–118.5 |
| Sperm structure | | | | | | | | | | | | |
| Percent normal morphology* | 154 | 19.8 ± 8.5 | 18.3 | 1.0–53.5 | 62 | 15.9 ± 5.5 | 14.7 | 3.0–27.3 | 46 | 13.2 ± 6.5 | 13.5 | 1.0–28.5 |
| Percent morphologically normal heads* | 154 | 39.3 ± 10.9 | 39.0 | 10.7–76.0 | 62 | 30.3 ± 6.5 | 29.4 | 16.7–45.7 | 46 | 35.2 ± 6.8 | 35.0 | 23.5–48.0 |
| SCSA COMP$\alpha_t$* | 158 | 19.2 ± 12.2 | 15.6 | 2.7–67.1 | 61 | 16.2 ± 9.3 | 14.5 | 2.0–45.6 | 47 | 28.8 ± 20.4 | 25.7 | 2.9–81.0 |
| Quality of sperm motion—CASA[b] | | | | | | | | | | | | |
| VSL* | 139 | 43.3 ± 10.0 | 44.1 | 21.5–66.2 | 58 | 44.6 ± 9.5 | 43.8 | 20.1–72.0 | 31 | 48.3 ± 7.4 | 50.4 | 27.9–59.9 |
| VCL* | 139 | 91.4 ± 21.7 | 90.1 | 51.7–139.3 | 58 | 84.2 ± 17.6 | 79.8 | 48.6–129.0 | 31 | 107.8 ± 12.1 | 105.2 | 87.6–131.6 |
| Linearity* | 139 | 47.6 ± 8.2 | 48.0 | 28.0–68.0 | 58 | 53.2 ± 6.5 | 53.0 | 39.0–69.0 | 31 | 44.7 ± 5.6 | 45.0 | 32.0–57.0 |

[a]Total progressive = Total motile × percent sperm with VSL > 25 µm/sec. [b]Only for samples with at least 25 sperm tracks; VSL and VCL are in µm/sec. *Different by Kruskal-Wallis test, $p < 0.05$.

lower in men exposed to periods of medium or high pollution (Table 6). These differences remained after control of confounding by season. The decrement in percent normal sperm shows an increasing exposure–response relationship. However, the percent of normal sperm heads, albeit significantly decreased for both exposed periods, did not show an increasing exposure–response pattern, suggesting that alterations of sperm head shape may be a significant component of the sperm morphology effect but do not account for all of it.

Analysis of the SCSA data focused on the COMP$\alpha_t$ variable, the percent of sperm with abnormal chromatin (i.e., demonstrating increased susceptibility to DNA denaturation *in situ*). COMP$\alpha_t$ was significantly higher in samples obtained after the period of high, but not medium, air pollution (Table 5) and this association remained significant in the multivariable analysis (Table 6). A longitudinal study with monthly semen samples for 45 men for a total of 8 sequential months suggests that SCSA measures do not vary by season (*25*); therefore, the analysis for COMP$\alpha_t$ did not include control for potential confounding by season.

## Discussion

This study was undertaken to obtain a preliminary characterization of reproductive health in 18-year-old men who live in either of two districts in the Czech Republic. It was initiated because of community concern that living in the Teplice district, an area with periods of elevated air pollution during the winter (compared with the Prachatice district, an area with considerably lower air pollution), may be associated with increased abnormal reproductive health and/or poor semen quality. The data obtained are unusual in that all the men were young and of the same age when sampled. In addition, during these young men's lifetimes, movement between communities was uncommon. These demographics are advantageous when looking for effects related to environmental exposures because the young men in this study group would have had similar lifetime exposures to environmental pollution and would be less likely than older men to have experienced significant occupational exposures to reproductive toxicants. Furthermore, changes in semen quality known or suspected to occur with advancing age are not an issue in this study group. On the other hand, few comparison databases on semen quality exist for men of this age and they were too young to evaluate their fertility.

Data obtained from the physical examination and questionnaire indicated that the young men living in Teplice were similar to those living in Prachatice with respect to physical characteristics, lifestyle, and general health. The physical examination revealed no evidence of delayed puberty; all men were sexually mature based on secondary sex characteristics. Furthermore, there were no differences by district in testicular volume or self-reported age at first semen appearance.

We categorized exposure in this study based on mean levels of pollutants monitored during the 90 days preceding the sample collection. Monitoring methods have been described in detail elsewhere (*6*) along with more detailed analyses of source (industry vs. home heating) and components (specific PAHs and metals). These efforts documented that periods of elevated air pollution occurred in Teplice in the winters of 1993 and 1994, with conditions being worse in 1993. Analysis of representative samples of particulate matter for metal content indicated that ambient lead and cadmium levels were well below the existing standards for these metals. However, because internal measures of metal exposure were not available in the study participants and exposure to these metals has been associated with adverse effects on semen quality (*11*), a potential association between metals in the air pollution and adverse semen outcomes cannot be ruled out. However, the specific component(s) of the air pollution that may account for any adverse effects observed in this study were not identified.

**Table 6.** Results of adjusted regression analyses, beta (95% CI).[a]

| | Preliminary analyses | | Final analyses | | | |
|---|---|---|---|---|---|---|
| | | | Medium vs. low | | High vs. low | |
| Measure | Teplice versus Prachatice | Season | Adjusted[a] | With season | Adjusted[a] | With season |
| Production of viable sperm | | | | | | |
| Semen volume (mL) | −0.07 (−0.16–0.02) | 0.04 (−0.06–0.14) | −0.11 (−0.22–0.00) | −0.16 (−0.29–−0.03) | 0.06 (−0.06–0.18) | 0.01 (−0.12–0.15) |
| Adjusted[a] | A | A | A | A | A | A |
| Concentration (millions/mL) | 0.08 (−0.03–0.18) | 0.01 (−0.10–0.12) | 0.08 (−0.05–0.20) | 0.10 (−0.05–0.25) | 0.05 (−0.09–0.19) | 0.07 (−0.08–0.23) |
| Adjusted[a] | A | A | A | A | A | A |
| Total count (millions/sample) | 0.04 (−0.08–0.16 ) | 0.04 (−0.08–0.16) | 0.02 (−0.125–0.17) | −0.003 (−0.17–0.16) | 0.11 (−0.05–0.27) | 0.09 (−0.09–0.26) |
| Adjusted[a] | A, H | A, H | A, C | A, C | A, C | A, C |
| Percent motile | −3.82 (−7.94–0.29) | −3.62 (−8.01–0.77) | −8.12 (−12.95–−3.30)* | −8.03 (−13.57–−2.49)* | −3.02 (−8.99–2.95) | −2.93 (−9.49–3.64) |
| Adjusted[a] | B, H, S | B, H, S | B, H, S, Smk | B, H, S, Smk | B, H, S, Smk | B, H, S, Smk |
| Total motile (in millions) | −0.07 (−0.20–0.06) | −0.09 (−0.22–0.05) | −0.16 (−0.32–−0.01)* | −0.14 (−0.32–0.03) | −0.02 (−0.21–0.16) | −0.01 (−0.21–0.20) |
| Adjusted[a] | A, H | A, H | A, H | A, H | A, H | A, H |
| Total progressive[b,c] (in millions) | −0.04 (−0.17–0.08) | −0.11 (−0.24–0.02) | −0.15 (−0.30–−0.01)* | −0.11 (−0.27–0.05) | 0.03 (−0.14–0.21) | 0.07 (−0.12–0.27) |
| Adjusted[a] | A, H, S | A, H, S | A, H | A, H | A, H | A, H |
| Sperm structure | | | | | | |
| Percent normal morphology | −0.35 (−0.59–−0.11)* | −0.21 (−0.47–0.04) | −0.42 (−0.69–−0.14)* | −0.54 (−0.86–−0.22)* | −0.84 (−1.15–−0.53)* | −0.96 (−1.31–−0.62)* |
| Adjusted[a] | — | — | — | — | — | — |
| Percent morphologically normal heads | −0.38 (−0.58–−0.18)* | −0.17 (−0.39–0.05) | −0.73 (−0.96–−0.50)* | −0.87 (−1.13–−0.60)* | −0.30 (−0.56–−0.04)* | −0.44 (–0.73–−0.14)* |
| Adjusted[a] | — | — | — | — | — | — |
| SCSA COMP$\alpha_t$ | −0.02 (−0.09–0.05) | 0.04 (−0.03–0.12) | −0.14 (−0.34–0.06) | NA[d] | 0.30 (0.08–0.52)* | NA[d] |
| Adjusted[a] | Smk | Smk | Smk | | Smk | |
| Quality of sperm motion—CASA[c] | | | | | | |
| VSL | 0.32 (−2.12–2.76) | 5.80 (3.19–8.40)* | 0.88 (−2.00–3.75) | −2.10 (−5.24–1.03) | 4.21 (0.56–7.86)* | 1.38 (−2.42–5.17) |
| Adjusted[a] | C, S | C, S | C, Smk | C, Smk | C, Smk | C, Smk |
| VCL | −0.01 (−0.03–0.02) | 0.20 (−0.11–0.50) | −0.04 (−0.06–−0.01)* | −0.05 (−0.08–−0.01)* | 0.07 (0.03–0.11)* | 0.06 (0.02–0.10)* |
| Adjusted[a] | B, C, S, Smk | C, S | B, C, S, Smk | B, C, S, Smk | B, C, S, Smk | B, C, S, Smk |
| LIN | 1.03 (−1.04–3.10) | 5.46 (3.29–7.64)* | 5.60 (3.25–7.95)* | 3.28 (0.73–5.83)* | −3.67 (−6.61–−0.74)* | −5.88 (−8.93–−2.82)* |
| Adjusted[a] | M | – | A, M | A, M | A, M | A, M |

Abbreviations: A, abstinence; B, wearing briefs; C, caffeine; H, high fever; M, work/hobbies with metals; S, work/hobbies with solvents; Smk, packs of cigarettes.
[a]Factors considered for adjustment include those listed in Table 3. [b]Total progressive = Total motile × percent sperm with VSL ≥ 25 µm/sec. [c]Only for samples with at least 25 sperm tracks; VSL and VCL are in µm/sec. [d]Not applicable; because SCSA was not associated with season in a longitudinal study (*25*), this outcome was not tested for potential confounding by season. *$p < 0.05$.

Although air measurements were made in one central location in each community, each individual's precise exposure to any component of the air pollution during the time period would be expected to depend on his location within the community, his activity patterns, and the weather conditions. The magnitude of exposure in the different sampling periods varied widely and was used to define periods of high, medium, or low air pollution for the purpose of analysis. If the important exposures are the peak levels, error may be introduced given the timing of these peaks within the 90-day period. Both the number of peaks and the time between the peaks and semen sampling varied between the two winters in Teplice. Because the ability to detect an effect of an acute exposure on a susceptible germ cell stage depends on the time the semen is sampled, such effects can be missed in a study such as this one, with only one sampling time. Also, an effect detected on a particular semen measure might be stronger one winter than the other, based on differences in the duration of the pollution episodes and the time between exposure and sampling. Thus, strict exposure–response relationships might not be expected. With these problems inherent in the study design, we considered each winter exposure period separately when making comparisons with the samples obtained after periods of relatively low exposures.

Sperm concentrations and total sperm counts in these men, although not significantly associated with district, season, or exposure to periods of elevated air pollution, were at the low end of the ranges reported for populations of men worldwide (*41*). With respect to reference values for these measures provided by the WHO (*39*), 54% of these samples fell below 2.0 mL for semen volume, 21% fell below 20 million/mL for sperm concentration, and 28% fell below 40 million sperm/sample for total sperm count. Short abstinence intervals in young men could account for these observations. However, even after omitting samples from men reporting < 2 days sexual abstinence, the respective percentages are still relatively high: 51% below 2.0 mL, 19% below 20 million sperm/mL, and 21% below 40 million sperm/sample. This sperm concentration statistic is similar to that reported recently for a cohort of men in the United States consisting of partners of women presenting for infertility evaluation: 18% of the men had sperm concentrations < 20 million/mL (*42*). However, the mean sperm concentration of the Czech men (61.2 million/mL) was lower than that determined for this group of American men (94.4 million/mL) (*42*). Nevertheless, the median sperm concentration for the Czech men in this study (44.0 million/mL) is comparable to that recently reported for a cohort of unselected young Danish men [41.0 million/mL (*43,44*)], and both cohorts have lower median sperm concentrations when compared to a cohort of Finnish men without proven fertility [126.8 million/mL (*41*)]. These reports strengthen the evidence for geographic (possibly genetic) differences in sperm concentrations in Europe, as have been reported in the United States (*45*).

Exposure to environmental pollution may contribute to a perceived decline in sperm counts world wide (*41–47*). One recent report showed progressive declines in sperm production in Greece during a time period when air pollution increased (*48*). However, our results do not support a relationship between either district of residence or exposure to periods of elevated air pollution and decreased sperm production (concentration or sperm per sample) in the Czech Republic.

Evidence from laboratory and wildlife populations indicates that exposure to endocrine-disrupting chemicals during fetal or neonatal development may alter sexual differentiation, sperm production, and/or epididymal storage capability in adulthood (reviewed elsewhere by Toppari et al. (*41*) and Kavlock et al. (*49*). Air pollution since 1975–1996, when these men were born, has followed the same patterns by district and season observed during the years under study. Therefore, sperm counts might also differ by district if potential endocrine disruptors were present in the air pollution (*49*). Although our sample size is limited for detecting such effects, we found no evidence to support this hypothesis. However, this study does not address the possibility that other types of pollution present in the environment, or lifestyle factors such as consumption of alcohol and cigarettes from a relatively young age, might impact in general upon sperm production potential in young Czech men.

Nevertheless, when sampled at the end of winter after periods of elevated air pollution, men from Teplice showed evidence of lower sperm motility (1994 samples) and poorer morphology (both years) when compared with men sampled after periods of lower air pollution (Teplice in the fall and Prachatice the late winter or fall). These observations provide preliminary evidence that periods of elevated air pollution may be associated with decrements in sperm quality. While fertility data for this group of young men are not available, low values for sperm motility and normal morphology have been associated with infertility (*50–53*).

Decreases in the percentage of motile sperm (a measure of sperm viability) were observed in the men sampled late winter 1994 but not late winter 1993 despite the fact that levels of air pollutants were higher in 1993. The apparent lack of an exposure–response relationship for this measure could be due to differences in the dynamics of the exposure or, alternatively, to other factors not assessed in this study. Additional studies in which men are sampled at various times between exposure and sampling could further examine this association.

We used CASA to evaluate the quality of sperm motion, and these results, albeit somewhat difficult to interpret, did not demonstrate any consistent negative associations between the quality of sperm motion and periods of high air pollution. However, positive associations were observed between VSL and LIN and season (winter). CASA has not been applied widely in field studies, in part because the logistics of recording the sample promptly (to avoid degradation of sperm motility over time) and controlling the temperature precisely (*13*) are challenging in the field setting. Despite careful attention to these details, the recording equipment failed on 1 study day, and CASA data for 10 samples were unfortunately lost. Furthermore, the difficulty of recording a sufficient number of motile sperm in samples with very low percentages of motile sperm is widely recognized (*13*). The extent to which missing CASA data could have biased the results is difficult to determine. Nevertheless, based on the data available in this study, the quality of sperm motion did not appear to be negatively impacted in any consistent manner after periods of elevated air pollution. Limited information is available suggesting seasonal variations in sperm velocity measures (*33*) and this topic merits further study.

We found highly significant associations between exposure to periods of medium (1994) and high (1993) air pollution and poor sperm morphology in this study. For both sperm morphology and sperm head morphology, no association was observed with season alone, and the negative associations with periods of pollution in both years remained strong after controlling for potential confounding by season. This finding may be important for public health because in clinical studies, sperm morphology is a relatively good predictor of fertility status (*53*).

The SCSA was included in this study to provide a measure of the genetic integrity of sperm. Periods of high air pollution encountered in winter 1993 were associated with increased percent sperm with abnormal chromatin structure ($COMP\alpha_t$). High $COMP\alpha_t$ (> 30) has been associated with infertility and spontaneous abortion (*24*) in clinical studies, but this measure has only recently been applied to epidemiology studies. One such study found an association between exposure to cigarette smoke (which, like air pollution,

also contains genotoxic PAHs) and elevated COMP$\alpha_t$ (*54*). It may be relevant, therefore, that smoking was retained in the COMP$\alpha_t$ model in this report. In this regard, it is noteworthy that another measure of genetic integrity, namely sperm aneuploidy, was significantly elevated in a subset of the same Czech men exposed to periods of high air pollution (nonsmokers from the Teplice winter 1993 group) compared to men exposed to periods of low air pollution (nonsmokers from the Teplice summer 1993 group) (*54*). Interestingly, a subset of active smokers (1 pack/day, selected from the Teplice winter 1994 group) also exhibited sperm with elevated levels of aneuploidy compared to nonsmokers in the same group (*56*). Taken together, these intriguing observations suggest further consideration of the potential impact of air pollution and/or smoking on the genetic integrity of human sperm.

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