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Research Article

The Effect of Urban Air Pollution from Traffic on Semen Quality

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- Air Pollution; Sperm Quality; DNA Fragmentation; Urban Agglomeration

Abstract

Background: We examined sperm quality in a cohort of city policemen in the capital city of Prague, an area experiencing elevated air pollution levels as a result of a substantial rise in traffic. As the control group, policemen working in Ceske Budejovice were selected due to this city's location in a nonindustrial area characterized by substantially lower pollution levels compared to Prague.

Methods: Semen samples from 63 Prague and 16 Ceske Budejovice city policemen were examined for standard semen parameters, sperm motion characteristics and sperm DNA fragmentation. Average daily air pollutant concentrations were recorded by stationary monitoring for 90 days preceding the collection of semen samples.

Results: Sperm volume, concentration, DNA fragmentation index and the percentage of immature sperm in Prague policemen did not differ significantly between the monitoring periods. The percentage of total and progressive motility, % vitality, % normal sperm morphology and % acrosome-intact sperm were significantly lower in autumn. In policemen from Ceske Budejovice, there were no significant differences in any of the evaluated parameters of semen quality between the monitoring periods. During both spring and autumn samplings, a significant decrease in sperm volume was observed in the policemen from Prague compared to Ceske Budejovice. In autumn, significantly lower values of sperm viability percentage and motility were detected in Prague.

Conclusion: City policemen working in a large city with high air pollution levels exhibited significantly poorer sperm quality parameters than policemen from a smaller city with lower traffic intensity. This difference was observed in the summer months

INTRODUCTION

With a population of approximately 1.3 million, the capital city of Prague is the largest city in the Czech Republic, covering an area of 496 km^2. This region in the Czech Republic is characterized by high levels of air pollution due to the substantial rise in traffic volume, complicated by the geographical and architectural aspects of the inner city. Mobile sources, including road vehicles, are one of the largest contributors to air pollution in Prague, with nitrogen oxides, especially nitrogen dioxide (NO2), being the most hazardous to population health. Vojtisek-Lom et al. [1], demonstrated that NO2 concentrations in Prague in 2019 were significantly correlated (R2 = 0.76) with average daily traffic. NO2 is associated with serious adverse health effects on humans, such as respiratory and cardiovascular diseases. Numerous studies have shown that NO2 can also significantly impair central nervous system (CNS) function, including neurobehavioural development, in children [2,3]. Furthermore, multiple authors have confirmed the detrimental effects of NO2 exposure on sperm quality [4-7]. However, these results are still controversial because some authors failed to demonstrate an association between NO2 and sperm quality [8,9]. The aim of this study was to assess the sperm quality of a cohort of city policemen in Prague at the end of the winter season and the same cohort at the end of the summer season. The city policemen spend the majority of their working time outdoors actively patrolling the city streets, thereby experiencing considerable exposure to exhaust fumes from vehicular traffic.

As the city of Ceske Budejovice exhibited markedly lower pollution levels compared to Prague, policemen working there were selected as the control group. Ceske Budejovice, with a population of approximately 100 000 inhabitants, is the capital of the South Bohemian Region in the southern part of the country and is characterized by its nonindustrial environment. The region has limited natural resources and lacks substantial energy resources. Agricultural activities constitute the primary source of pollution in the region. Although the pollution level in Ceske Budejovice is also influenced by factors such as industry, local heating, and car traffic, their impact is considerably lower than that in Prague [10].

MATERIALS AND METHODS

Study design and population

The study group consisted of 63 nonsmoking city policemen working in Prague, Czech Republic. The average age of the policemen was 39.5±9.5 years (median 38.5, range 23–63 years). The average length of employment with the city police in Prague was 11.8±7.1 years (median 11.6, range 1–26).

The control group consisted of 16 nonsmoking city policemen working in Ceske Budejovice. The average age of the policemen was 37.1±6.5 years (median 38.0, range 22–48 years).

The average length of employment with the city police in Ceske Budejovice was 9.7±7.6 years (median 7.8, range 1–27).

Data on each participant's reproductive and general health and on factors that might impact his semen quality were collected by a questionnaire. Policemen with chronic or andrological diseases and long-term treatment were excluded from this study. No participant in this study was diagnosed with diabetes, varicocele, accessory gland infection or chlamydial infection. Alcohol and drug abuse were also monitored. All participants signed an informed consent form and could withdraw their participation at any time during this study, in accordance with the Helsinki II declaration. This study was approved by the ethical committee of the Institute of Experimental Medicine AS CR in Prague (approval number: 2018/09).

Inhalation exposure

The Czech Hydrometeorological Institute, Prague, performed stationary monitoring. Air quality monitoring data from publicly available tabulated data provided by air quality monitoring stations within a nationally validated air quality database were used. Average daily air pollutant concentrations recorded by stationary monitoring for 90 days preceding the collection of the semen samples were evaluated for different city districts and the whole territory of Prague and Ceske Budejovice.

Semen collection and analysis

Semen samples were collected on site by masturbation into clean glass containers. The sampling was carried out at the end of March and September 2019. An abstinence interval of 2–7 days was requested. After liquefaction at room temperature, standard semen parameters were assessed according to the guidelines of the World Health Organization [11]. The parameters included semen volume, sperm concentration, sperm morphology (head shape, midpiece and tail defects), sperm motility, acrosomal reaction and sperm plasma membrane integrity. Sperm counts were determined using a Neubauer chamber. Sperm motility was evaluated under a light microscope at 200x magnification. Acrosome-intact sperm rates were analysed by *Pisum sativum* (PSA) lectin staining of fixed semen smears [12]. Sperm vitality was estimated by assessing the percentage of sperm with plasma membrane damage detected by staining with eosin-nigrosin [11]. The percentage of morphologically normal sperm was determined by examining 200 sperm per sample stained with a Diff-Quik rapid staining kit at 1000× magnification under oil immersion and classifying them according to strict criteria as described by the World Health Organization [11].

Sperm DNA damage was analysed after acridine orange staining by the sperm chromatin structure assay (SCSA) using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA) as previously described [12,13]. Semen samples were exposed to 488-nm monochromatic laser light, and red (ssDNA) and green (dsDNA) fluorescence values were recorded in 5000 spermatozoa per sample. SCSA-Soft software (SCSA Diagnostics, Inc., Brookings, SD, USA) was used to assess the rates of sperm with fragmented DNA (DNA fragmentation index, DFI) and high-density staining (representing mainly immature sperm, HDS).

Computer-assisted semen analysis (CASA)

The collected ejaculates were allowed to undergo liquefaction at room temperature for 45 minutes prior to analysis. Leja standard 20-micron depth chambers in the format of 8 chambers per slide (Leja Products B.V., GN Nieuw Vennep, The Netherlands) were used for the analysis as previously described [14]. Two microlitres per sample with a concentration of 30 million sperm/mL was placed into the chambers. Dense samples were diluted with saline at 37°C and a pH of 7.2. Sperm motion characteristics were determined in at least 300 sperm using CEROS II Version 1.0 hardware and evaluated by HT CASA II Version 1.3 software (Hamilton Throne, Inc., Beverly, MA). The evaluated standard parameters included total sperm motility, progressive sperm motility and sperm concentration. Sperm kinematic parameters were analysed by means of the distance of the average path (DAP), distance of the straight line path (DSL) and distance of the curvilinear path (DCL). To express sperm velocity over specific paths, we used the velocity in the average path (VAP), velocity in a straight line (VSL) and curvilinear velocity (VCL); sperm trajectories were represented by straightness ((STR) VSL/VAP), linearity ((LIN) VSL/VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF) and wobble ((WOB) VAP/VCL).

Cotinine assay

The level of tobacco smoke exposure reported in the lifestyle questionnaires was verified by detection of the urinary levels of cotinine as the major nicotine metabolite by radioimmunoassay [15]. Subjects with cotinine levels higher than 200 ng/mg of creatinine were considered active smokers and were excluded.

Statistical analysis

Due to the nonnormal distribution of certain variables (Kolmogorov–Smirnov test), nonparametric analyses were applied. Statistical analysis was conducted using nonparametric exact tests with the SPSS software package, version 18 for Windows (SPSS, Inc., Chicago, IL, USA). The Wilcoxon matched pairs test for dependent samples was used. The Mann–Whitney U test was used for bivariate comparison of environmental pollution values obtained in the 90-day period prior to sampling. Correlation analyses with continuous variables were performed using Spearman's rank correlation. Differences were adjusted for age.

RESULTS

Air pollution

Data were acquired by automatic air pollution monitoring in each city. In 2019, a network of 17 stationary monitoring stations was distributed across different locations in Prague. Of these, 14 stations collected comprehensive monthly data for particulate matter with a diameter of 10 microns or less (PM10) and nitrogen oxides (Czech Hydrometeorological Institute). In addition, oxygen (O2) and B(a)P data could be obtained from five stations. Comparing the results from all 14 stations, there were significantly higher concentrations of PM10, NO and NOx during the winter season [Table 1]. In particular, NO2 emissions from cars varied across the urban environment depending on the urban structure. Comparing the nitrogen oxide data from seven stations in the city centre and seven in the peripheral areas separately (Figure 1), there was no significant difference in NOx, NO or NO2 concentrations between the winter and summer months in the city centre. At stations situated in the periphery, the concentration was significantly lower in the summer months [Table 2]. In Ceske Budejovice, there is only one stationary station located among urban buildings in the central part of the city, which monitors NO2. The average NO2 concentration measured by this station was higher in the first quarter of the year than in the third quarter, including the summer season (17.3±10.1 vs. 9.6±7.3, P <0.001). The same applied to PM2.5 concentrations (15.5±11.9 vs. 9.6±5.2, P <0.001). To compare the two locations, the Czech Hydrometeorological Institute calculated the average immission concentrations of the monitored pollutants using a spatial analysis for the entire area of Prague and Ceske Budejovice using available data [Table 3].

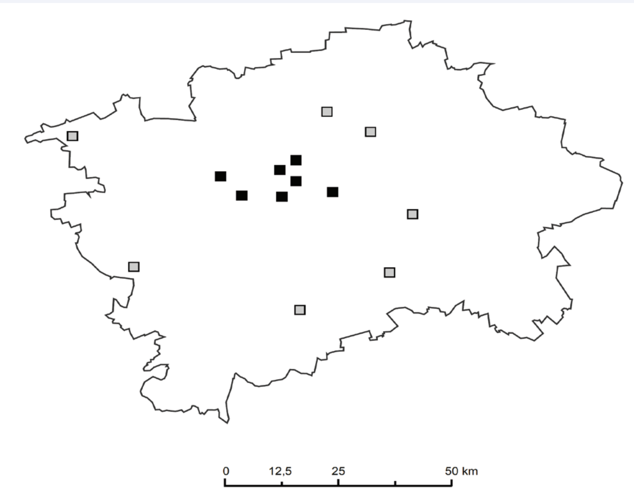

Figure 1 Locations of the air quality monitoring stations in Prague used in this study.
Black square: monitoring station in the central part of the city
Grey square: monitoring station in the peripheral part of the city
Black line: city boundary

Table 1: Air pollution recorded by monitoring stations for 90 days preceding the collection of the semen samples in Prague

Variable	Season	Mean±SD	Median (Min.Max)	*p*-value
PM10 ($\mu g/m^3$)	Winter	23.8±3.75	24.9 (17.8, 28.1)	
	Summer	18.6±4.1	17.3 (13.8, 27.4)	0.001
NO_x ($\mu g/m^3$)	Winter	50.5±25.1	38.6 (17.7, 100.9)	
	Summer	39.1±26.4	23.8 (16.8, 95.5)	0.007
NO ($\mu g/m^3$)	Winter	14.6±10.9	9.9 (3.0, 37.2)	
	Summer	9.5±9.3	3.3 (1.8, 27.4)	0.004
NO_2 ($\mu g/m^3$)	Winter	27.6±7.4	26.5 (18.8, 43.5)	
	Summer	24.6±10.4	19.4 (13.7, 46.6)	NS
O_3 ($\mu g/m^3$)	Winter	43.9±5.8	47.7 (34.4, 48.8)	
	Summer	60.4±7.6	63.8 (47.5, 66.2)	NS
B[a]P (ng/m^3)	Winter	1.7±1.2	1.22 (0.9, 3.6)	
	Summer	0.97±0.4	0.98 (0.6, 1.3)	NS

B[a]P: benzo[a]pyrene, *PM:* particulate matter, *NO:* nitric oxide, *NO_2:* nitrogen dioxide, *NO_x:* nitrogen oxides, *NS:* nonsignificant, *O_3:* ozone

Table 2: Oxides of nitrogen recorded by monitoring stations in Prague city centre and periphery 90 days preceding the collection of the semen sampling

Variable	Monitoring stations	Season	Mean±SD	Median (Min. Max)	*p*-value
NO_x ($\mu g/m^3$)	Central	Winter	57.9±23.8	56.3 (37.4, 100.9)	
		Summer	46.9±31.4	36.5 (23.1, 95.5)	NS
NO_x ($\mu g/m^3$)	Peripheral	Winter	43.1±26.0	35.1 (17.7, 80.0)	
		Summer	31.3±19.4	22.5 (16.8, 65.0)	0.02
NO ($\mu g/m^3$)	Central	Winter	17.2±11.4	15.8 (6.5, 37.2)	
		Summer	12.6±10.8	7.0 (2.3, 27.4)	NS
NO ($\mu g/m^3$)	Peripheral	Winter	12.0±10.7	8.0 (3.0, 29.7)	
		Summer	6.8±7.4	3.2 (1.8, 20.8)	0.02
NO_2 ($\mu g/m^3$)	Central	Winter	31.2±6.5	31.3 (24.6, 43.5)	
		Summer	28.3±11.6	24.8 (18.7, 46.6)	NS
NO_2 ($\mu g/m^3$)	Peripheral	Winter	24.0±7.0	20.5 (18.5, 34.1)	
		Summer	20.9±8.2	17.5 (13.7, 33.1)	0.02

NO: nitric oxide, *NO_2:* nitrogen dioxide, *NO_x:* nitrogen oxides

Semen outcomes

Our results showed that the study groups primarily comprised normospermic men [11] in terms of semen volume and sperm concentration, morphology, vitality and motility. The findings of the respective semen parameters at the end of the winter and summer seasons are shown in Tables 4 and 5. Sperm volume, concentration, DNA fragmentation index (%DFI) and the percentage of immature sperm (HDS) of Prague policemen did not differ significantly between the monitoring periods. The percentages of total and progressive motility, % vitality, % sperm morphology (normal form) and % acrosome-intact sperm were significantly lower in autumn after the summer season. However, examination using computer-assisted semen analysis (CASA) showed differences in sperm movement kinematics between samples collected in the spring and autumn seasons. In contrast, a significantly higher percentage of straightness (p=0.001) and linearity (p=0.001) was found in autumn [Table 6].

Table 3: Air pollution levels for the first and the third quarter of 2019 in Prague and Ceske Budejovice. Average concentrations related to the whole territory of Prague and Ceske Budejovice

Variable	Prague		Ceske Budejovice	
	Q1	Q3	Q1	Q3
PM_{10} ($\mu g/m^3$)	23.2	17.6	14.7	10.6
$PM_{2.5}$ ($\mu g/m^3$)	11.7	10.0	9.2	5.7
NO_2 ($\mu g/m^3$)	21.5	18.2	10.6	5.3
Benzene ($\mu g/m^3$)	1.2	0.6	1.0	0.4
B[a]P (ng/m^3)	1.7	0.1	0.6	0.2

B[a]P: benzo[a]pyrene, *NO2:* nitrogen dioxide, *PM2.5* particulate matter with an aerodynamic diameter smaller than or equal to 2.5 μm, *PM10* particulate matter with an aerodynamic diameter smaller than or equal to 10 μm

Table 4: Results of the semen analysis in Prague

Variable	March	September	p-value
Sperm volume (mL)	2.8± 1.52	2.8± 1.46	NS
	2.5 (1.7, 3.6)	2.5 (1.8, 3.6)	
Sperm concentration (x10⁶/mL)	93.4± 69.4.	94.8± 69.6	NS
	69 (42, 133)	81 (40, 128)	
Total motility (%)	58± 6.62	50.5± 8.17	0.001
	59 (54, 61)	52 (48, 55)	
Progressive motility (%)	51.6± 7.85	44.7± 8.34	0.001
	52 (48, 56)	45 (42, 50)	
Vitality (%)	73.6± 8.36	69.2± 9.71	0.001
	74 (68, 80)	71 (64, 77)	
Sperm morphology, normal form (%)	10.4± 5.78	9.3± 3.81	0.007
	10 (6, 14)	9 (7, 11)	
Sperm head morphology, normal form (%)	14.1± 7.85	12.2± 5.18	0.021
	13 (8, 18)	12 (9, 13)	
Acrosome-intact sperm (%)	85.0± 5.99	82.8± 6.65	0.001
	85 (80, 91)	84 (80, 86)	
DFI (%)	22.0± 10.30	20.6± 12.91	NS
	20 (9, 55)	18 (6, 66)	
HDS (%)	12.9± 6.38	12.9± 5.55	NS
	12 (5, 29)	12 (3, 27)	

Note: Data presented as mean ± standard deviation and median (IQR).
DFI DNA fragmentation index, *HDS* high DNA stainability, *NS* nonsignificant

There was no significant difference in any of the evaluated parameters of semen quality between the monitoring periods for policemen from Ceske Budejovice. Values of movement characteristics as measured by CASA were significantly higher in autumn for % straightness (p=0.039) but not for linearity (p=0.065) [Table 7]. The percentage of sperm with fragmented chromatin (% DFI) was considerably lower in autumn (17.2 vs. 22.3%, p=0.056).

While conducting the statistical analysis of differences in findings between Prague and Ceske Budejovice, age and length of employment with the police were used as confounding factors. In both spring and autumn sampling, semen volume was significantly lower in Prague (p=0.03). No significant difference was found in any other parameter in spring. During the autumn season in Prague, significantly lower values were observed for the percentage of viable sperm (69.2 vs. 74.9, p=0.047), total motility (50.5 vs. 55.6, p=0.03) and progressive motility (50.3 vs. 44.7, p=0.04). There was no significant difference in % DFI between policemen in Prague and Ceske Budejovice. In Ceske

Table 5: Results of the semen analysis in Ceske Budejovice

Variable	March	September	p-value
Sperm volume (mL)	3.7±1.06	3.6±0.89	NS
	3.4 (3.0, 4.6)	3.8 (2.9, 4.2)	
Sperm concentration (x10⁶/mL)	117.7±102.7	104.1±69.6	NS
	88 (56, 116)	76 (46, 156)	
Total motility (%)	56.4±6.9	55.6±4.66	NS
	57 (53, 62)	55 (52, 58)	
Progressive motility (%)	50.3±7.2	49.6±3.5	NS
	51 (47, 57)	50 (47, 52)	
Vitality (%)	72.9±8.9	74.9±7.2	NS
	74 (65, 81)	76 (70, 81)	
Sperm morphology, normal form (%)	8.9±2.9	10.1±3.5	NS
	9 (6, 12)	10 (7, 13)	
Sperm head morphology, normal form (%)	11.2±4.5	12.7±3.9	NS
	11 (7, 15)	12 (9, 16)	
Acrosome-intact sperm (%)	83.7±6.6	84.0±5.37	NS
	81 (78, 90)	84 (81, 88)	
DFI (%)	22.3±9.6	17.2±8.37	0.056
	21 (11, 47)	16 (6, 34)	
HDS (%)	11.4±3.2	11.2±3.21	NS
	11 (7, 17)	10 (7, 18)	

Note: Data presented as mean ± standard deviation and median (IQR).
DFI DNA fragmentation index, *HDS* high DNA stainability, *NS* nonsignificant

Table 6: Range of computer-assisted semen analysis parameters in the Prague study group

Variable	March	September	p-value
VCL (μm/s)	81.5 (45.8-132.2)	76.2 (38.5-118.4)	0.009
VSL (μm/s)	41.3 (21.6-60.6)	42.1 (17.1-59.9)	NS
VAP (μm/s)	50.9 (33.4-74.4)	50.0 (21.9-71.3)	NS
DSL (μm)	17.7 (10.3-28.3)	17.9 (8.1-25.8)	NS
ALH (μm)	4.9 (2.1-7.9)	4.4 (2.2-7.0)	0.004
LIN (%)	54.0 (37.3-75.2)	58.1 (41.2-77.1)	0.001
STR (%)	79.5 (65.0-93.1)	83.5 (70.5-96.8)	0.001
BCF (Hz)	18.9 (12.5-27.4)	19.6 (14.2-27.1)	NS

Note: Data presented as median (min,max).
VCL curvilinear velocity, *VSL* straight line velocity, *VAP* average path velocity, *DSL* length of straight-line path, *ALH* amplitude of lateral head displacement, *LIN* linearity (VSL/VCL), *STR* straightness (VSL/VAP), *BCF* beat cross frequency.

Table 7: Range of computer-assisted semen analysis parameters in the Ceske Budejovice study group

Variable	March	September	p-value
VCL (μm/s)	73.4 (47.6-105.4)	82.6 (62.1-128.5)	NS
VSL (μm/s)	38.1 (25.0-46.5)	45.4 (31.7-69.9)	0.001
VAP (μm/s)	48.5 (30.2-59.5)	54.9 (40.9-76.7)	0.008
DSL (μm)	16.2 (10.6-19.9)	18.5 (14.0-31.6)	0.006
ALH (μm)	4.8 (3.0-6.4)	4.8 (3.1-7.3)	NS
LIN (%)	50.4 (39.9-67.8)	59.7 (42.0-71.8)	NS
STR (%)	79.7 (62.5-89.5)	83.0 (69.4-91.8)	0.039
BCF (Hz)	20.0 (15.0-22.3)	21.5 (16.8-2)	0.016

Note: Data presented as median (min,max).
Abbreviations: see table 6

Budejovice, the index of DNA fragmentation was lower in autumn (17.2 vs. 20.6%), but the number of policemen examined there was quite lower compared to Prague (16 vs. 63).

A negative correlation of DFI with vitality, motility and acrosome-intact sperm was observed in Prague policemen both in spring and autumn but with concentration and sperm morphology only in spring. In Ceske Budejovice, a significant correlation between DFI and motility, sperm morphology and acrosome-intact sperm and sperm morphology was found in spring, but with sperm morphology only in autumn. In both Prague and Ceske Budejovice, a negative correlation with HDS and morphologically normal head forms was found in spring (-0.348, p=0.005 and -0.675, p=0.004, respectively).

DISCUSSION

The presence of traffic-related air pollutants in cities has an adverse effect on sperm quality [16]. However, the results of several studies regarding the effect of air pollution on the structure and function of sperm have been controversial. When comparing the impact of air pollution on sperm quality across different locations, multiple external and internal factors come into play, potentially influencing the final outcome. The primary factors implicated are professional exposure and lifestyle-related elements. In our study, all participants were nonsmokers who completed a detailed questionnaire focusing on lifestyle and any existing health conditions. Participants who had a medical condition or were undergoing treatments that could potentially impact their reproductive organs and sperm development were excluded from this study. This study included city policemen who were actively serving within the same city, ensuring that their professional exposure was similar. The statistical analysis accounted for the factors of age and length of employment with the city police. Concentrations of air pollutants can fluctuate between winter and summer seasons [14], and seasonal effects on some sperm quality parameters, such as ambient temperature and variations in daylight hours throughout the year, have also been documented [17,18]. Therefore, we conducted the examination during the spring season after winter and in the autumn season after summer. Only policemen who participated in both sampling periods were included in this study.

Regarding the basic parameters of sperm quality of policemen from Prague and Ceske Budejovice, semen volume and total concentration did not differ significantly between spring and autumn sampling in either location. However, semen volume and total sperm concentration were significantly lower in both seasons in Prague policemen than in Budejovice policemen ($p<0.05$). These findings are consistent with the results of a meta-analysis conducted by Zhang et al. [19], demonstrating that elevated levels of air pollution were associated with significant declines in semen volume, sperm concentration, normal sperm morphology and sperm motility. In Ceske Budejovice, no significant difference between spring and autumn sampling was observed for any of the abovementioned parameters. In Prague, we found a significantly lower percentage of viable, acrosome-intact, morphologically normal and overall progressively motile sperm in autumn. During this season, viability and total and progressive sperm motility were significantly impaired in Prague compared to Ceske Budejovice. The fact that some sperm quality parameters in Prague were better in spring than in autumn, despite higher concentrations of some pollutants in spring, was also probably influenced by other factors. Seasonal variations in semen parameters may have played a role in this observation. Kabukcu et al. [18], in an extensive 8-year study involving over 6,000 semen samples, found that the progressively motile sperm count was 23.6% lower in October than in May (p=0.026). Examining 5,131 men, Santi et al. [17] found that the temperature 30-60 days prior to sampling was inversely proportional to total sperm number, motility and normal sperm forms. The total sperm count was lower in summer/autumn and was inversely proportional to daylight hours.

As observed across all 14 monitoring stations in Prague, the concentrations of PM10, NOx and NO in air were significantly increased in spring, whereas NO2, O3 and B[a]P did not show a significant increase [Table 1]. However, the concentrations of nitrogen oxides from stations in the central part of Prague did not show any differences between the monitoring periods [Table 2]. Although NO2 comes from a variety of sources, road traffic is its most important source in the urban environment and is associated with adverse health effects [2,3].

To elucidated the impact of car traffic on NO2 concentrations in Prague, Vojtisek-Lom et al. [1], determined NO2 concentrations using membrane-closed passive samplers at 65 locations in Prague in March-April and September-October 2019. The annual average NO2 level exceeded the EU limit of 40 μg/m3 at 32 locations. These data complement those calculated using values from the CHMU stationary monitoring stations for the period of spermatogenesis in policemen included in this study.

The impact of traffic pollutants on sperm quality has been described in numerous studies. Sperm motility seems to be most affected, but sperm concentration is also reduced, and sperm morphology is disturbed. Guven et al. [4], found significant differences regarding reduced sperm count, motility, and sperm with normal morphology in men working as highway toll collectors compared to a control group of clerks (P = 0.002 and P = 0.003, respectively). Boggia et al. [5], found a significantly lower percentage of motile sperm in workers exposed to traffic pollutants. Based on residential air pollution data, Chen et al. [6], found that NOx exposure is a risk factor for reduced sperm concentration and motility. The adverse effect of NO2 exposure on total sperm count, concentration and normal sperm morphology was also described by Santi et al. [17]. Furthermore, the association of NO2 exposure with decreased concentration, total number and progressive motility was reported by Wang et al. [7]. Belladelli et al. [20], found a negative association of PM 10, PM 2.5 and NO2 with sperm morphology in a large homogeneous cohort of infertile men. Zhang et al. [21] described a negative correlation between NO2 and O3 exposure and total and progressive motility in sperm donors. They also reported that sulfur dioxide (SO_2) might be a major contributor to the adverse effects of air pollution on sperm quality in the general population by reducing sperm motility, concentration and total sperm count.

In a meta-analysis, Xu et al. [22], analysed the association between PM2.5, PM10, SO2, NO2, CO, and O3 exposure and semen quality. Exposure to ambient PM2.5, PM10 and SO2 90 days prior to semen collection was associated with decreased sperm concentration, total sperm count, total motility and/or progressive motility. Significantly higher concentrations of PM10 could be one of the factors adversely affecting these sperm quality parameters in Prague compared to Ceske Budejovice, but SO2 concentrations were relatively low in Prague. The average annual concentrations in 2019 at the two monitoring stations were 1.9± 0.83 and 2.3± 0.97 μg/m3, with no significant difference between spring and autumn. In Ceske Budejovice, the average annual SO2 concentration was higher (4.4±1.38 μg/m3), with the third quarter exhibiting a higher concentration than the first quarter (2.9 vs. 5.1 μg/m3). Ma et al. [23], also found that PM2.5 and PM10 were inversely associated with sperm concentration, total sperm count, total motility and progressive motility.

Although in spring, the percentage of motile sperm was higher in Prague, CASA showed impaired sperm movement kinematics. The percentages of straightness (STR) and linearity (LIN) were significantly decreased in spring (p=0.001) compared with autumn. For policemen in Ceske Budejovice, only the percentage of STR was lower in spring (p=0.04). A meta-analysis performed by Zhang et al. [19] showed that air pollution causes only a nonsignificant decrease in VCL, VSL and LIN. Reductions in some variables measured with CASA systems (VCL, VSL, VAP and ALH) following increased exposure to PM2.5 and PM10 were described by Wu et al., [24].

In Prague, we found no significant difference either in sperm chromatin integrity disturbances (%DFI) or in the proportion of immature sperm with defects in the histone-to-protamine transition (%HDS). In Ceske Budejovice, the DFI was lower in autumn, reaching the border of statistical significance (p=0.056). In spring, the DFI was comparable in both locations (22%); in autumn, it was lower in Ceske Budejovice than in Prague (17.2 vs. 20.6%). However, these differences were not significant given the small number of policemen in Ceske Budejovice. Osaka et al. [25], set a cut-off value of 24.0% for sperm DFI to predict fertility. This value is very similar to the 25% threshold reported by Evenson et al. [26], for fair pregnancy outcomes. In our study, 24 (30.4 %) and 16 (20.3%) policemen exceeded the 25% cut-off in spring and autumn, respectively. These percentages are relatively high. Calogero et al. [27], found that exposure to vehicle engine exhaust fumes among highway toll station workers significantly increased the percentage of sperm with DNA fragmentation. This may lead to a decrease in fertility in men exposed to exhaust fumes, as El-Shorbagy et al. [28], reported that sperm DNA fragmentation is an important cause of recurrent implantation failure.

The majority of published studies suggest that sperm DFI is negatively correlated with sperm concentration, motility, vitality and percentage of normal morphology [14,25,29,30]. This is consistent with our observations. Moreover, our study revealed a negative correlation with acrosome-intact sperm. Zini et al. [31], reported that HDS values are essential for the morphology of the sperm head with incomplete sperm chromatin condensation and contribute to sperm head defects. This is in agreement with our findings. In both Prague and Ceske Budejovice, we observed a negative correlation between HDS and the percentage of morphologically normal sperm heads (-0.348, p=0.005 and -0.675, p=0.004, respectively).

CONCLUSION

City policemen working in a large city with high air pollution levels exhibited significantly poorer sperm quality parameters than policemen from a smaller city with lower traffic intensity. This difference was observed especially during in the summer months as a result of the high traffic intensity. The results demonstrate the risk of impaired sperm quality for the male population of large cities.

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Ethics approval and consent to participate

Written consent has been obtained from each participant after full explanation of the purpose and nature of all procedures used in accordance with the Helsinki II declaration. This study was approved by the ethical committee of the Institute of Experimental Medicine AS CR in Prague (approval number: 2018/09)

AUTHOR CONTRIBUTIONS

JR contributed to the overall concept and planning of the study, JS and VK performed benchtop work and analysis of samples, MV and PM contributed to sample evaluation and data processing, all authors contributed to the substantial revisions of the manuscript.

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