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Original article

Ozone decreases sperm quality in systemic lupus erythematosus patients

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ABSTRACT

Objective: To investigate the deleterious effects of air pollutants exposure in the Sao Paulo metropolitan region on semen quality in systemic lupus erythematosus (SLE).
Methods: A seven-years longitudinal repeated-measures panel study was performed at the Laboratory of Experimental Air Pollution and Rheumatology Division. Two semen samples from 28 post-pubertal SLE patients were analyzed. Daily concentrations of air pollutants exposure: PM_{10}, SO_2, NO_2, ozone, CO, and meteorological variables were evaluated on 90 days before each semen collection dates using generalized estimating equation models.
Results: Intravenous cyclophosphamide (IVCYC) and ozone had an association with a decrease in sperm quality of SLE patients. IVCYC was associated with decreases of 64.3 million of spermatozoa/mL (95% CI 39.01–89.65; $p = 0.0001$) and 149.14 million of spermatozoa/ejaculate (95% CI 81.93–216.38; $p = 0.017$). With regard to ozone, the most relevant adverse effects were observed from lags 80–88, when the exposure to an interquartile range increase in ozone 9-day moving average concentration led to decreases of 22.9 million of spermatozoa/mL (95% CI 5.8–40.0; $p = 0.009$) and 70.5 million of spermatozoa/ejaculate (95% CI 12.3–128.7; $p = 0.016$). Further analysis of 17 patients that never used IVCYC showed association between exposure to ozone (80–88 days) and decrease of 30.0 million of spermatozoa/mL (95% CI 7.0–53.0; $p = 0.011$) and 79.0 million of spermatozoa/ejaculate (95% CI 2.1–155.9; $p = 0.044$).
Conclusion: Ozone and IVCYC had a consistent adverse effect on semen quality of SLE patients during spermatogenesis. Minimizing exposure to air pollution should be taken into account, especially for patients with chronic systemic inflammatory diseases living in large cities.

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O ozônio diminui a qualidade do sêmen em pacientes com lúpus eritematoso sistêmico

Palavras-chave:
Lúpus eritematoso sistêmico
Poluição do ar
Qualidade do sêmen
Fertilidade
Ciclofosfamida

RESUMO

Objetivo: Investigar os efeitos deletérios da exposição aos poluentes do ar na Região Metropolitana de São Paulo sobre a qualidade do sêmen de pacientes com lúpus eritematoso sistêmico (LES).

Métodos: Foi feito um estudo longitudinal de painel com medidas repetidas de sete anos no Laboratório de Poluição Atmosférica Experimental e Reumatologia. Foram analisadas duas amostras de sêmen de 28 pacientes com LES pós-púberes. Foram avaliadas as concentrações diárias de exposição aos poluentes do ar PM_{10}, SO_2, NO2, ozônio e CO e variáveis meteorológicas 90 dias antes de cada data de coleta de sêmen com o uso do método de equações de estimativas generalizadas.

Resultados: A ciclofosfamida intravenosa (CICIV) e o ozônio estiveram associados a uma diminuição na qualidade do sêmen dos pacientes com LES. A CICIV esteve associada a um decréscimo de 64,3 milhões de espermatozoides/mL (IC 95% 39,01-89,65; p = 0,0001) e 149,14 milhões de espermatozoides/ejaculado (IC 95% 81,93-216,38; p = 0,017). Em relação ao ozônio, os efeitos adversos mais relevantes foram observados entre os lags (intervalo de tempo) 80 e 88, quando a exposição a uma concentração média de ozônio um intervalo interquartil maior em nove dias móveis levou a um decréscimo de 22,9 milhões de espermatozoides/mL (IC 95% 5,8-40; p = 0,009) e 70,5 milhões de espermatozoides/ejaculado (IC 95% 12,3-128,7; p = 0,016). Uma análise mais aprofundada dos 17 pacientes que nunca usaram CICIV mostrou associação entre a exposição ao ozônio (80-88 dias) e o decréscimo de 30 milhões de espermatozoides/mL (IC 95% 7-53; p = 0,011) e 79 milhões de espermatozoides/ejaculado (IC 95% 2,1-155,9; p = 0,044).

Conclusão: O ozônio e a CICIV tiveram um efeito adverso consistente sobre a qualidade do sêmen de pacientes com LES durante a espermatogênese. Deve-se considerar a minimização da exposição à poluição do ar, especialmente para pacientes com doenças inflamatórias sistêmicas crônicas que vivem nas grandes cidades.

Introduction

Gonad function is severely affected in male SLE patients. Recently, severe sperm abnormalities with testicular atrophy, high follicle-stimulating hormone (FSH) levels and testicular Sertoli cell dysfunction associated with intravenous cyclophosphamide (IVCYC) treatment were reported by our group in systemic lupus erythematosus (SLE).[1–3]

Exposure to air pollutants has also been correlated with male reproductive outcomes, especially sperm quality,[4–9] however this environmental factor was not studied in male SLE population with gonadal function assessment.

In fact, air pollution is composed of a heterogeneous mixture of gases and particles that include ozone (O_3), particulate matter (PM_{10}), nitrates (NO), sulfur dioxide (SO_2), toxic by-product of tobacco smoke and carbon monoxide (CO) and may trigger systemic inflammation and autoimmunity in SLE.[10,11]

Therefore, the objective of this study was to investigate prospectively the correlation of air pollutants exposure concentrations and semen quality in Sao Paulo metropolitan region in SLE patients.

Material and methods

A longitudinal repeated-measures panel study was performed with 35 nonsmokers post-pubertal male SLE patients regularly followed at the Pediatric Rheumatology Unit and the Lupus Clinics of the Rheumatology Division. All patients fulfilled the American College of Rheumatology classification criteria for SLE.[12] None of them had cryptorchidism, hydrocele, hypospadia, testicular infection (e.g., mumps), orchitis, testicular vasculitis, testicular cancer, ureteral impairment and previous history of any scrotal or inguinal surgery (e.g., varicocelectomy, vasectomy and hernia repair). Nine SLE patients were excluded since they did not reside within the metropolitan region of the city of Sao Paulo, presented azoospermia or had only one sample of sperm collected. Therefore, from January 2000 to January 2006, 26 SLE patients resident of Sao Paulo metropolitan region performed a global reproductive health evaluation including two sperm samples of each patient with a median interval of 1 month (range 0.7–8 months).

The Ethics Committee of our University Hospital approved this study and an informed consent was obtained from all participants.

Global reproductive health evaluation

Demographic data and life style habits: Current age, disease duration, years of education, smoking and alcohol consumption were recorded.

Urological evaluation and testicular doppler ultrasound: A clinical examination of the genitalia included evaluation of testicles, epididymis, vas deferens, scrotum and penis was performed blinded to the semen analysis. The patients were examined in a warm room (temperature not inferior to 22 °C), with and without Valsalva manoeuver and in both the standing and supine positions to assess clinical varicocele.[13,14] Testicular volumes were measured using the Prader orchidometer. Testicular ultrasound was performed in all SLE patients by an expert sonographer blinded to the semen analysis to assess radiographic varicocele and testicular volumes. The largest measurement in each dimension was recorded and used to calculate the testicular volume according to the formula to an ellipsoid (length × width × thickness × 0.52). The normal mean value in male post-pubertal adolescents and adults is 15 ± 8 mL.[15] Low testicular volume was defined if a SLE patient had a reduced testicular volume by Prader's orchidometer and/or ultrasound. Varicocele was defined if a SLE patient had presented clinical or radiographic enlargement of the pampiniform venous plexus in the scrotum.[13]

Semen analysis and anti-sperm antibodies: Fifty-two sperm analysis were performed by two expert medical technologists who were blinded to the other parameters. Sperm volume, concentration, total sperm count (total spermatozoa per ejaculate), progressive motility were carried out based on guidelines of the World Health Organization (WHO).[16] All patients collected at least two semen samples (median interval of 1 month, range 0.7–8 months) after 48–72 h of sexual abstinence. The spermatozoa were analyzed by manual hand count as well as by a computer-assisted semen analysis system under 400× magnification, using an HTM-2030. Each slide was scanned to estimate the number of spermatozoa per field equivalent to 1 mL, to obtain an approximate sperm concentration in millions of spermatozoa per mL of semen. The motility of each spermatozoa was graded 'a' (rapid progressive motility), 'b' (slow or sluggish progressive motility), 'c' (non-progressive motility) and 'd' (no motility).[17] The presence of antisperm antibodies was determined by direct Immunobead test using Immunobea^{dR} rabbit anti-human Ig (IgA, IgG, and IgM) kits (Irvine Scientific, Santa Ana, CA, USA) in all patients.

Hormonal status: Hormone determinations were performed at study entry blinded to the other parameters of gonadal function: FSH (normal value: 1–10.5 IU/L) and morning total testosterone (271–965 ng/dL) were detected by fluoroimmunoassay using DELFIA time-resolved fluoroimmunoassay kits (Wallac, Turku, Finland). Intra- and inter-assay coefficients of variation were limited to 3.5% and 2.1%, respectively. Inhibin B levels [normal value: 74–470 pg/mL (12–17 years old) and 60–300 pg/mL (18–50 years old)] were measured by enzymatically amplified, two-site, two-step, sandwich-type immunoassay (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Intra- and inter-assay coefficients of variation were limited to 3.5–5.6% and 6.2–7.6%, respectively. Gonadal hormonal abnormality was considered if the testosterone and/or inhibin B serum levels were reduced or the FSH serum levels were increased.

Clinical evaluation and treatment

SLE disease activity and cumulative damage at the time of study entry were measured in all patients, using the SLE Disease Activity Index (SLEDAI)[18] and the Systemic Lupus International Collaborating Clinics/ACR (SLICC/ACR) Damage Index.[19] Data concerning the therapy were determined.

Air quality and meteorological data

Daily records of studied pollutants, including O_3 (the highest hourly average), SO_2 (24-h average), NO_2 (the highest hourly average), PM_{10} (24-h average) and CO (the highest 8-h moving average), were obtained for the entire study period from the Sao Paulo State Environmental Agency (CETESB) by 13 automatic monitoring station spread around the city. PM_{10} concentration (beta radiation – FH62l N Graseby Andersen) was measured in 12 of these stations. The highest hourly average of O_3 (ultraviolet – Thermo E.I.I-Model 49) and NO_2 (chemiluminescence – Thermo E.I.I-Model 42) was measured at four stations. The highest 8 h moving average was measured at five stations for CO (non-dispersive infrared – Thermo E.I.I-Model 48) and at 13 stations for SO_2 (pulse fluorescence-ultraviolet – Thermo E.I.I-Model 43).[20] All pollutants were measured from 00:01 AM to 12:00 PM. The average of all stations that measured each pollutant was adopted as an exposure status throughout the city, since air pollutants levels recorded in each station were highly correlated with the others. The daily minimum temperature and mean relative air humidity were obtained from the Institute of Astronomy and Geophysics at the University of São Paulo. Pollutants' concentration and meteorological variables were evaluated daily on 90 days before semen collection dates.

Statistical analysis

We used the generalized estimating equation model (GEE), considering fixed effects for repeated measurements to estimate the association and effect of pollutants on sperm concentration, sperm count and sperm progressive motility. S-Plus 2000 Professional Release 3 software (MathSoft Inc., Seattle, WA, USA) was employed, adjusting the model for the independent variables by way of an exchangeable correlation as a working matrix, which assumes equal correlation for measurements in each subject. The dependent variables were defined based on the fifth percentile of the reference limits of WHO guidelines for sperm analysis: sperm concentration (minimum value 15 million per mL), total sperm count (minimum value 39 million per ejaculate) and sperm progressive motility (sum of percentage of "a", "b" of spermatozoa motility – minimum value 32%). The regressions were adjusted for independent variables: current age, years of education, smoking, alcohol consumption, time of sexual abstinence, reduced testicular volume, presence of varicocele, gonadal hormonal abnormality, presence of antisperm

Table 1 – Demographic data, disease activity, hormone evaluation, urological evaluation, sperm analysis and treatment of male systemic lupus erythematosus (SLE) patients.

Characteristics	Reference values	Patient values (n = 26)	Without IVCYC use (n = 17)	With IVCYC use (n = 9)	p
Demographic data					
Age at study entry, years		29.8 (8.9)	29.5 (2.1)	30.4 (3.2)	0.8
Age at SLE onset, years; mean (SD)		20.4 (9.6)	20.3 (10.4)	19.7 (8.5)	0.8
Age at spermarche, years; mean (SD)		12.9 (1.0)	12.9 (1.2)	12.78 (0.6)	0.7
Disease duration, years; mean (SD)		9.3 (6.5)	8.7 (6.9)	10.6 (5.8)	0.5
Educational level, years; mean (SD)		10.0 (3.3)	10.1 (2.9)	9.8 (3.0)	0.8
Smoking, *n* (%)		4 (15)	4 (28.6)	0 (0)	0.3
Alcohol consumption, *n* (%)		8 (31)	5 (29.4)	3 (33.3)	0.5
SLEDAI scores, n (%)					
>4		5 (19.2)	3 (17.6)	2 (22.2)	0.46
>8		3 (11.5)	1 (5.9)	2 (22.2)	
Hormone evaluation					
Follicle stimulating hormone, IU/liter; mean (SD)		7.6 (5.8)	6.3 (5.9)	10.0 (5.2)	0.16
Elevated levels; *n* (%)		7 (26.7)	3 (17.6)	4 (44.4)	0.34
Inhibin B, pg/mL; mean (SD)		125.5 (78.0)	138.8 (73.6)	97.3 (84.5)	0.22
			3 (17.6)		
Decreased levels; *n* (%)		7 (26.7)	500.5 (264.8)	4 (44.4)	0.34
			2 (11.8)		
Total testosterone, ng/dl; mean (SD)		526.3 (243.3)		576.8 (201.2)	0.5
Decreased levels; *n* (%)		2 (7.7)		0 (0)	0.5
Intravenous cyclophosphamide (IVCYC) use				0	
Current use; *n* (%)				9 (34.6)	
Previous use; *n* (%)				8.5 (12.01)	
Cumulative dose (g); (mean/SD)				9 (34.6)	
Number of pulse therapy; *n* (%)				1.69 (1.47)	
Duration of treatment; (mean years/SD)					
Others drugs that alter sperm quality n (%)		10 (38.5)	5 (29.4)	5 (55.6)	0.2
Urological evaluation testicular					
Reduced volume (clinical and US); *n* (%)		7 (26.9)	5 (29.4)	2 (22.2)	0.4
Clinical or radiographic varicocele; *n* (%)		11 (42.3)	7 (41.2)	4 (44.4)	1.0
Sperm analysis	WHO 2010				
Sexual abstinence, days; (median/IQR)	≥2	3 (2.5)			
Sperm volume, mL; (mean/SD)	≥1.5	2.3 (1.2)	2.1 (0.9)	2.9 (1.5)	0.1
Sperm pH; (mean/SD)	≥7.2	7.6 (0.3)	7.6 (0.3)	7.6 (0.3)	1.0
Sperm concentration, $\times 10^6$ /mL; (mean/SD)	>15	63.2 (72.0)	86.3 (77.8)	19.6 (28.7)	0.02
Total sperm count, $\times 10^6$ per ejaculate (mean/SD)	>39	147.3 (223.2)	209.9 (302.4)	46.1 (55.6)	0.1
Progressive motility, %; (mean/SD)	>32	53.0 (23.0)	57.6 (17.7)	47.0 (26.4)	0.2
Antisperm antibodies, %; (mean/SD)	<20	29.0 (14.4)	29.3 (14.8)	28.8 (14.7)	0.9

Results are presented in *n* (%), mean ± standard deviation or median (interquartile range).

antibodies, SLE disease activity and cumulative damage, prednisone use, immunosuppressive use (IVCYC, azathioprine, mycophenolate mofetil and methotrexate), use of others medications that alter sperm quality (angiotensin-converting-enzyme inhibitor, spironolactone, cimetidine, haloperidol, carbamazepine and thalidomide), and factors regarding air pollutants: temperature, relative humidity and daily concentration of the pollutants. The lag structure between air pollutant exposure and the dependent variables was assessed using lags of 0–90 days and moving averages of 2, 8, and 9 days for pollutants that was statistically significant on single models. For instance, a 2 day moving average is the mean of the pollutants levels in the concurrent and previous day assigned for the concurrent day. Changes were analyzed, mainly, in respect to specific periods of spermatozoa development, which correspond to epididymal storage, development of sperm motility and total duration of spermatogenesis (0–9, 10–14 and 70–90 days before collection, respectively).[21,22] Single-pollutant models were used for the analysis. However, if more than one pollutant had a significant effect on the outcome then two-pollutant models were adopted. Pearson correlation coefficients were estimated for air pollutant variables. Effects were reported as decreases in the outcomes [with the respective 95% confidence interval (CI)] for an interquartile range (IQR) increase in each pollutant.

Results

Demographic data, disease activity, hormone evaluation, urological evaluation, sperm analysis and treatment of male SLE patients are included in Table 1. Only six patients received

Table 2 – Descriptive statistics of air pollutants, temperature and humidity of study period.

	Minimum	Maximum	Mean	Standard deviation	Quartiles 25–75%
O_3 (μg/m^3)	59.67	106.94	83.3	12.73	71.04–94.61
CO (p.p.m.)	1.32	2.91	1.90	0.49	1.18–1.99
NO_2 (μg/m^3)	84.4	159.44	64.45	16.60	77.28–85.13
SO_2 (μg/m^3)	6.94	18.28	11.27	3.46	7.49–14.80
PM_{10} (μg/m^3)	30.33	55.87	38.36	7.30	31.71–45.41
Temperature (°C)	11.4	18.6	15.6	2.18	13.28–17.39
Humidity (%)	53.79	98.02	77.04	8.92	79.2–82.11

O_3, ozone; CO, carbon monoxide; NO_2, nitrogen dioxide; SO_2, sulfur dioxide; PM_{10}, particulate matter.

corticosteroids and chloroquine diphosphate treatment before puberty. The mean time interval between the last dose of IVCYC and sperm collection was 5.4 ± 3.7 years.

Sperm evaluations of SLE patients showed that the median of sexual abstinence, means of sperm volume, pH, sperm concentration, total sperm count and sperm progressive motility values were at the parameters regarded as percentile 5% by WHO guidelines (Table 1).

The humidity and the temperature and the range of variation in pollutant concentrations and weather conditions during the assessed period are presented in Table 2. None of the pollutant surpassed the Brazilian national standards for air quality limits in the studied period.

Primary air pollutants were highly correlated with each other, with Pearson's coefficients varying from 0.48 (SO_2 and NO_2) to 0.77 (PM_{10} and CO) $p < 0.001$ and $p < 0.001$ respectively. Ozone's lowest Pearson's coefficient was observed with CO (0.28), and ozone's highest coefficient was with NO_2 (0.60) $p = 0.04$ and $p < 0.001$ respectively. Since NO_2 measurements were performed on a daily basis, on those days with high NO_2 concentrations, there was a high formation of the secondary pollutant as ozone.

In the regression models, only IVCYC use and ozone had an association with decrease in sperm quality.

IVCYC use was associated with decreases in sperm concentration (64.3 million/mL; 95% CI, 39.01–89.65; $p = 0.0001$), total sperm count (149.14 million per ejaculate; 95% CI, 81.93–216.38; $p = 0.017$) and progressive sperm motility (20.94%; 95% CI, 4.75–37.05; $p = 0.001$) in the evaluated periods (Table 3).

With regard to ozone, an increase of interquartile range (IQR) of 23.57 μg/m^3 in this pollutant averaged over the 0–90 day period was associated with a decrease of 30.6 million of spermatozoa/mL (95% CI, 2.0–59.3; $p = 0.040$) in sperm. The specific analysis of daily exposure to ozone revealed that the critical period was 80–88 days before the date of sample collection with a cumulative decrease of 22.9 million of spermatozoa/mL (95% CI, 5.8–40.0; $p = 0.009$) and 70.5 million of spermatozoa/ejaculate (95% CI, 12.3–128.7; $p = 0.016$) associated with ozone cumulative effect exposure to an IQR of 23.57 μg/m^3 (Fig. 1). No effects were observed with the others pollutants on sperm concentration or total count.

No pollutants' effects on progressive sperm motility were observed in the two weeks after exposure (period of motility

Table 3 – Decrease in total sperm count and sperm concentration associated with exposure to ozone 80–90 days before the date of sperm sample collect.

	Decrease (95% CI[a]) O_3 (IQR[b] = 23.57 μg/m^3)			
	Total sperm count (million per ejaculate)		Sperm concentration (million per mL)	
	Decrease	(95% CI)[a]	Decrease	(95% CI)[a]
Lag80	21.3	(4.8; 37.8)[c]	5.1	(0.2; 10.0)[e]
Lag81	21	(7.8; 34.1)[d]	2.8	(−1.4; 7.1)
Lag82	3	(−18.7; 24.8)	1.6	(−10.3; 7.1)
Lag83	8.9	(−10.9; 28.8)	2	(−4.0; 7.9)
Lag84	5.2	(−30.2; 19.8)	2.9	(−10.7; 4.9)
Lag85	13.8	(−16.3; 43.9)	3.4	(−6.7; 13.6)
Lag86	26.8	(4.3; 49.4)[c]	6.1	(−1.24; 13.4)
Lag87	12.6	(−3.6; 28.7)	4.1	(−0.8; 9.0)
Lag88	9.9	(−7.72; 27.5)	5.1	(0.2; 9.9)
9 day moving average (80–88)	70.5	(12.3; 128.7)[c]	22.9	(5.8; 40.0)[e]

Lag n = n days after exposure.

[a] Confidence interval.
[b] Interquartile range.
[c] $p = 0.01$.
[d] $p = 0.001$.
[e] $p = 0.03$.

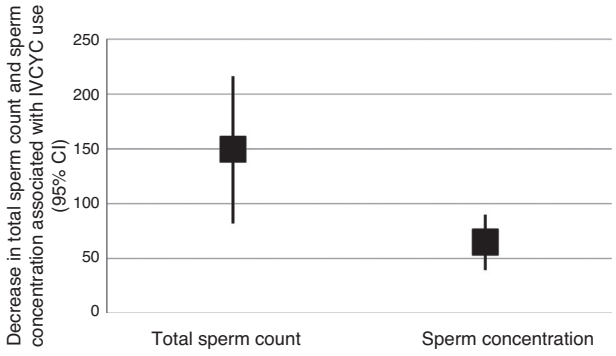

Fig. 1 – Effects of intravenous cyclophosphamide on semen quality.

development), but we found a negative effect in weeks six and seven after exposure to ozone (from lag 31 to lag 38). The eight-day cumulative effect of exposure to an IQR increase of 23.57 $\mu g/m^3$ in ozone was associated with 5.7% decrease (95% CI, 0.6–10.7; $p = 0.023$) in the progressive sperm motility.

Further analysis of 17 patients that never used IVCYC showed nine-day cumulative effect (80–88 days) of exposure to an IQR increase in ozone was associated with a decrease of 30.0 million of spermatozoa/mL (95% CI, 7.0–53.0; $p = 0.011$), 79.0 million of spermatozoa/ejaculate (95% CI, 2.1–155.9; $p = 0.044$). The eight-day cumulative effect (31–38 days) was associated with a decrease of 6.6% (95% CI, 2.9–10.3; $p = 0.001$) in the progressive sperm motility.

No statistically significant associations were found between other pollutants and other dependent variables at the specific periods of spermatozoa development.

Discussion

To our knowledge, this was the first study to identify the lag structure effects of exposure to tropospheric pollution on the sperm quality in SLE patients. The ozone and IVCYC had significant deleterious effect on spermatogenesis.

The main strength of the present study was a complete assessment of gonadal parameters in post-pubertal lupus patients and an evaluation of air pollutants in a metropolitan area of a large city. In addition, the study design of repeated-measures of sperm analysis had advantages to require a smaller number of individuals than a completely randomized study and it provides more suitable conditions of co-variables that may influence the sperm quality, without the need of a healthy control group evaluation.[23] Moreover, the analysis of a subgroup patient not exposed to IVCYC, a known gonadotoxic treatment, allowed a more accurate definition of ozone sperm harmful effect. The main limitations of the present study were the small sample size, particularly in those SLE patients that were previously exposed to cyclophosphamide, and the fact that fixed monitoring stations did not fully reflect the individual variation in exposure to pollutants. In addition, the city of São Paulo has a population of 10,886,518 people with more than 6 million vehicles. This automotive fleet is the main source of air pollution and it is observed that the ozone concentration has progressively increased.[20] Air pollutants levels recorded in the stations in São Paulo were highly correlated with each other and even ozone presented positive statistically significant correlations to primary pollutants as observed in this study. The lack of effect of others pollutants on sperm quality may result from the high correlation among air pollutants observed herein. In fact, each analyzed pollutant can be considered a good independent marker for complex mixture of air pollution. In this way, it is possible to assume that the effects on the semen quality were due to the action of all criteria air pollutants.

Air pollution consists of a heterogeneous mixture of gases and particles that include O_3. Oxidative stress and inflammation induced by this pollutant may result in respiratory disorders,[24–26] as well as contribute to a state of systemic inflammation[27,28] and autoimmunity.[10]

Additionally, ozone can induce testicular oxidative stress and excessive generation of free radicals that can provoke damage on mature spermatozoa and germ cells apoptosis, resulting in reduced sperm concentration and motility,[29] as observed herein. In this regard, Sokol et al.[8] observed an association between sperm quality and exposure to ozone and Hansen et al.[9] reported the same association with increase of $PM_{2.5}$. Air pollution may also influence male fertility[7–9] due to endocrine disruption, sperm DNA damage and toxicity mediated by the aryl hydrocarbon receptor.[30,31]

Interestingly, exposure to air pollutants may affect germ cell development including the entire period (90 days) or specific periods of sperm development before sample collection (epididymal storage or development of sperm motility).[4,8,9] Hammoud et al.[4] found that air pollution lead to a decreased in progressive motility after more than 4 weeks of the exposure. Indeed, the sperm motility is a late event in spermatogenesis and during epididymis transit, the spermatozoa undergo changes in morphology, chemistry and motility. Our study suggested that exposure to pollutant could be deleterious for progressive motility in SLE patients, even before the sperm reach the epididymis (0–14 days before semen collecting).

Moreover, we confirmed and extended through a complex statistical model that IVCYC was an important cause of injury to spermatogenesis[1–3,13,14,32–37] emphasizing the relevance of cryopreservation of semen for post-pubertal males in order to guarantee the possibility of reproduction after this immunosuppressive therapy.[38,39]

In conclusion, ozone and the use of IVCYC had a consistent adverse effect on semen quality of SLE patients. We further identified that this abnormality is not restricted to early spermatogenesis but also occurs in later stages. Consequently, minimizing exposure to air pollution should be taken into account, especially for patients residing in large cities.

Conflicts of interest

The authors declare no conflicts of interest.

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