Original Article

A comparative study of semen parameters of men undergoing fertility treatment from urban population residing in Delhi/NCR region and semi-urban population from adjoining states

Sweta Gupta, Vikram Jeet Singh, Ashish Fauzdar, Ajay Srivastava, Kamlesh Sharma

Medicover Fertility, New Delhi, India

Abstract

Objective: To analyze the difference in semen parameters of men from urban areas of Delhi/NCR with those residing outside Delhi region. **Material and Methods:** A retrospective cross-sectional study was undertaken at tertiary level center located in New Delhi region between the period of August 2019 to January 2020. A total of 657 men undergoing fertility workup during their infertility treatment were included in the study with 521 (79.2%) men from Delhi/NCR region (urban) and another 136 (20.7%) men from outside Delhi region (semi-urban) with mean age of both the group was 35.6 ± 5.7 years. **Results and Discussion:** It was observed in both the groups that there was no statistical difference noted in mean ± SD of in semen volume (1.79 ± 0.84 *vs* 1.78 ± 0.83), sperm concentration (49.9 ±32.4 *vs* 49.8 ± 32.4), total motility (58 ± 22.5 *vs* 58.1 ± 22.5), progressive motility (32.3 ±13.8 *vs* 32.2 ± 13.8) $P = 0.95$ and sperm morphology (2.3 ± 2.2 *vs* 2.3 ± 2.2). The prevalence of male factor subfertility was higher in men residing outside Delhi as compared to men of Delhi 53.1% versus 56.6% respectively and overall combined male factor in study population including the men from both the group was 58%. **Conclusion:** There were no significant differences in semen parameters including sperm concentration, total motility progressive motility, total motile concentration and sperm gross morphology in the men residing in urban area of Delhi and those residing in semi-urban area outside Delhi/NCR for up to three years period.

Keywords: Air pollution, male infertility, reproductive health, semen analysis, sperm, semen parameters

Address for correspondence: Dr. Sweta Gupta, Clinical Director, Medicover Fertility, E-20, Panchsheel Park, New Delhi, PIN-110017, India.
E-mail: sweta.gupta@medicoverfertility.com
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INTRODUCTION

There have been several studies in past decade that have focused on the investigation of seminal quality including a meta-analysis that showed that sperm density had declined globally by about 50% during the second half of the last century and attracted significant attention and have been a matter of debate. The review article published by Carlsen *et al*[1] analyzing sperm concentrations in fertile men and men of unknown fertility published between 1938 and 1990 and showed a significant decrease in sperm

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concentrations (from 113 million/ml to 66 million/ml) and in semen volume (from 3.40 ml to 2.75 ml). It is suggested that the increased frequency of male reproductive abnormalities is due to adverse effects of environmental or lifestyle factors, such as occupational and environmental exposures, medications, and sexually transmitted diseases.[2-3] In last quarter of century there has been intensive industrialization, urbanization, population growth and extensive usage of pesticides in agriculture. There are also many studies that suggest that the living condition of males play a significant role in spermatogenesis and hormonal metabolism disorders due to pollution of air, water and earth.[4-6]

Pollution has become major problem affecting overall health of residents. Reproductive health is affected by multiple factors general health, age, environment. Women offer the rate-limiting step in fertility and productiveness because of the regulated production of eggs and other barriers during and around pregnancy[7] Simultaneously men are regular producers and always fertile during their reproductive life and can potentially father millions of children if fecundity is to be tested.[7,8] A threat to sperm production would therefore be potentially dangerous for species renewal and propagation. Successful conception of a healthy offspring depends on proper functioning of multiple biological processes, including oogenesis, spermatogenesis, fertilization, implantation of the embryo, and development of the foetus.[8,9] This multistep, complex process of reproduction is a result of natural selection with adaptive significance.

India being the second most populated country in the world is the third most air polluted country worldwide and can contribute significantly in variation of semen parameters. Air pollution is the area of concern especially in urban cities including cities like Delhi due to rapid urbanization, vehicular traffic and due to stubble burning by farmers from neighboring states during end of harvesting season particularly during winter season. All these contribute to raise in particulate matter (PM) in the respirable range PM2.5 and PM10. PM2.5 is of particular interest, because it can carry multiple trace elements and polycyclic aromatic hydrocarbons (PAHs), a group of compounds that include several endocrine disruptors which may affect both the hypothalamic pituitary axis and testicular spermatogenesis and have the potential for causing sperm alterations.[10,11] It is hypothesized that in urban areas and industrial areas have more air pollution as compared to semi-urban or rural areas that can ultimately affect semen quality, but the current evidences are contradictory. Urban population is defined as towns, cities that have at least 1,00,000 persons or a high density of population and categorized with minimum of following three criteria: (i) minimum population of 5,000; (ii) at least 75 percent of male main working population engaged in non-agricultural pursuits; and (iii) population density of at least 400 persons per sq. km (1,000 persons per sq. mile). An urban agglomeration or suburban population is a continuous urban spread constituting a town and its adjoining outgrowths (OGs). An Urban Agglomeration consist of at least a statutory town and its total population should not be less than 20,000 as per the 2011 Census of government of India.

Ambient air pollution has been associated with a variety of health effects including cardiovascular[12] and respiratory diseases,[13] adverse pregnancy outcome or impaired neurodevelopment in children.[14] However, a limited amount of research has been conducted to examine the association between air pollution and male reproductive outcomes, specifically semen parameters. Male semen parameters are of considerable public health importance. One of the well-established semen parameters is the sperm count that is closely linked to male fecundity and is a crucial component of semen analysis. This is first step to identify male factor infertility as per World Health Organization, 2010 standards.[15] The economic and societal burden of male infertility is high and increasing all around the world.[16] There are world-wide many studies on sperm count and other semen parameters that have shown association with multiple environmental influences, including endocrine disrupting chemicals such as pesticides, air pollution, lifestyle factors, eating habits, stress, smoking and BMI.[17-19] Unfortunately, being a complicated and lengthy biological process, human fertility is vulnerable to disruption by changes in environmental components like air pollution and lifestyle factors.[3] Spermatogenesis disruption can occur with occupational exposure to air pollution level in urban cities. Therefore, evaluation of semen parameters including sperm count and motility sensitively reflect the impact of the modern environment including pollution on male health throughout the life course.

In the present study we tested the hypothesis for possible association of semen parameters and in men residing in the urban areas of Delhi/National capital region (NCR) in comparison to men residing in the neighboring cities of semi-urban areas. We also observed the Air Quality Index (AQI) of the both the study group giving mean of spatial distributions of the atmospheric pollutants $PM_{2.5}$, PM_{10}, SO_2, and NO_2 of urban and sub-urban cities. The main

objective of the study was to study variation in semen parameters of subfertile males from men residing in urban areas of Delhi/NCR with that of semi-urban areas from neighboring states and to rule-out the possible role of air pollution.

MATERIAL & METHODS

Study design

A retrospective analysis was undertaken at tertiary level fertility center located in Delhi between the period of six months between August 2019 to January 2020. All the male partners of couples that attended the infertility clinic as part of evaluation of infertility and treatment were evaluated for conventional semen parameters. The institutional review board that approved the study, and signed informed consent was obtained from all patients recruited.

The retrospective cross-sectional study done in two stages in the first stage a detailed questionnaire designed by the authors of the study and in the second stage physical examination and evaluation of male semen parameters respectively. The questionnaire used in the study consisted included complete infertility workup of all male patients attending the infertility clinic with detailed information about the male partner including information on respondent's age, employment type, fast-food eating habits with past and present history of smoking and alcohol habit along with place of residence with city and state. In the second stage of the study semen analysis was performed for each male for diagnosing male factor of infertility. The sample was collected after a minimum of 2-7 days of sexual abstinence through masturbation. The specimen was kept for liquefaction at ambient temperature, between 25°C and 37°C before routine semen analysis was done as per defined 2010 WHO criteria. Based on the study group and semen analysis parameters patients were allocated into different study groups.

Semen parameters and definition

Routine semen analysis is done by analysis of semen parameters under brightfield microscope for measuring sperm concentration (SC), total sperm count (TSC), total sperm motility and progressive motility (PR), total motility (TM) and sperm morphology. All these semen parameters are defined and analyzed as per WHO, Manual 2010. **Sperm concentration (SC)** is number of spermatozoa per unit volume of semen and is a function of the number of spermatozoa emitted and the volume of fluid diluting them. **Total sperm concentration (TSC)** is defined as total sperm number refers to the total number of spermatozoa in the entire ejaculate and is obtained by multiplying the sperm concentration by the semen volume. **Total motility (TM)** is defined as total motile sperm count including both progressively motile (PR) and non-progressive (NP) motile sperms. **Progressive motility (PM)** includes spermatozoa moving actively, either linearly or in a large circle, regardless of speed and **non-progressive motility (NP)** all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head. **Sperm morphology (SM)** is defined to assess the defects on the sperm head, midpiece or tail for labelling as normal or abnormal sperm morphology.

Depending on the varying concentration and percentage of various semen parameters, semen analysis for male partner is reported as **Normozoospermia** total number concentration of spermatozoa, and percentages of progressively motile (PR) and morphologically normal spermatozoa, equal to or above the lower reference limits, **Oligozoospermia** total number or concentration of spermatozoa, below the lower reference limits**,** **Asthenozoospermia** percentage of progressively motile (PR) spermatozoa below the lower reference limit, **Asthenoteratozoospermia** total percentages of both progressively motile (PR) and morphologically normal spermatozoa, below the lower reference limits)**,** **Oligoasthenotertaozoospermia** total number or concentration of spermatozoa, and percentages of both progressively motile (PR) and morphologically normal spermatozoa, below the lower reference limits)**,** **Teratozoospermia** percentage of morphologically normal spermatozoa below the lower reference limit, **Azoospermia** no spermatozoa or sperms in the ejaculate.

Exclusion & Inclusion Criteria of Study

The study inclusion criteria included male partners of the sub-fertile couple with age between 20 and 50 years with primary or secondary infertility. Residence area was noted for the each of the participant included in the study and was divided into two study groups (i) Men from urban area residing in Delhi/NCR (ii) men residing sub-urban areas outside Delhi for minimum of 1 year and at least of 3 years continuously. As per exclusion criteria, study excluded male partners with medical diseases such as diabetes mellitus, hypertension, mumps, tuberculosis, STD's hydrocele, varicocele, undescended testis, inguinal hernia repair or surgery for hypospadias, congenital absence of vas deferens or some kind of genetic or chromosomal abnormalities. Further study

Table 1: Comparison of semen parameters of males from residing in Delhi/NCR region versus the males residing in neighboring states along with *P*-value (95% confidence interval)

Variables/range (Mean ± SD)	Delhi/NCR region	Outside Delhi/NCR region	*P*-value (95% CI)
Number of Participants (*n*)	521	136	
Age (years)	20–54 (35.6 ± 5.7)	23–52 (35.6 ± 5.7)	1.00 (−1.17 to 1.17)
Abstinence (days)	2–30 (5.2 ± 6.1)	1–20 (5.1 ± 6.0)	0.87 (−1.31 to 1.11)
Semen parameters			
Volume (ml)	0.1–5.8 (1.79 ± 0.84)	0.4–6 (1.78 ± 0.83)	0.90 (−0.18 to 0.16)
Sperm concentration (SC) 10^6/ml	0.1–210 (49.9 ± 32.4)	0.8–120 (49.8 ± 32.4)	0.97 (−6.60 to 6.40)
Total sperm concentration (TSC) 10^6/ml	0.2–420 (93.0 ± 70.7)	0.8–390 (92.5 ± 70.7)	0.94 (−14.85 to 13.85)
Total motility (TM) %	1–99 (58 ± 22.5)	5–90 (58.1 ± 22.5)	0.96 (−4.50 to 4.70)
Total motile concentration (TMC)%	1–399 (64.6 ± 54)	1–154 (63.0 ± 54.2)	0.80 (−14.39 to 11.19)
Progressive motile (PM) %	1–81 (32.3± 13.8)	2–52 (32.2 ± 13.8)	0.95 (−3.81 to 3.61)
Sperm morphology (%)	1–4 (2.3 ± 2.2)	1–8 (2.3 ± 2.2)	1.00 (−0.44 to 0.44)

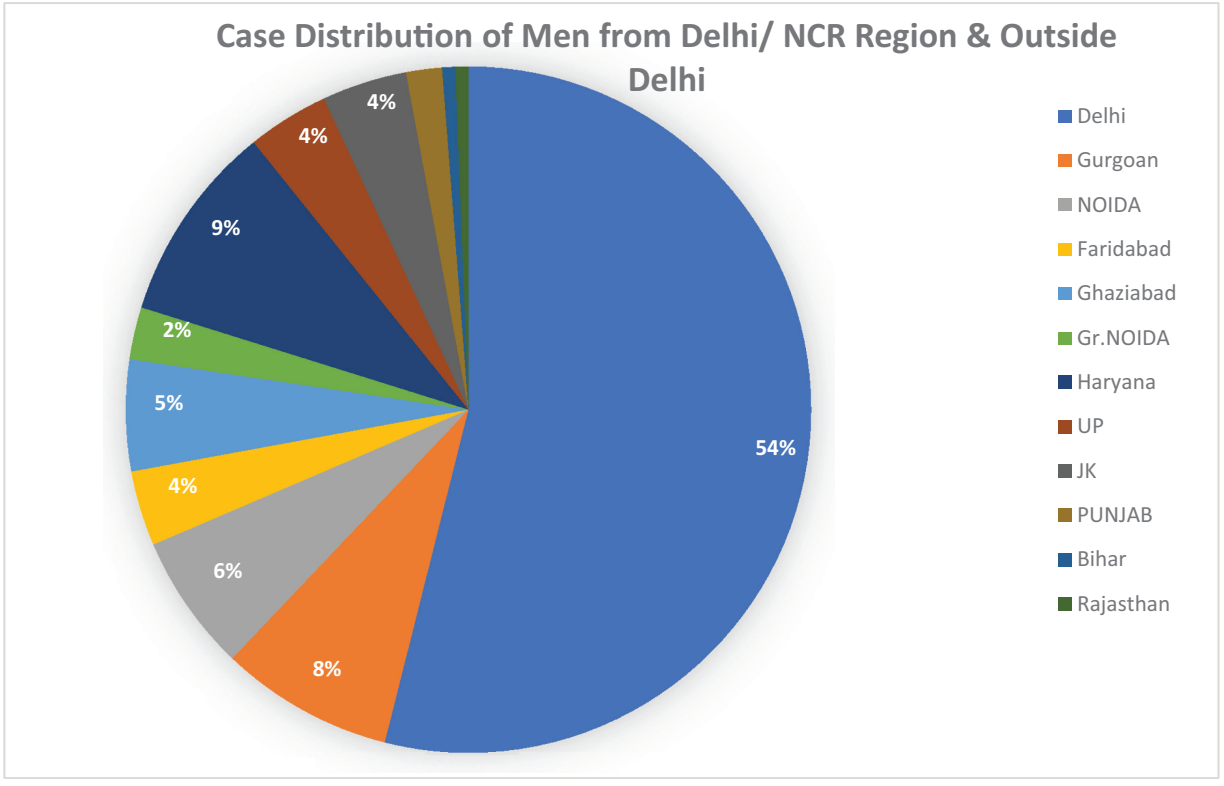

Figure 1: Pie chart showing percentage (%) of men included in the study from Delhi/NCR region and those living outside Delhi

also excluded males taking any psychotropic, anti-epileptics or cardiotropics drugs males having tumors or undergoing chemotherapy that could impair semen parameters.

Air pollution data and exposure assessment

Air pollution data for the study period was obtained from the website of Central Pollution Control Board, Government of India (https://cpcb.nic.in/national-air-quality-index) executing a nationwide program of ambient air quality monitoring known as National Air Quality Monitoring Program (NAMP). Under N.A.M.P., four air pollutants *viz*., Sulphur Dioxide (SO_2), Oxides of Nitrogen as NO_2, Respirable Suspended Particulate Matter (RSPM / PM10) and Fine Particulate Matter ($PM_{2.5}$) have been identified for regular monitoring at all the locations. The monitoring of pollutants is carried out for 24 hours (4-hourly sampling for gaseous pollutants and 8-hourly sampling for particulate matter) with a frequency of twice a week, to have one hundred and four (104) observations in a year. Overall AQI is monitored for all the cities and calculated if data are available for minimum three pollutants out of which one should necessarily be either PM2.5 or PM10. According to the WHO Air Quality Guidelines, 2005,[57] the recommended annual mean is 20 mg/m3 for PM_{10},

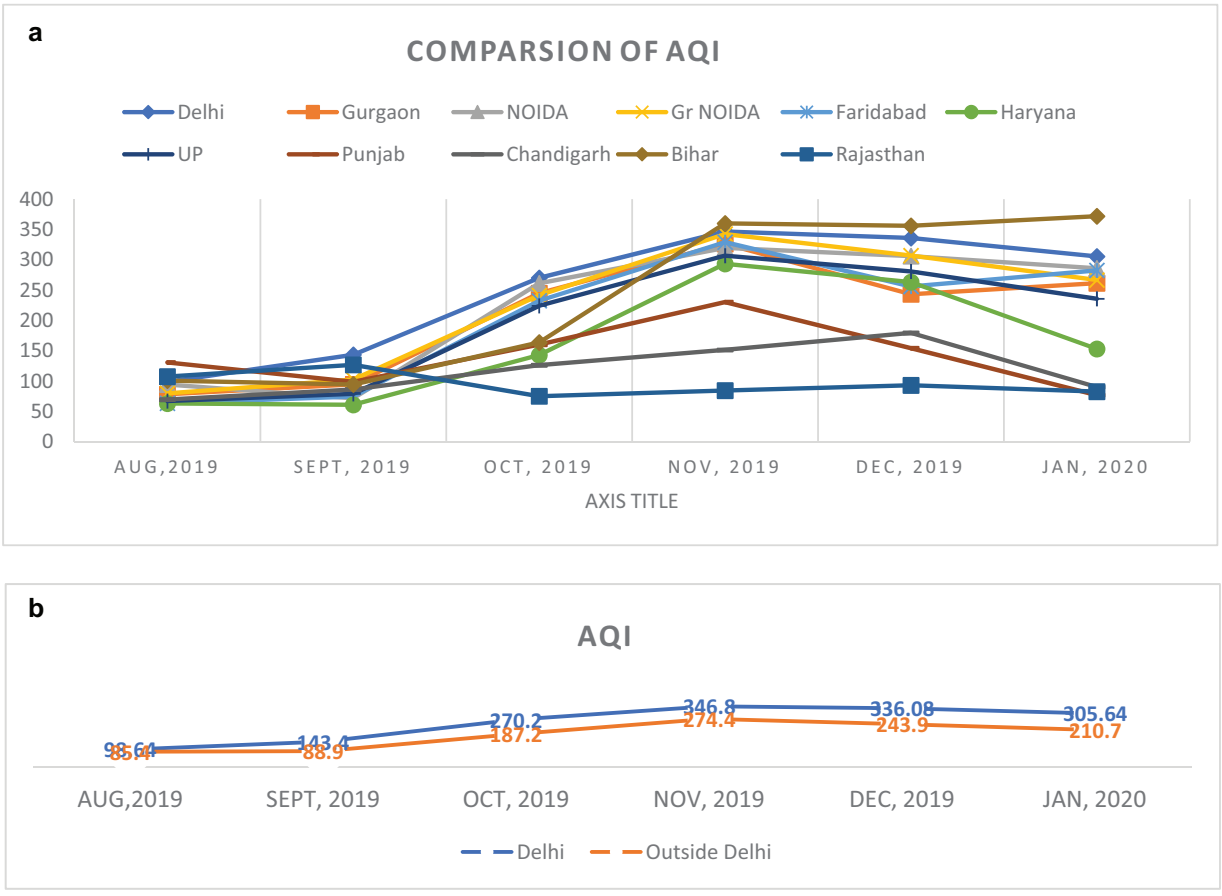

Figure 2: (a) Line diagram showing Air Quality Index (AQI) of Delhi and neighboring cities and states during the study period and (b) comparison of AQI between Delhi and outside Delhi states

40 mg/m3 for NO2, and 20 mg/m3 for SO2. Urban residents are all exposed to higher concentrations of PM $_{2.5}$ PM_{10}, SO_2 and NO_2 than residents in the sub-urban area [Figure 2b].

Statistical analysis

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Qualitative variables were correlated using Chi-Square test/Fishers Exact test. A *P*-value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spread sheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

RESULTS

A total of 657 men undergoing fertility workup during their infertility treatment were included in the study with 521 (79.2%) men from Delhi/NCR region and another 136 (20.7%) men from outside Delhi region with mean age of both the group was 35.6 ± 5.7 years. More than half (54%) of participants were from Delhi followed by adjoining cities of National Capital region and then neighboring states as shown in the pie chart (Figure 1). The average AQI of month for urban area of Delhi/NCR and sub-urban area of nearby adjoining cities and states was obtained from web-based automatic system designed on real time basis during the study period as shown in Figure 2a, b.

A comparison of semen parameters was done between samples analyzed from Delhi/NCR region with those of men residing outside Delhi. The mean average with ± standard deviation and *P*-value was calculated with 95% confidence interval between the group for identifying any significant semen parameter in both the groups. A mean ± standard deviation was calculated for all the analyzed semen parameters for both the groups along with semen volume, abstinence days, sperm concentration (SC), total sperm concentration (TSC), total motility (TM), progressive motility (PR), total motile concentration (TMC) and morphology [Table 1]. In the present study, we found that the spatial distributions of Air Quality Index (AQI) giving the average of atmospheric pollutants PM10, SO2, and NO2 were

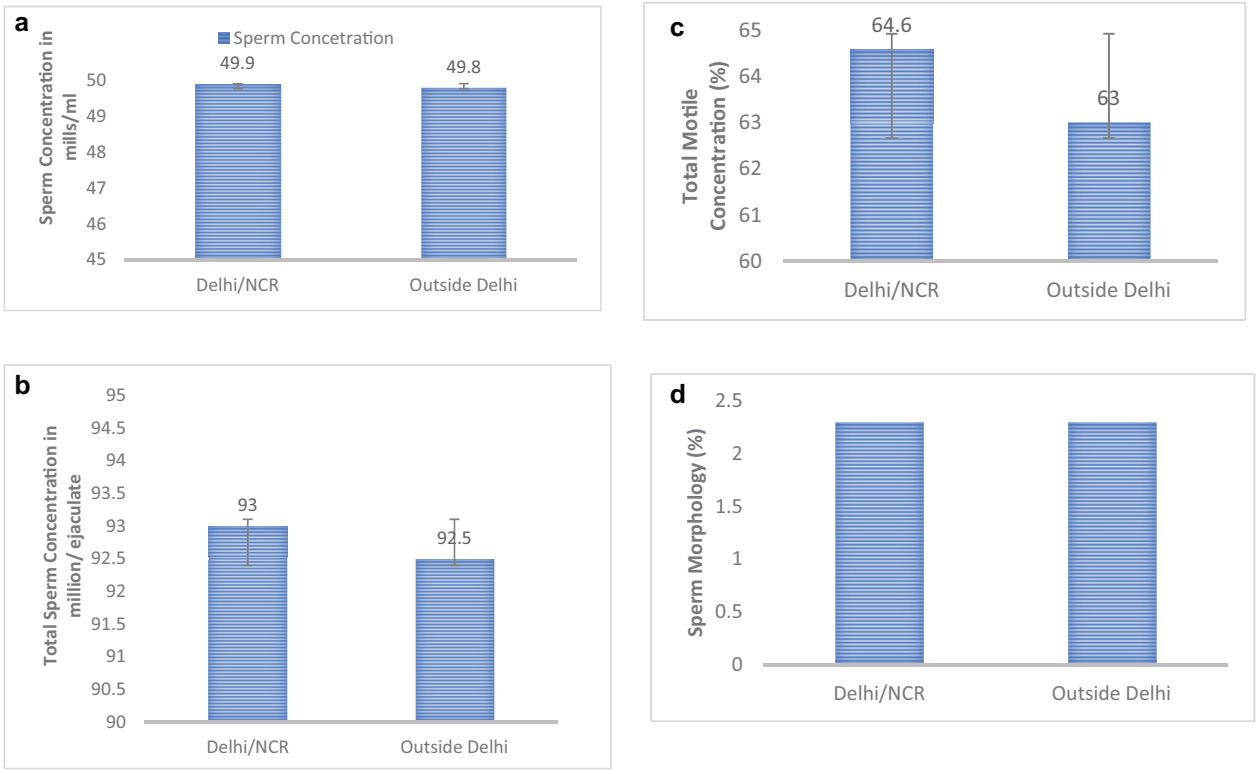

Figure 3: Comparison of semen parameters of men residing in the Delhi/NCR region versus men's residing outside Delhi (a) Sperm concentration (B) Total Sperm Concentration (C) Total Motile Concentration (TMC) (D) Sperm Morphology

significantly higher in urban areas than in the rural areas. It was finally observed in both the groups there was no difference observed in mean ± SD of in semen volume (1.79 ± 0.84 *vs* 1.78 ± 0.83) *P* = 0.90, sperm concentration (49.9 ±32.4 *vs* 49.8 ± 32.4) p-0.97, total motility (58 ± 22.5 *vs* 58.1 ± 22.5) *P* = 0.96, progressive motility (32.3 ±13.8 *vs* 32.2 ± 13.8) *P* = 0.95 and sperm morphology (2.3 ± 2.2 *vs* 2.3 ± 2.2) *P* = 1.00. A slight difference in mean ± SD was observed for total sperm concentration (93.0 ± 70.7 *vs* 92.5 ± 70.7) *P* = 0.94 and total motile concentration (64.6 ± 54 *vs* 63.0 ± 54.2) *P* = 0.80 with no significance difference in *P*-value between both the groups [Table 1, Figure 2a-d].

The prevalence of abnormal semen analysis findings was compared between both the groups and it was observed that there were a greater number of Oligozoospermia men from Delhi /NCR region 16% vs 13.2 % from men residing outside Delhi. It was further observed that there were increased number of men with asthenozoospermia 13.2% vs 10.5% and teratozoospermia 30.1% vs 26.6% in men residing outside Delhi as compared to men from Delhi/NCR region. There was no difference in prevalence observed for severe-oligo-astheno-teratozoospermia (5.5% vs 5.8%) in both the groups. The overall prevalence of male factor was higher in men residing outside Delhi as compared to men of Delhi/NCR 53.1% vs 56.6% respectively and the overall combined male factor in both the study group was 58% [Table 2, Figures 3 and 4].

DISCUSSION

In the present study it has been observed that sperm concentration, total motility progressive motility and sperm morphology has no difference from the men residing in Delhi/NCR region population residing in the urban area in comparison to semi-urban population. Whereas a slight difference in mean ±SD was observed for total sperm concentration and total motile concentration with no significance difference in both the study group. Though there has been seasonal variation in air pollution especially in winter in Delhi/NCR region, but it seems that such a short-term variation in air pollution has no impact on the semen parameters of urban population of Delhi/NCR region and neighboring states. In the same study, we found that the spatial distributions of Air Quality Index (AQI) were significantly higher in urban areas than in sub-urban areas. It was observed from the AQI data that there was a strong seasonal variation in urban area of Delhi and sub-urban areas outside Delhi region. The highest health risk due to particulates was also found in the city zone during winter. In our study, the same seasonal

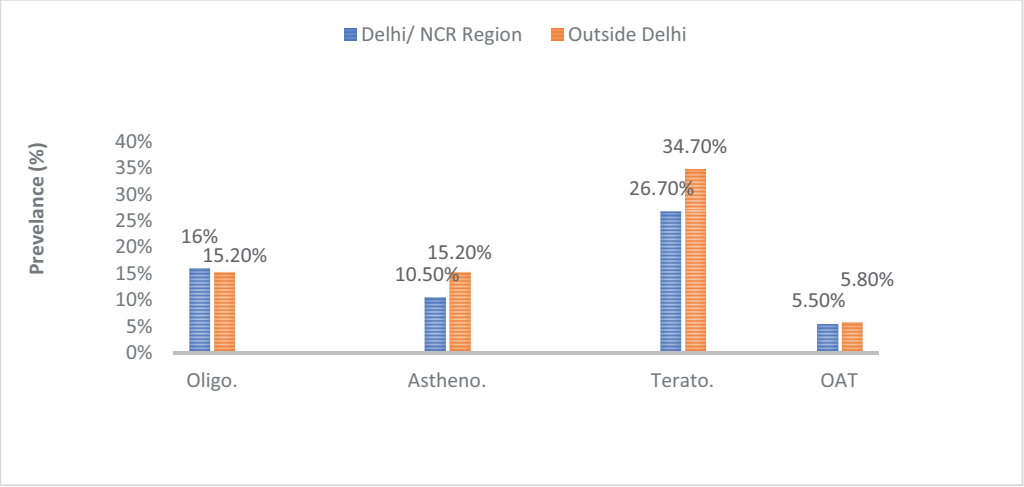

Figure 4: Comparison of Prevalence of Oligozoospermia, Asthenozoospermia, Teratozoospermia and Oligoasthenotertaozoospermia of males from Delhi/NCR region and men's residing outside Delhi

variation for the air pollutants PM_{10}, SO_2, and NO_2 was found in urban areas. The urban air pollution distribution pattern for PM_{10}, SO_2, and NO_2 all showed the highest concentrations in winter months and the lowest concentrations in summer months.

A study from Czech Republic and the authors noticed that men exposed to air pollution were more likely to have lower percentage of motile sperm and lower percentage of sperm with normal morphology (fewer sperm with normal morphology or normal head shape) than were those lived in a city with less air pollution.[20] Later study among the same participants did not find the association between high air pollution and sperm concentration, volume, motility and morphology.[21] There are also reported studies from urban and rural areas in China performed among 1346 volunteers and observed that the concentration of PM10, SO2 and NO2 were negatively associated with normal sperm morphology percentage. In another study among 1759 men from Wuhan city undergoing assisted reproductive technology procedures found that exposure to PM2.5 was inversely associated with sperm concentration. The Authors concluded that the inconsistency between studies could be due to differences in the air exposure level among the different populations. Though fundamental researches supported the negative effects of air pollution on sperm quality, several epidemiologic studies demonstrated nonsignificant or contrary results. For instance, about sperm count, several studies have demonstrated that air pollution can result in significant reduction in sperm concentration and total sperm count.[22-26] But other studies did not demonstrate significant results. Some of the other published reports on the impact of air pollution on semen quality are increasingly numerous, but the findings vary greatly. This is certainly due to the differences in research models, on the one hand, and to the presence of other harm-ful environmental factors in the areas where air pollutants abound, on the other.

Exposure to air pollution is the main cause of global threats to the environment and to the human population, and contributes to a range of adverse health outcomes, also affecting reproductive health.[27-29] The adverse impact of air pollution on semen quality was confirmed by Wu *et al.*,[30] WHO reported a deterioration in sperm morphology with exposure to PM10 and SO2. In turn, Santi *et al.*[31] only demonstrated an impact of PM10 on sperm morphology. In the present study we did not find evidence for the adverse impact of air pollution on sperm density in the male patients first reporting for fertility treatment. This is con-sistent with the report by Santi *et al.*[30] WHO found no association between PM10 and sperm count per 1 ml of ejaculate, as opposed to Wu *et al.*[31] WHO claimed that exposure to PM10 did reduce sperm density. The negative impact of environment pollution on sperm quality has been suggested by several studies,[32,33] despite that data on this matter are still controversial.[34-36] Several studies attempted to address the role of specific air pollutants on sperm quality, showing no conclusive results. Accordingly, NO_2 concentration was found to positively correlate with total sperm count,[31] but this was not further confirmed.[35] Similarly, particulate matter $(PM)_{2.5}$ and PM_{10} have been reported to affect sperm quality only in a single study,[37] while multiple data do not show any association within the sperm quality.[31,35, 38] Radwan *et al.*[39] studied the effects of $PM_{2.5}$, SO_2, NO_2, and

Table 2: Prevalence of semen analysis findings of men residing in Delhi/NCR region and outside Delhi

Semen analysis	Oligozoospermia	Asthenozoospermia	Teratozoospermia	Severe-Oligo-astheno-teratozoospermia	Male factor
Delhi/NCR Region	16% (83/521)	10.5% (55/521)	26.6% (139/521)	5.5% (29/521)	53.1% (306/521)
Outside Delhi	13.2% (18/136)	13.2% (18/136)	30.1% (41/136)	5.8% (8/136)	56.6% (77/136)
Overall	15.3% (101/657)	11.1% (73/657)	27.3% (180/657)	5.6% (37/657)	58% (383/657)

CO separately and reported morphologic alterations associated with all air pollutants. Santi *et al.*[31] found a decrease in normal sperm morphology only with PM_{10} but not with $PM_{2.5}$. Rubes *et al.*,[40] Hansen *et al.*[41] and Hammoud *et al.*[38] did not find any statistically significant relationship.

There are possibly numerous factors contributing to the divergent results between the studies. The various endpoints of semen quality (sperm concentration, motility, morphology, sperm DNA damage, sperm aneuploidy) used may be a possible explanation for the different study results. Also different level of exposure to air pollutants may impact on the differences between studies. The choice of covariates for statistical models may also impact the results. Further issue is a possibility of concomitant exposure to other environmental or occupational factors which may also have an impact on semen quality. Other potential explanations for the differences among studies include the type and timing of exposure, dose, measurement of the exposure or an outcome. The biological mechanisms linking ambient air pollution to decreased sperm quality have yet to be determined. Rubes *et al.*[40] concluded that the reactive metabolites of PM10 can reach the testes and react with sperm DNA to form adducts; this toxic effect occurs in late spermatogenesis, when there is no repair mechanism to correct it, resulting in increased DNA fragmentation.[41] Additionally, Hammound *et al.*[38] suggest that PM2.5 could act as an endocrine disruptor affecting late synthesis of proteins necessary for sperm motility.[19] Additionally, the reactive oxygen species damage the integrity of DNA in the sperm nucleus which may affect sperm count and motility.[42,43] Excessive air pollution may impair the body's antioxidant defense systems. The antioxidant defense system of semen includes enzymatic and non-enzymatic factors that interact to provide optimum protection against reactive oxygen species (ROS).[44] A deficiency of any of these factors may reduce the overall antioxidant capacity. As ROS are highly reactive, they quickly react with proteins, lipids and nucleic acids, also initiating chain reactions, and induce the formation of other free radical products. Oxidative stress results in damage to cellular macromolecules, leading to dysfunction and, ultimately, to cell death.[43,45]

In study population subfertile men undergoing treatment were exposed to moderate levels of ambient air pollution and there was no clear evidence to suggest an association between ambient air pollution exposure and adverse changes in semen quality as observed by other.[46-48] This suggests that air pollution may not be a major contributor to differences in semen quality among men. But due to rapid economic development and urbanization in large cities has been reported to lead to severe air pollution in urban areas.[49,50] Because of the increasing traffic, industrial activity, and energy consumption in urban areas, higher emissions of air pollutants usually occur there. Regional variability of air pollutant concentration in urban and rural areas has also been identified.[51-55] There are very few studies being reported from India on the investigated area and one of the study by Pant *et al.*[56] on urban population of Lucknow, India, observed to have significantly lower percentage of sperm motility than the rural population in India.

There are certain limitations about the present study as potential confounders that could impact the results are not ruled out nor considered in the statistical analysis so there is possibility that residual or unmeasured confounding factors partly may have contributed to the observed association. The major potential confounders in studies of exposure to air pollution and semen quality are well-known and most studies at least try to assess them. The potential risk factors or well-known confounders in such studies are abstinence period (days before semen collection), age, smoking status and drinking season, temperature, BMI and ethnicity. However, there were some limitations to this study. First, we used monitoring data for the study sites to measure ambient air pollution instead of precisely measuring each individual's exposure level. Secondly, the measurement and prediction of PM_{10}, SO_2 and NO_2 exposure were performed outside, and population exposures were also influenced by indoor environments and other confounders as discussed. Third, using only a single semen sample to predict male reproductive function over a longer period is a potential limitation of the present study.

CONCLUSION

Our study did not show any significant change in semen parameter of men on comparing with residence in urban or semi-urban area upto period of three years. Although interesting, these results are still preliminary and warrant further follow-up in the future. To the best of our knowledge, this study is the first attempt to investigate the effects of outdoor exposure to air pollutants on semen quality in both urban and semi-urban areas around Delhi/NCR region. Future research is still necessary, using a better characterization of exposure models in order to validate fully the ongoing effects of air pollution on human sperm that were found in this study. Future studies should also consider other cofounding factors which may interfere with male reproductive health and should incorporate different seasonal variation to generate a more accurate and full assessment of adverse effect of air pollution on male fertility. There is need to have long-term study over period of years on air pollution level (air quality) and then correlating with semen parameters in men living in urban cities like Delhi and adjoining cities.

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Research involving human participants and/or animal

This article does not contain any studies with human participants or animals performed by any of the authors.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. BMJ 1992;305:609-13.
2. Sharpe RM. Declining sperm counts in men-is there an endocrine cause? J Endocrinol 1993;136:357-60.
3. Forti G, Serio M. Male infertility: is its rising incidence due to better methods of detection or an increasing frequency? Hum Reprod 1993;8:1153-4.
4. Kumar S. Occupational exposure associated with reproductive dysfunction. J Occup Health 2004;46:1-19.
5. Sheiner ETK, Sheiner E, Hammel R, Potashnik G, Carel R. Effect of occupational exposures on male fertility: literature review. Ind Health 2003;41:55-62.
6. Wdowiak A, Wdowiak L, Wiktor H. Evaluation of the effect of using mobile phone on male fertility. Ann Agric Environ Med 2007;14:169-72.
7. Marimuthu P, Kapilashrami MC, Misro MM, Singh G. Evaluation of trend in semen analysis for 11 years in subjects attending a fertility clinic in India. Asian J Androl 2003;5:221-225.
8. Mishra P, Negi MPS, Srivastava M, Singh K, Rajender S. Decline in seminal quality in Indian men over the last 37 years. Reprod Biol Endocrinol 2018;16:103.
9. Dama MS, Rajender S. Secular changes in the semen quality in India during the past 33 years. J Androl 2012;33:740-744.
10. Kado NY, Okamoto RA, Kuzmicky PA, Kobayashi R. Emissions of toxic pollutants from compressed natural gas and low sulfur diesel-fueled heavy-duty transit buses tested over multiple driving cycles. Environ Sci Technol 2005;39:7638-49.
11. Jeng HA, Yu L. Alteration of sperm quality and hormone levels by polycyclic aromatic hydrocarbons on airborne particulate particles. J Environ Sci Health A Tox Hazard Subst Environ Eng 2008;43:675-81.
12. Stafoggia RB, Raaschou-Nielsen M. Long-term exposure to air pollution and cardiovascular mortality: an analysis of 22 European cohorts. Epidemiology 2014;25:368-78.
13. Sava F, Carlsten C. Respiratory health effects of ambient air pollution: an update. Clin Chest Med 2012;33:759-69.
14. Yorifuji T, Kashima S, Higa Diez M, Kado Y, Sanada S, Doi H. Prenatal exposure to traffic-related air pollution and child behavioral development milestone delays in Japan. Epidemiology 2016; 27:57-65.
15. World Health Organization. WHO laboratory manual for the examination and processing of human semen, 2010.
16. Skakkebaek NE, Rajpert-De Meyts E, *et al*. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol Rev 2016;96:55-97
17. Bloom M, Whitcomb B, *et al*. Associations between urinary phthalate concentrations and semen quality parameters in a general population. Hum Reprod 2015;30:2645-2657.
18. Nordkap L, Jensen TK, *et al*. Psychological stress and testicular function: a cross-sectional study of 1, 215 Danish men. Fertil Steril 2016;105:74-187.e1–e2.
19. Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization Laboratory methods for the examination of human semen. Eur Urol 2016;70:635-645.
20. Selevan SG, Borkovec L, Slott VL, Zudova Z, Rubes J, Evenson DP, Perreault SD. Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Persp 2000;108:887-94.
21. Rubes J, Selevan SG, Evenson DP, Zudova D, Vozdova M, Zudova Z, Robbins WA, Perreault SD. Episodic air pollution is associated with increased DNA fragmentation in human sperm without changes in semen quality. Hum Reprod 2005; 20:2776-83.
22. Guven A, Kayikci A, Cam K, *et al*. Alterations in semen parameters of toll collectors working at motorways: does diesel exposure induce detrimental effects on semen? Andrologia 2008;40:346-51.
23. Calogero AE, La Vignera S, Condorelli RA, *et al*. Environmental car exhaust pollution damages human sperm chromatin and DNA. J Endocrinol Invest 2011;34:e139-43.
24. Wu L, Jin L, Shi T, *et al*. Association between ambient particulate matter exposure and semen quality in Wuhan, China. Environ Int 2017;98:219-28.
25. Liu Y, Zhou Y, Ma J, *et al*. Inverse association between ambient sulfur dioxide exposure and semen quality in Wuhan, China. Environ Sci Technol 2017;51:12806-14
26. Selevan SG, Borkovec L, Slott VL, *et al*. Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Perspect 2000;108:887-94.
27. Lelieveld J, Evans JS, Fnais M, Giannadaki D, Pozzer A. The contribution of outdoor air pollution sources to premature mortality on a global scale. Nature 2015;525:367-71

28. Chen EK, Zmirou-Navier D, Padilla C, Deguen S. Effects of air pollution on the risk of congenital anomalies: a systematic review and meta-analysis. Int J Environ Res Public Health.
29. Jurewicz J, Radwan M, Wielgomas B, Kałużny P, Klimowska A, Radwan P, *et al*. Environmental levels of triclosan and male fertility. Environ Sci Pollut Res Int 2018;25.
30. Wu L, Jin L, Shi T, Zhang B, Zhou Y, Zhou T, *et al*. Association between ambient particulate matter exposure and semen quality in Wuhan, China. Environ Int 2017;98:219-28.
31. Santi D, Vezzani S, Granata AR, Roli L, De Santis MC, Ongaro C, *et al*. Sperm quality and environment: A retrospective, cohort study in a Northern province of Italy. Environ Res 2016;150:144-53.
32. Calogero AE, La Vignera S, Condorelli RA, Perdichizzi A, Valenti D, Asero P, Carbone U, Boggia B, De Rosa N, Lombardi G, D'Agata R, Vicari LO, Vicari E, De Rosa M. Environmental car exhaust pollution damages human sperm chromatin and DNA. J Endocrinol Investig 2011;34:e139-e143
33. Oliveri Conti G, Calogero AE, Giacone F, Fiore M, Barchitta M, Agodi A, Ferrante M. B(a)P adduct levels and fertility: a crosssectional study in a Sicilian population. Mol Med Rep 2017;15:3398-404
34. Hansen C, Luben TJ, Sacks JD, Olshan A, Jeffay S, Strader L, Perreault SD. The effect of ambient air pollution on sperm quality. Environ Health Perspect 2010;118:203-209
35. Zhou N, Cui Z, Yang S, Han X, Chen G, Zhou Z, Zhai C, Ma M, Li L, Cai M, Li Y, Ao L, Shu W, Liu J, Cao J. Air pollution and decreased semen quality: a comparative study of Chongqing urban and rural areas. Environ Pollut 2014;187:145-52
36. Nobles CJ, Schisterman EF, Ha S, Kim K, Mumford SL, Buck Louis GM, Chen Z, Liu D, Sherman S, Mendola P. Ambient air pollution and semen quality. Environ Res 2018;163:228-236
37. Zhou N, Jiang C, Chen Q, Yang H, Wang X, Zou P, Sun L, Liu J, Li L, Li L, Huang L, Chen H, Ao L, Zhou Z, Liu J, Cui Z, Cao J. Exposures to atmospheric PM10 and PM10-2.5 affect male semen quality: results of MARHCS study. Environ Sci Technol 2018;52:1571-81
38. Hammound A, Douglass TC, Gibson M, Sanderson M, Parker-Jones K, Peterson M. Decreased sperm motility is associated with air pollution in Salt Lake City. Fertil Steril 2010;93:1875-9.
39. Radwan M, Jurewicz J, Polanska K, Sobala W, Radwan P, Bochenek M, Hanke W. Exposure to ambient air pollution-does it affect semen quality and the level of reproductive hormones? Ann Hum Biol 2016;43:1-7.
40. Rubes J, Selevan SG, Sram RJ, Evenson DP, Perreault SD. GSTM1 genotype influences the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutation Res 2007;625:20-8.
41. Hansen C, Luben TJ, Sacks JD, Olshan A, Jeffay S, Strader L, Perreault SD. The effect of ambient air pollution on sperm quality. Environ Health Perspect 2010;118:203-9
42. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril 2003;79:829-43.
43. Aitken J, Fisher H. Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. Bioessay 2004;16:259-67.
44. Sokol RZ, Kraft P, Fowler IM, Mamet R, Kim E, Berhane KT. Exposure to environmental ozone alters semen quality. Environ Health Persp 2006;114:360-5
45. Rubes J, Rybar R, Prinosilova P, Veznik Z, Chvatalova I, Solansky I, Sram RJ. Genetic polymorphism influence the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutat Res 2010;683:9-15.
46. Jurewicz J, Dziewirska E, Radwan M, Hanke W. Air pollution from natural and anthropic sources and male fertility. Reprod Biol Endocrinol 2018;16:09
47. Wdowiak A, Wdowiak E, Bień A, Bojar I, Iwanowicz-Palus G, Raczkiewicz Air pollution and semen parameters in men seeking fertility treatment for the first time. Int J Occup Med Environ Health 2019;32:387-99
48. Zhang J, Cai Z, Yang B, Li H. Association between outdoor air pollution and semen quality: Protocol for an updated systematic review and meta-analysis. Medicine (Baltimore) 2019;98:e15730
49. Liu S, Tao S, Liu WX, Dou H, Liu YN, Zhao JY, Little MG, Tian Z, Wang J, Wang L, Gao Y. Seasonal and spatial occurrence and distribution of atmospheric polycyclic aromatic hydrocarbons (PAHs) in rural and urban areas of the North Chinese Plain. Environ Pollut 2008;156:651e656.
50. Guéguen F, Stille P, Millet M. Air quality assessment by tree bark biomonitoring in urban, industrial and rural environments of the Rhine Valley: PCDD/Fs, PCBs and trace metal evidence. Chemosphere 2011;85:195e202.
51. Ma WL, Qi H, Li YF, Liu LY, Sun DZ, Wang DG, Zhang Z, Tian CG, Shen JM. Seasonal and spatial variations of air concentrations of polycyclic aromatic hydrocarbons in Northeastern Chinese urban region. Bull Environ Contam Toxicol 2011;86:43e49.
52. Hoek G, Meliefste K, Cyrys J, Lewné M, Bellander T, Brauer M, Fischer P, Gehring U, Heinrich J, van Vliet P, Brunekreef B. Spatial variability of fine particle concentrations in three European areas. Atmos Environ 2002;36:4077e4088.
53. Lewné M, Cyrys J, Meliefste K, Hoek G, Brauer M, Fischer P, Gehring U, Heinrich J, Brunekreef B, Bellander T. Spatial variation in nitrogen dioxide in three European areas. Sci Total Environ 2004;332:217e230.
54. Van der Zee SC, Hoek G, Harssema H, Brunkekreef B. Characterization of particulate air pollution in urban and non-urban areas in the Netherlands. Atmos Environ 1998;32:3717e3729.
55. Zhu YF, Hinds WC, Kim S, Shen S, Sioutas C. Study of ultrafine particles near a major highway with heavy-duty diesel traffic. Atmos Environ 2002;36:4323e4335.
56. Pant N, Shukla M, Kumar Patel D, Shukla Y, Mathur N, Kumar Gupta Y, Saxena DK. Correlation of phthalate exposures with semen quality. Toxicol Appl Pharmacol 2008;231:112e116.
57. WHO 2005. Air Quality Guidelines: Global Update 2005: Particulate Matter, Ozone, Nitrogen Dioxide and Sulfur Dioxide. World Health Organization/Cambridge University Press, Cambridge, UK.