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ORIGINAL ARTICLE

Sperm DNA damage—the effect of stress and everyday life factors

M Radwan[1], J Jurewicz[2], D Merecz-Kot[3], W Sobala[1], P Radwan[1], M Bochenek[4] and W Hanke[2]

The clinical significance of sperm DNA damage lies in its association with natural conception rates and also might have a serious consequence on developmental outcome of the newborn. The aim of the present study is to determine whether stress and everyday life factors are associated with sperm DNA damage in adult men. The study population consisted of 286 men who attended the infertility clinic for diagnostic purposes and who had normal semen concentration of 20–300 M ml^{-1} or with slight oligozoospermia (semen concentration of 15–20 M ml^{-1}) (WHO, 1999). Participants were interviewed and provided a semen sample. The sperm chromatin structure assay was assessed using flow cytometry. In the present study, we found evidence for a relationship between sperm DNA damage parameters and everyday life factors. High and medium level of occupational stress and age increase DNA fragmentation index ($P = 0.03$, $P = 0.004$ and $P = 0.03$, respectively). Other lifestyle factors that were positively associated with percentage of immature sperms (high DNA stainability index) included: obesity and cell phone use for more than 10 years ($P = 0.02$ and $P = 0.04$, respectively). Our findings indicate that stress and lifestyle factor may affect sperm DNA damage. Data from the present study showed a significant effect of age, obesity, mobile phone radiation and occupational stress on sperm DNA damage. As DNA fragmentation represents an extremely important parameter indicative of infertility and potential outcome of assisted reproduction treatment, and most of the lifestyle factors are easily modifiable, the information about factors that may affect DNA damage are important.

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INTRODUCTION

DNA fragmentation is an important factor in etiology of male fertility.[1,2] However, it is still under evaluated and its inclusion in routine semen analysis is debated. DNA fragmentation has been shown to be a robust indicator of fertility potential, more than conventional semen parameters. DNA damage may be present in men with both abnormal and normal semen parameters[1] and routine semen parameters are not robustly predictive of infertility or outcome of assisted reproduction treatment.[3,4]

Men with high DNA fragmentation levels have significantly lower odds of conceiving, both naturally and through procedures such as intrauterine insemination and IVF, sustained pregnancy and infertility problems.[4–6] Also there is growing evidence associating sperm DNA damage with mutation development risks and offspring defects.[7,8]

Semen quality in the adult male can be affected by a number of environmental and lifestyle factors. However, most studies to date have only considered how sperm concentration, motility and morphology are affected by such exposures. More recent studies have begun to examine the effect of environmental and lifestyle factors on sperm DNA integrity. Important factors include: chemical agents such as cigarette smoke,[9,10] biological factors including, increasing male age,[11] elevated body mass index (BMI)[12–14] and physical agents such as mobile phone radiation.[15] A number of hypotheses have been proposed to account for how sperm DNA might be damaged by these factors.

There is, however, uncertainty about the most appropriate laboratory test(s) to identify and quantify such DNA damage and no convincing evidence on possible therapeutic measures. The aim of the present study was to examine the association between everyday life factors (age, BMI, cell phone radiation exposure, physical activity, smoking, alcohol and coffee consumption), level of stress (occupational stress, life stress) and male sperm DNA damage measures: the percentages of DNA fragmentation index (DFI), medium of DNA fragmentation index (M DFI), high DNA fragmentation index (H DFI) and high DNA stainability index (HDS—percentage of immature sperms).

MATERIALS AND METHODS

Study population

Study subjects were a subset of 286 men from a parent study of 344 men assessing the impact of environmental, lifestyle and occupational exposure on semen quality. In the parent study men aged under 45 years of age (range: 22.7–44.8 years) who attended infertility clinic in Lodz, Poland for diagnostic purposes with normal semen concentration of 20–300 M ml^{-1} or with slight oligozoospermia (semen concentration of 15–20 M ml^{-1})[16] between 2008 and 2011 from the study 'Environmental factors and male infertility' were eligible for inclusion. The Nofer Institute of Occupational Medicine Bioethical Committee Board had approved the study (Resolution No. 9/2007 (04.06.2007)) and written informed consent was obtained from all subjects before their participation. All participants completed questionnaire which collected demographic information, lifestyle factors and

[1]Department of Gynecology and Reproduction, 'Gameta' Hospital, Rzgów, Poland; [2]Department of Environmental Epidemiology, Nofer Institute of Occupational Medicine, Lodz, Poland; [3]Department of Health and Work Psychology, Nofer Institute of Occupational Medicine, Lodz, Poland and [4]Department of Biotechnology of Animal Reproduction, National Research Institute of Animal Production, Kraków Balice, Poland. Correspondence: Dr M Radwan, Department of Gynecology and Reproduction. 'Gameta' Hospital, Rzgów 95-030, Poland or Dr J Jurewicz, Department of Environmental Epidemiology, Nofer Institute of Occupational Medicine, Lodz 91-348, Poland.
E-mail: mradwan@gameta.pl or joannaj@imp.lodz.pl
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medical history. Full details of the parent study have been described elsewhere.[17] Study participants provided urine, saliva and semen samples on the same day. Urine samples were used for the assessment of biomarkers of exposure to environmental factors: phthalates, polycyclic aromatic hydrocarbons, synthetic pyrethrois. In addition, the level of cotinine was measured in saliva. As samples from the parent study had been used for other semen analysis research, eligibility for this study was based on availability of semen in the biorepository. Of the 344 men enrolled in the parent study, sufficient semen samples were available for 286 (83.14%).

Assessment of stress and everyday life factors

All study participants were asked to complete self-administered questionnaires: Subjective Work Characteristics Questionnaire (SWCQ) by Dudek *et al.*,[18] which assesses the occupational stress and Perceived Stress Scale (PSS), which assesses the life stress. SWCQ is a widely used scale for diagnosis of occupational stress, which consists of 55 items describing potential occupational stressors. The general indicator of the level of stress was the sum of the points that were marked by each respondent. The higher the sum of points, the higher was the level of stress. According to the above-mentioned norms, there is a low level of stress when the sum of the points is in the range of 65–80, the medium stress level is 81–101, a high one is 102–152 and over.[18] PSS is the most widely used psychological instrument for measuring one's perception of general life stress. Those 10-items self-administered scale was used to measure an individual's level of perceived stress. The results are the sum in points from the statements in the PSS. The higher the score obtained by respondents, the higher the level of their global stress.

A detailed questionnaire about the lifestyle factors was performed among the study participants.

The lifestyle factors were coded positively if they occurred during the 3-month window before semen collection.

Height and weight were collected during the physical examination. The BMI was calculated according to WHO 2012.[19]

The smoking status was verified by measuring cotinine level in saliva in a laboratory in Nofer Institute of Occupational Medicine. The saliva cotinine level was measured using high performance liquid chromatography coupled with tandem mass spectrometry/positive electrospray ionisation (LC-ESI+MS/MS) and the isotope dilution method (ISO 17025; criteria and accredited by the Polish Center of Accreditation (Certificate AB215)). Men were recognized as smokers when their cotinine level in saliva was higher than 10 ng ml^{-1}.[17] Metabolic Equivalent Task (MET) method was used to calculate the leisure time activity. Leisure time physical activity was divided into three categories based on the intensity of activity: light activity (that is, walking), moderate activity (that is, biking), vigorous activity (that is, swimming). To each category of leisure time physical activity, the ranges were attributed: 3.3, 4 and 8, respectively.[20,21] To calculate the MET indicator, first the number of hours spent on each type of activity weekly were multiplied by the appropriate range and then those products were summed for each of the study participants. Two categories of MET indicator categories were chosen: < 24 and $\geqslant 24$ based on the MET indicators distribution in study population.

Study subjects were grouped according to the frequency of their alcohol drinking (< 1 per week, 1–3 per week, 4–7 per week). Also the consumption of coffee was based on the frequency of drinking per week (< 1 per week, 1–6 per week, every day).

Cell phone use was based on the years of usage of this equipment (0–5 years, 6–10 years, 11–25 years).

All categories of variables were based on their distribution in study population.

Semen analysis

All men provided a semen sample. Ejaculate was obtained by masturbation into a sterile standard plastic container after a period of sexual abstinence about mean 5 days as a part of fertility investigation. Semen analysis was performed after 30 min of liquefaction at 37 °C. The semen analysis included determination of ejaculate volume, sperm concentration and sperm motility according to WHO guidelines (World Health Organization, 1999).[16] Sperm morphology was quantified using strict Kruger criteria[22] the semen smears have been air-dried, fixed and stained according to Papanicolaou. The assessment of the sperm chromatin structure assay (SCSA) was performed using flow cytometry.[23] SCSA data resolve three different cell populations: (1) % sperm without DNA fragmentation, (2) % sperm with DNA fragmentation and (3) high DNA stainability index—percentage of immature sperms (HDS). The cells with abnormal chromatin structure (that is, fragmented DNA) showed a distinct shift of alpha *t* parameter value (alpha-*t* = red/(red+green) fluorescence). The DFI was calculated according to the formula: DFI = (cells with shift of alpha-*t* parameter/speramtozoa) × 100. DFI = M DFI+H DFI. The medium and high DFI informed us about the degree of chromatin fragmentation. In SCSA method, the spermatozoa with lack of chromatin compaction ('immature' spermatozoa) had higher acridine orange stainabilities than ones with normally condensed chromatin. It resulted in stronger green fluorescence. The threshold is a DFI of 30%. The % of sperm with high DNA stainability (% HDS) related to retained nuclear histones consistent with immature sperm.[24] High DNA stainability (% HDS) was calculated on the basis of the percentage of sperm with high levels of green fluorescence, which are thought to represent immature spermatozoa with incomplete chromatin condensation.[25] Results were reported as the percentage of DFI, medium DFI (part of sperm with medium DFI), high DFI part of sperm with high DFI) and as the percentage of high DNA stainability index (HDS).

Statistical analysis

Descriptive statistics on subject demographics were calculated, along with sperm DNA damage measures. Bivariate analysis were conducted between all sperm DNA damage, stress, everyday life factors and demographic variables to investigate differences between distributions or categories and the potential for confounding. Differences were tested statistically using parametric or non-parametric methods where appropriate. Confounders identified: age (continuous variable), smoking (yes/no), alcohol (none or < 1 drink per week, 1–3 drinks per week, every day), past diseases (yes/no), BMI (25 and $\geqslant 25$ kg m^{-2}), duration of couple's infertility (years) (1–2, 2–3, 3–5, > 5), time of sexual abstinence, level of stress (low, medium, high), cell phone use (0–5 years, 6–10 years, 11–25 years). Some dependent variables were transformed if they were not a normal distribution. DFI, M DFI, H DFI, HDS were log transformed. Multiple linear regression were used to assess associations between stress, everyday life factors and sperm DNA damage. R 2.15.1 statistical program was used to analyze data.[26] Pearson correlations between DFI and semen quality parameters were examined.

The observations with missing data were excluded based on variables used in model. The significance level 0.05 was used for statistical inference.

RESULTS

The study population consisted of 286 men who attended infertility clinics for diagnostic purposes. The mean age of men participating in this study was 32.2 years. Most of them had higher (42.7%) or secondary (38.1%) education, while about 19% had only vocational education. Past diseases that may have impact on semen quality (for example, mumps, cryptorchidism, testes surgery, testes trauma) was reported by 13% of participants. The abstinence before the semen analysis was mostly 3–7 days among 74.13% of study participants, mean 5 ± 2.3 days. Most of the study participants were overweight (BMI 25–29.9 kg m^{-2}) 47.6 and 21% were obese (BMI 30–40 kg m^{-2}). 52.1% of study population used cell phone from 6 to 10 years. The leisure time physical activity was reported by 69.3% of study population. Most of the participants were nonsmokers (70.3%) and drank alcohol 1–3 drinks per week (51.4%). Occupational stress was moderate (mean 95 points (range: 59–160)).[27] The level of life stress as measured by the PSS was also medium—22 points (range: 8–40). The detailed characteristics of the study participant are presented in Table 1.

Table 1 also presents the semen quality and sperm DNA damage among the study subjects. The semen quality among the study participants were in the normal range of the WHO 1999[26] semen quality indicators. The mean percentage of DFI was 16.2% (s.d. = 11.0%, median 13.3%), DFI medium 8.5% (s.d. = 7.5%, median 6.5%), DFI high 7.9% (s.d. = 6.6%, median 6.0%) and the percentage of immature sperms (HDS) 8.8% (s.d. = 4.3%, median 8.3%) (Table 1). A negative correlation was found between sperm DNA fragmentation and the sperm concentration ($r = -0.39$, $P = 0.007$) and sperm motility ($r = -0.55$, $P = 0.0007$).

Table 1. Characteristics of the study population

Characteristics	N *(%)*	*Mean ± s.d.*	*Median (range)*
Education			
Vocational	55 (19.2)		
Secondary	109 (38.1)		
Higher	122 (42.7)		
Past diseases, which may have impact on semen quality			
No	248 (86.7)		
Yes	38 (13.3)		
Duration of couple's infertility (years)			
1–2	104 (36.4)		
2–3	95 (33.2)		
3–5	44 (15.4)		
>5	43 (15.0)		
Age (years)		32.3 ± 4.4	32.0 (22.0–44.26)
22–30	94 (32.9)		
31–40	180 (62.9)		
41–45	12 (4.2)		
Time of sexual abstinence (days)		5.0 ± 2.3	5.0 (0.0–20.0)
<3	14 (4.90)		
3–7	212 (74.13)		
>7	17 (5.94)		
Missing data	43 (15.03)		
Alcohol use			
None or <1 drink per week	94 (32.9)		
1–3 drinks per week	147 (51.4)		
Every day	45 (15.7)		
Smoking determined by cotinine level			
None	201 (70.3)		
Smoker	85 (29.7)		
BMI (kg m^{-2})		27.2 ± 3.80	27.4 (18.5–39.5)
18–24.9	91 (31.8)		
25–29.9	136 (47.6)		
30–40	59 (20.6)		
Coffee (days per week)			
< 1	67 (23.4)		
1–6	81 (28.3)		
Everyday	138 (48.3)		
Leisure time activity (MET)			
No	88 (30.7)		
<24	104 (36.4)		
⩾24	94 (32.9)		
Cell phone (years)			
0–5	45 (15.7)		
6–10	149 (52.1)		
11–25	68 (23.8)		
Missing data	24 (8.4)		
The level of occupational stress (points)		94.9 ± 25.0	88.0 (59.0–200.0)
Low (65–80 points)	78 (27.3)		
Medium (81–101 points)	87 (30.4)		
High (>102 points)	121(42.3)		
The level of life stress (points)		22.1 ± 5.8	22.5 (8.0–40.0)
8–20 points	95 (33.2)		
21–30 points	92 (32.2)		
>31 points	99 (34.6)		
Sperm DNA fragmentation index (%)			
DFI (%)	286 (100.0)	16.2 ± 11.0	13.3 (2.7–71.2)
M DFI (%)	286 (100.0)	8.5 ± 7.5	6.5 (1.7–60.5)
H DFI (%)	286 (100.0)	7.9 ± 6.6	6.0 (0.5–44.5)
HDS (%)	286 (100.0)	8.8 ± 4.3	8.3 (0.7–30.7)
Main semen parmeters			
Concentration (M ml^{-1})	286 (100.0)	53.6 ± 52.4	33.6 (15–360)
Motility (%)	286 (100.0)	57.4 ± 19.8	54 (30–99)
Sperm with abnormal morphology (%)	286 (100.0)	47.2 ± 19.7	46 (20–96)

Abbreviations: BMI, body mass index; DFI, DNA fragmentation index; H DFI, high DNA fragmentation index; HDS, high DNA stainability index; M DFI, medium DNA fragmentation index; N, number of participants.

The association between everyday life factors, level of stress and sperm DNA damage

A positive association was observed between medium and high level of occupational stress and H DFI ($P = 0.03$ and $P = 0.004$, respectively; Table 2). On the other hand, life stress (assessed by PSS) was not related to any of the examined DNA damage parameters.

Age category >40 years increased the H DFI ($P = 0.03$). Obesity (BMI 30–40 kg m^{-2}) and using cell phone more than 10 years was positively related to HDS ($P = 0.02$ and $P = 0.04$, respectively) (Table 2). Other examined lifestyle factors: smoking, alcohol consumption, coffee drinking were not related with any of the examined parameters of sperm DNA damage and high DNA stainability. The result were adjusted for potential confounders.

DISCUSSION

DNA fragmentation is underevaluated in male infertility, and represents an extremely important parameter indicative of infertility and potential outcome of assisted reproduction treatment.

In the present study, we found evidence for a relationship between stress and lifestyle factors and sperm DNA damage parameters. Occupational stress increases DFI (M DFI, H DFI). Other lifestyle factors that were positively associated with sperm DNA damage parameters include: age (H DFI), obesity (HDS), cell phone using for more than 10 years (HDS).

The results of the studies that have assessed the relationship between exposure to occupational stress and semen quality are inconsistent. Sheiner *et al.*[28] observed that male infertility group had higher marks in all of the measures of burnout as compared with the controls.[28] Similarly, no associations between semen characteristics, sexual hormones and any job strain variables were found by Hjollund *et al.*[27] Contrarily, El-Helaly *et al.*[29] in a case–control study, showed a significant relationship between job stress and male infertility. Also in study by Jurewicz *et al.*[30,31] an association between occupational stress and the percentage of progressive spermatozoa was observed. This study had a smaller sample size and took into account only a few semen parameters: volume, motility, percentage of atypical sperm and progressive spermatozoa.[30,31]

The relationship between stress and semen quality reached a level of significance only in the case of chronic occupational stress exposure (SWCQ). The PSS does not allow to assess the chronicity of life stress, and a stress indicator based on a 1-month assessment may not reflect the overall stress burden properly.

Table 2. The association between stress and lifestyle factors and DNA fragmentation index—multivariate analysis

	DFI		*M DFI*		*H DFI*		*HDS*	
	Correlation coefficient	P-*value*	*Correlation coefficient*	P-*value*	*Correlation coefficient*	P-*value*	*Correlation coefficient*	P-*value*
Level of stress[1]								
Subjective Work Characteristics Questionnaire (occupational stress)								
Medium (81–101 points)	0.04	0.13	0.03	0.55	0.46	**0.03**	0.22	0.33
High (102–152 points)	0.03	0.23	0.03	0.45	0.15	**0.004**	0.02	0.20
Perceived Stress Scale (life stress)								
21–30 points	0.06	0.22	0.04	0.63	0.02	0.55	0.06	0.55
> 31 points	0.08	0.18	0.01	0.83	0.03	0.63	0.02	0.88
Lifestyle factors								
Age[2]								
30–39.9	0.10	0.19	0.12	0.14	0.12	0.24	0.05	0.40
>40	0.09	0.68	0.11	0.42	0.09	**0.03**	0.22	0.16
BMI[3]								
25–29.9	0.00	0.95	0.02	0.85	0.01	0.98	0.04	0.57
30–40	−0.11	0.30	−0.07	0.55	0.17	0.20	0.17	**0.02**
Smoking[4]								
Yes	0.04	0.69	−0.02	0.85	0.07	0.54	0.04	0.56
Alcohol drinking[5]								
1–3 per week	−0.07	0.55	−0.04	0.73	−0.05	0.69	0.02	0.84
4–7 per week	0.04	0.88	−0.08	0.68	−0.85	0.26	0.17	0.35
Coffee drinking[6]								
1–6 per week	0.03	0.78	0.04	0.69	0.03	0.82	0.10	0.24
Everyday	0.02	0.82	−0.03	0.74	0.07	0.57	0.02	0.77
Leisure time physical activity[7]								
< 24	−0.06	0.51	−0.03	0.74	−0.10	0.40	−0.06	0.17
⩾24	−0.10	0.28	−0.03	0.76	−0.15	0.44	−0.04	0.60
Cell phone use[8]								
6–10	0.01	0.97	−0.11	0.48	0.08	0.66	0.06	0.57
11–25	0.20	0.22	0.08	0.65	0.31	0.15	0.08	**0.04**

Abbreviations: BMI, body mass index; DFI, DNA fragmentation index; H DFI, high DNA fragmentation index; HDS, high DNA stainability index; M DFI, medium DNA fragmentation index; N, number of participants. All models were adjusted for: past diseases, duration of couple's infertility, time of sexual abstinence additionally: model 1 was adjusted for: age, smoking, alcohol consumption, BMI and cell phone use; model 2 was adjusted for: stress, BMI, smoking, alcohol consumption and cell phone use; model 3 was adjusted for: stress, age, smoking, alcohol consumption and cell phone use; model 4 was adjusted for: stress, BMI, age, alcohol consumption and cell phone use; model 5 was adjusted for stress, BMI, age, smoking and cell phone use; model 6 was adjusted for: stress, BMI, age, smoking, alcohol consumption and cell phone use; model 7 was adjusted for: stress, BMI, age, smoking, alcohol consumption and cell phone use; model 8 was adjusted for: stress, BMI, age, smoking and alcohol consumption. Reference groups: level of stress, ref. low; age 22–29.9 years; BMI 18–24.9kg m^{-2}; smoking no-ref; alcohol drinking < 1 per week; coffee drinking < 1 per week; leisure time physical activity no-ref; cell phone use 0–5. Bold values signify that significance level 0.05 was used for statistical interference.

In the present study obesity increases the HDS. Farriello *et al.*[10], LaVignera *et al.*[13], Dupont *et al.*[14] and Chavarro *et al.*[32] observed higher sperm DNA damage in obese, but not in overweight men. Also Kort *et al.*[33] found a positive correlation between BMI and DFI, with the mean BMI rising from 19.9% in normal BMI men to 27% in obese men.

Age category >40 years was associated with increase in H DFI. Age-related infertility has been linked with DNA damage where age correlates positively with DNA fragmentation.[34,35]

In our study, using a cell phone for more than 10 years increases HDS. A recent study showed that DNA fragmentation was the only parameter altered in mobile phone users, in a group of high usage (>4 h daily) that stored their phone in the trouser pocket.[36] In addition, in the study performed in Poland using cell phone more than 10 years was negatively associated with the percentage of motile sperm cell.[17]

Although tobacco smoke contains high concentrations of ROS including O_2- and OH·, and participate in Fenton reactions to produce H_2O_2,[37] cadmium and lead derived from cigarette smoke also cause DNA strand breaks[38] and nicotine is oxidative, and can induce double-stranded DNA breaks in sperm DNA *in vitro*.[39] In the present study, we found no association between smoking and sperm DNA damage. In a number of studies, the correlations between smoking and DNA damage indices have been demonstrated in a group of fertile men,[9,40] men with varicoceles[10] and men with idiopathic infertility.[41] The absence of an effect of tobacco smoke in the present study is consistent with some previous studies assessing the semen quality parameters.[42,43]

In addition, no effect of alcohol, coffee consumption and physical activity on DNA damage was observed, but there have been no human studies to date that measured DNA fragmentation as an outcome.

Moderate exercise has correlated with improved semen parameters in a limited number of studies.[44,45] Studies on caffeine intake and semen quality have shown contradictory results. Some suggested no association,[46,47] whereas others found reduced sperm concentration, total sperm count and motility.[48,49] An association between alcohol consumption and semen quality parameters was observed in numerous studies.[50,51] Although alcohol may have effects on sperm, there is little conclusive evidence linking alcohol with oxidative stress and infertility. Oxidative stress has been found to be systemically increased with alcohol consumption,[52,53] but there is not yet a clear link between sperm oxidative stress and alcohol.[54]

Exposure to a variety of environmental, lifestyle factors (age, obesity, mobile phone radiation) and emotional stress connected with work may impact on sperm chromatin structure. All those factors can disturb biochemical events that occur during spermatogenesis, which can ultimately lead to abnormal chromatin structure.[55] Exact molecular mechanisms by which those factors lead to sperm DNA damage and/or chromatin abnormalities are not fully understood.[56] There are currently three main theories: chromatin packing abnormalities, reactive oxygen species (ROS) and apoptosis.[56] Oxidative stress is considered to be the major cause of DNA damage in spermatozoa.[57] Decreased levels of individual and total antioxidant capacity and high concentrations of seminal ROS have been detected in men with elevated DNA damage in numerous studies.[58–60] Lifestyle factors such as age, obesity, mobile phone radiation can contribute to oxidative stress.[61] Eskiocak *et al.*[62] found that psychological stress may affect the L-arginine-nitric oxide (NO) pathway. Seminal plasma NO was higher in stressful situation compared with non-stress period.[63] In addition, during stress period, stress scores and superoxide dismutase activities increased significantly compared with the non-stress period, and catalase activities showed no change.[63] Whereas Darzynkiewicz *et al.*[64] found that stress can cause sperm chromatin abnormalities by inducing chromatin structural problems such as apoptosis and necrosis.

The men in this study were from a fertility clinic as opposed to the general population. We were not able to examine a representative sample of general male population. We tried to overcome this disadvantage by selection among infertility male patients only men with normal semen parameters or with slight oligozoospermia according to WHO 1999 classification (WHO, 1999).[16] According to the WHO manual 2010, all our study subjects show semen parameters that fall within the range of fertile subjects.[65] Although they may differ from men in the general population, there is currently no evidence showing that they would differ in ways that would alter their response to lifestyle factors.

Our study had several methodological strengths. The chromatin structure had been evaluated using SCSA. Several methods are employed to routine diagnostic laboratories in the investigation of DNA fragmentation, among them the most common are the SCSA, the TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL) and Comet assays, and the sperm chromatin dispersion test (SCD),[66] but according to the literature, the SCSA, giving a DFI value, is perceived as the most statistically robust and reproducible test and is a valuable predictor of fertility.[61,66] Although the use of different methodologies to assess sperm DNA damage has been widely discussed, few reports have compared the clinical utility and the correlation between the most common methods in a comprehensive manner.[67–69] Different methodologies might detect different aspects of the sperm DNA fragmentation, as SCD and SCSA might be detecting some aspects related to chromatin fragmentation, and Comet and TUNEL assays could be detecting DNA breaks directly.[70–72] As different techniques may measure different aspects of chromatin integrity, a double analysis using more than one sperm DNA fragmentation technique would allow confirmation of the diagnosis.

Although few studies have explored the association between exposure to stress and life factors, and male reproductive function, none have carefully assessed the percentage of medium DFI and the percentage of immature sperms. A detailed questionnaire information on demographics, medical, lifestyle risk factors performed among study participants allowed for control of confounding in the statistical models. The relative homogeneity of study participants (educated, white) helped reduce the chance that our findings resulted from unmeasured health, behavioral or exposure factors. This homogenicity increases the internal validity of our study, but limits the generalization of study findings to more diverse population. Additional strength arise from the fact that smoking status was verified using the level of cotinine in saliva.

In conclusion, our findings indicate that stress and lifestyle factor may affect sperm DNA damage. Data from the present study showed a significant effect of age, obesity, mobile phone radiation and occupational stress on sperm DNA damage. As DNA fragmentation represents an extremely important parameter indicative of infertility and potential outcome of assisted reproduction treatment and most of the lifestyle factors are easily modifiable, information about factors that may affect DNA damage are important.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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