Lack of association between smoking and DNA fragmentation in the spermatozoa of normal men*

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Male factor infertility patients can have anomalies in their sperm nuclei, displaying high levels of loosely packaged chromatin and damaged DNA. The primary objectives of this study were to compare the extent of DNA fragmentation in the spermatozoa of healthy light and heavy smokers versus non-smokers, and to investigate its correlation with concentrations of the smoking markers cotinine and cadmium. A secondary objective was to compare the concentrations of blood cadmium and serum cotinine with corresponding concentrations in seminal plasma. Ninety-seven healthy male volunteers were divided into three groups: non-smokers, light and heavy smokers. There was no difference between the three groups with respect to age, number of ejaculations per week, serum testosterone concentration, and parameters of semen analysis. The percentages of DNA fragmentation in spermatozoa were not statistically different in the heavy smokers (12.11%), light smokers (11.66%) and non-smokers (20.41%). Serum and seminal plasma concentrations of cotinine were significantly higher in heavy smokers compared with the other groups (P < 0.0001). Median values for blood cadmium concentration were higher in heavy smokers (4.50 µg/l) than in light smokers (0.20 µg/l) and non-smokers (0.20 µg/l) (P < 0.001). Cadmium concentration in seminal plasma was significantly higher in heavy smokers (0.20 µg/l) than in light smokers (0.10 µg/l) and non-smokers (0.10 µg/l) (P < 0.05). In summary, our results indicate no association between smoking and DNA fragmentation in the spermatozoa of healthy men.

Key words: cadmium/DNA fragmentation/male infertility/smoking/spermatozoa

Introduction

Many studies have reported an association between cigarette smoking and a decrease in semen quality, most significantly for sperm concentration and motility (Stillman et al., 1986; Vine, 1996; Vine et al., 1996). This association is stronger in normal men (volunteers and donors) than in patients recruited from infertility clinic populations (Vine, 1996). Since men who smoke directly inhale a host of toxic substances which can be absorbed (Stillman et al., 1986), such as nicotine, carbon monoxide, benzo(a)pyrene, mutagenic pyrolysis-derived compounds and cadmium, a causal relationship is highly suspected (Vine et al., 1996; Zavos et al., 1998). In addition, it was reported that tobacco smoking, a source of reactive oxygen species, can increase the oxidative DNA damage in leukocytes. Most smoke constituents, however, have never been evaluated for toxicity and their effect on human spermatogenesis. In a group of male partners in an IVF programme, it was observed recently that smokers have a significantly higher percentage of spermatozoa with DNA fragmentation than non-smokers (Sun et al., 1997). This suggests that smoking could have a subtle impact on male reproduction that is not apparent in the usual parameters of semen analysis. It was thus of critical importance to confirm this result in a population of normal men in whom the effect could be even more apparent.

The primary objective of this study was to compare the degree of sperm DNA fragmentation in three groups of healthy men categorized as non-smokers, light or heavy smokers. To increase the statistical power of detecting an association between smoking and sperm DNA fragmentation, serum cotinine concentration was determined as a continuous measure of smoking dose. Since tobacco smoke represents a relatively important source of cadmium, the blood concentration of this element was included as an indicator of exposure to smoke. In addition, cadmium is recognized for its direct toxicity to the testis, at least in animal models (Xu et al., 1996) and in men exposed to high levels of this environmental agent (Xu et al., 1993).

Materials and methods

Study participants

Volunteers eligible for inclusion in this cross-sectional study were healthy men aged between 19 and 39 years (median = 27 years). Before participating in this project, each subject signed an informed consent form approved by the Ethics Committee of the Hospital Centre. The exclusion criteria were occupational exposure to heavy metals, any previous treatment affecting spermatogenesis (such as chemotherapy, radiotherapy and vasectomy), and the use of...
recreational drugs, including marijuana, cocaine or narcotic agents in the previous 3 months. In addition and for security reasons, all subjects testing positive for hepatitis B or HIV-1 were not eligible. The participants provided semen and blood samples, and completed a self-administered questionnaire describing their smoking habits and demographic characteristics. The study participants were not all of proven fertility. By definition, non-smokers had either never smoked or had smoked less than 100 cigarettes in their lifetime. Light smokers averaged between one and 19 cigarettes per day, and heavy smokers 20 or more cigarettes per day. All smokers had smoked cigarettes for at least 1 year before enrolment in this study.

**Questionnaire**

Information on smoking habits (e.g. number of cigarettes per day, number of years of smoking, brand of cigarette, and whether or not others smoked in the house) and demographic data were obtained through a questionnaire. Specific questions also sought to identify possible confounders and effect modifiers (e.g. diet, degree of exercise, previous genito-urinary tract infections, consumption of alcohol and caffeine). The questionnaire was reviewed with each participant by a physician at the time of the physical examination.

**Sample collection and storage**

Semen samples were collected in sterile polypropylene containers (Sarstedt, Montreal, Canada) at the Andrology Laboratory after 2–4 days of sexual abstinence. Standard clinical semen analysis was performed according to World Health Organization criteria (WHO, 1992) by a technician who was blinded to the identity of the donors; each sample was then divided into aliquots of 500 µl. Blood (45 ml) was drawn by venepuncture into K3 EDTA and SST Gel clot activator tubes (Becton Dickinson Vacutainer, Franklin Lakes, NJ, USA). Serum was recovered after centrifugation for 10 min at 9000 g and divided into 1 ml aliquots. Serum and seminal plasma were stored at −80°C until used.

**DNA fragmentation**

DNA fragmentation in spermatozoa was measured by a modification of the terminal deoxynucleotidyl transferase (TdT)-mediated deoxy-uridine triphosphate-biotin end labelling technique (TUNEL), essentially as described previously (Sun et al., 1997). After two washes in phosphate-buffered saline (PBS), 100 µl of TdT buffer containing single-strength 1 mol/l sodium cacodylate, 150 mmol/l Tris (pH 7.4), 25 mmol/l CoCl2, 1.25 mg/ml bovine serum albumin (BSA) and 0.1% Triton X-100 (Sigma Chemical Co., St Louis, MO, USA) were added to the sperm suspension. Following centrifugation (4 min at 10000 g), the TdT buffer was removed, and 50 µl of TdT buffer containing 3 µmol of biotin 16-deoxyuridine triphosphate (Boehringer Mannheim, Laval, Canada), 10 U of TdT (Boehringer Mannheim) and 0.1% Triton X-100 were added. The sperm suspension was then incubated at 37°C for 60 min. After two washes with TN buffer (30 mmol/l Tris, pH 7.4, 300 mmol/l NaCl and 1% Triton X-100), the fixed, permeabilized spermatozoa were treated with 100 µl of staining AP buffer (50 mmol/l Tris, pH 7.4, 150 mmol/l NaCl) containing 1% Triton X-100 and 2% streptavidin–fluorescein isothiocyanate (FITC), followed by incubation at room temperature in the dark for 45 min. The stained spermatozoa were washed twice with AP buffer before analysis. DNA fragmentation was measured using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with a 15 mW argon-ion laser for excitation. Flow during analysis was controlled at approximately 500 spermatozoa/s, and 10 000 cells were analysed in each sample.

Light-scattering and fluorescence data were obtained at a fixed gain setting in logarithmic mode. Debris were gated-out based on forward scatter versus side scatter dot plot, by drawing a region enclosing the spermatozoa population of interest; 10 000 events were collected. The percentage of labelled spermatozoa in each sample was determined. As negative controls, TdT was omitted from the reaction mixture. For positive controls, the spermatozoa were pre-treated with 0.1 IU deoxyribonuclease (DNase I, RNase-free) (Boehringer Mannheim) for 30 min at room temperature before labelling. Typical examples of such results are shown in Figure 1. Illustrations are presented of the typical frequency histograms obtained by flow cytometry with markers (M1) for the detection of fluorescence at 520 nm. The figure illustrates a typical negative control (10% of the spermatozoa are labelled) and a positive control treated with DNase I (98% labelling). A typical result for a healthy non-smoker with 16% of labelled spermatozoa is presented in panel C; a coefficient of variation of 13.2% (CV at 95% CI) was observed between assays.

**Hormone assays and blood chemistry**

Serum concentrations of free testosterone, total testosterone, thyroid-stimulating hormone (TSH) and prolactin were determined by radioimmunoassay as part of the institutionally approved protocol for semen donor candidates. Cholesterol, triglyceride and blood sugar were also measured. No measurement was made of germ cell markers (follicle stimulating hormone, inhibin, etc.) or of testicular volume.

**Cadmium assays**

All reagents were of high purity analytical grade suitable for trace element analysis. The cryovials were soaked overnight in 5% nitric acid, rinsed thoroughly with double-distilled water, and stored in deionized water until used. After semen analysis, the spermatozoa were separated from seminal plasma by centrifugation at 260 g for 10 min at room temperature, and the supernatant was aliquoted into 500 µl fractions in polypropylene plastic cryovials (Sarstedt) for storage at −80°C. The concentration of cadmium in blood and seminal plasma was determined by flameless atomic absorption spectrophotometry, using procedures described previously (Stoepepler and Brandt, 1980). The lower limit of detection for cadmium by this method is 0.2 µg/l for blood and 0.1 µg/l for seminal plasma.

**Cotinine assays**

Aliquots (1 ml) of serum and seminal plasma from each subject were analysed for cotinine by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The limit of detection for cotinine was 0.8 ng/ml of fluid. The laboratory technician performing the cotinine analyses was blinded to smoking status.

**Sample size**

We initially planned to include 200 subjects and to perform a mid-study analysis after the first 100 patients to re-evaluate the sample size. This number (n = 200) was a conservative estimate; it was based on the primary objective, which was to compare the three groups with respect to DNA fragmentation. Our intention was to show a difference of 20% in DNA fragmentation between non-smokers and heavy smokers (the groups that are more likely to present the largest difference if DNA fragmentation is linearly related to the number of cigarettes). Due to the lack of data available at the time, we estimated the standard deviation of DNA fragmentation to be 35%, a little less than twice the difference we wanted to detect. Supposing that DNA fragmentation was normally distributed, we found that a sample size of 65 patients per group was sufficient to reach a power of 80% with one-way analysis of variance at the 5% level. Our calculations were based on a previously described method (Cohen, 1988). Due to an overestimate of the standard deviation in the first place, the mid-study analysis finally demonstrated that with
Results

A total of 97 subjects was recruited through our sperm donor bank. These volunteers were divided into three groups: 69 non-smokers, 17 light smokers and 11 heavy smokers. There was no statistically significant difference between the three groups with respect to age, alcohol consumption, serum concentrations of free and total testosterone, cholesterol, triglyceride, glucose, TSH and prolactin (Table I).

The time period from specimen production to semen analysis, days of abstinence, and month of the year when semen samples were obtained did not differ significantly according to smoking status. The Kruskal–Wallis test indicated that smoking status was not significantly associated with any of the semen quality parameters (Table II).

The data for DNA fragmentation are presented in Figure 2. The percentages of labelled spermatozoa (median, 95% CI) in the heavy smokers [12.11% (CI 3.67, 17.87)], light smokers [11.66% (CI 7.64, 28.64)] and non-smokers [20.41% (CI 14.86, 24.92)] were not statistically different ($P = 0.476$). While this study was initially planned to include 200 subjects, mid-study analysis demonstrated that we could stop at 97 subjects without affecting the statistical power. This was due to an
compared with those of positive controls (DNase I-treated sperm) from smokers. Cotinine concentration in serum was higher in smokers, light smokers and heavy smokers. The results are serum samples and 90% of seminal plasma samples obtained from smokers than in non-smokers (0.20 (0.1, 1.0) µg/l and 4.5 (1.6, 10.9) µg/l in light smokers and heavy smokers respectively ($P < 0.0001$) (Figure 5). The overestimation of the standard deviation of DNA fragmentation was normal. This is because power computation for non-parametric tests is not as well developed as for parametric tests, and because the distribution of DNA fragmentation was not too severely non-normal (unlike cotinine or cadmium). Incidentally, we checked the parametric test to compare the three groups (one-way analysis of variance) and also obtained a non-significant value ($P = 0.7975$).

No correlation was found between DNA fragmentation and smoking markers: cadmium in blood ($r_s = -0.092, P > 0.05$) and seminal plasma ($r_s = -0.113, P > 0.05$); cotinine in seminal plasma ($r_s = 0.023, P > 0.05$) except for a weak negative correlation between DNA fragmentation and serum cotinine ($r_s = -0.240, P < 0.05$).

Detectable concentrations of cotinine were found in 95% of serum samples and 90% of seminal plasma samples obtained from smokers. Cotinine concentration in serum was higher than in seminal plasma ($P < 0.0001$). Median serum and seminal plasma concentrations of cotinine were significantly higher in heavy smokers compared with the other groups ($P < 0.0001$) (Figure 3). A highly significant correlation was found between cadmium concentration in blood and cotinine concentration in serum ($r_s = 0.661, P < 0.0001$) and seminal plasma ($r_s = 0.683, P < 0.0001$) (Figure 4). A weak but significant correlation was observed between cadmium concentration in seminal plasma and cotinine concentration in serum ($r_s = 0.395, P < 0.0001$) and seminal plasma ($r_s = 0.222, P < 0.05$).

Cadmium concentrations were higher in whole blood than in seminal plasma. Non-smokers had a median (min, max) blood cadmium concentration of 0.20 (0.2, 0.3) µg/l compared with 0.2 (0.2, 5.1) µg/l and 4.5 (1.6, 10.9) µg/l in light smokers and heavy smokers respectively ($P < 0.0001$) (Figure 5). The

![Figure 2](image-url)  
**Figure 2.** Median (95% CI) sperm DNA fragmentation in non-smokers, light smokers and heavy smokers. The results are compared with those of positive controls (DNase I-treated sperm) ($n = 10$) and negative controls (without terminal deoxynucleotidyl transferase) ($n = 10$). There was no statistically significant difference between the three groups. Kruskal–Wallis one-way analysis of variance on ranks ($P = 0.476$).

<p>| Table I. Median (min, max) values of hormone assays and blood chemistry according to smoking status |</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Free testosterone (pmol/l)</th>
<th>Total testosterone (nmol/l)</th>
<th>Cholesterol (nmol/l)</th>
<th>Triglyceride (mmol/l)</th>
<th>Glucose (mmol/l)</th>
<th>TSH (mU/l)</th>
<th>Prolactin (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>64.2 (18.7, 152.7)</td>
<td>14.5 (3.9, 32.1)</td>
<td>4.6 (2.2, 8.3)</td>
<td>1.4 (0.7, 10.4)</td>
<td>4.7 (2.6, 7.0)</td>
<td>1.5 (0.2, 2.9)</td>
<td>12.5 (5.5, 44.9)</td>
</tr>
<tr>
<td>Light smokers</td>
<td>63.1 (36.9, 100.0)</td>
<td>17.5 (8.0, 22.8)</td>
<td>4.8 (3.0, 6.8)</td>
<td>1.1 (0.5, 3.4)</td>
<td>4.5 (3.6, 5.9)</td>
<td>1.2 (0.8, 2.9)</td>
<td>12.1 (7.0, 26.4)</td>
</tr>
<tr>
<td>Heavy smokers</td>
<td>63.6 (44.0, 96.6)</td>
<td>16.0 (10.4, 24.7)</td>
<td>4.0 (2.9, 5.9)</td>
<td>1.2 (0.7, 2.9)</td>
<td>4.5 (2.7, 5.9)</td>
<td>1.3 (0.8, 2.2)</td>
<td>13.4 (5.7, 17.4)</td>
</tr>
</tbody>
</table>

<p>| Table II. Median (min, max) semen parameters according to smoking status |</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range/unit</th>
<th>Non-smokers ($n = 69$)</th>
<th>Light smokers ($n = 17$)</th>
<th>Heavy smokers ($n = 11$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (&lt;2 ml)</td>
<td>2.5 (0.4, 8.0)</td>
<td>2.2 (1.1, 3.7)</td>
<td>2.0 (0.9, 5.0)</td>
<td></td>
</tr>
<tr>
<td>Motility (%)</td>
<td>55.0 (6.0, 84.0)</td>
<td>60.0 (20.0, 78.0)</td>
<td>55.0 (11.0, 80.0)</td>
<td></td>
</tr>
<tr>
<td>Concentration (µg/ml)</td>
<td>57.0 (1.0, 412.0)</td>
<td>86.0 (3.5, 274.0)</td>
<td>82.0 (13.5, 311.0)</td>
<td></td>
</tr>
<tr>
<td>Abstinence (days)</td>
<td>3.0 (1.0, 4.0)</td>
<td>3.0 (2.0, 4.0)</td>
<td>3.0 (2.0, 4.0)</td>
<td></td>
</tr>
<tr>
<td>Normal forms (&gt;50%)</td>
<td>79.0 (55.0, 90.0)</td>
<td>77.0 (56.0, 91.0)</td>
<td>76.0 (61.0, 91.0)</td>
<td></td>
</tr>
</tbody>
</table>

| $^a$Kruskal–Wallis one-way analysis of variance on ranks. |

TSH = thyroid stimulating hormone.
Figure 3. Distribution of cotinine concentrations (ng/ml) by smoking status and in fluid (seminal plasma and serum).

(0.1, 0.3) µg/l versus 0.10 (0.1, 0.2) µg/l \( (P < 0.0005) \). When cotinine and cadmium concentrations in seminal plasma were tested for a possible relationship with the different parameters of semen analysis, no significant correlation was found.

Discussion

Epidemiological studies indicate an association between maternal smoking and decreased fertility (Zenes, 1995). This might be related in part to cadmium accumulation from tobacco smoking (Zenes et al., 1995). However, the impact of tobacco consumption on male fertility remains a controversial issue (Vine et al., 1996). The generally observed decrease in semen quality in smokers does not translate into a significant effect of paternal smoking on fertility indices among couples trying to conceive (Vine, 1996).

The three groups of subjects in our study were similar with respect to biological markers, anthropometric measurements and lifestyle, except, by definition, for smoking status. It was anticipated that selecting volunteers on the basis of smoking status—from non-smokers to light and heavy smokers—would allow the detection of dose–effect relationships with semen parameters and the extent of DNA fragmentation. Two methods are commonly used in the literature to evaluate the degree of exposure to cigarette smoke. The first comprises questionnaires which are retrospective, rapid, non-invasive and inexpensive. The second is an objective system based on the serum concentrations of a nicotine metabolite, cotinine, which is known to provide reliable correlations between the serum, urine and seminal plasma concentrations of this marker and the daily consumption of cigarettes (Vine et al., 1993). Our results reveal a significant correlation (not shown) between the concentration of cotinine in serum and seminal plasma and the number of cigarettes consumed per day, with values 10 to 35 times higher in smokers than in non-smokers. This result, and data from previous studies using the same radioimmuno-assay to measure cotinine (Vine et al., 1993, 1996), validate our stratification of smoking status.

Cadmium exposure, as determined by blood and seminal plasma concentrations, was significantly correlated with cotinine levels, with marked differences between smoking status, especially in serum. Any significant dose–effect relationship within this range of cadmium exposure should thus become readily apparent.

In the present study, we failed to demonstrate any statistically significant association between smoking status and the usual semen parameters, confirming previously published results (Vine et al., 1996). Several investigators (Evans et al., 1981; Stillman et al., 1986; Zavos, 1989; Tuormaa, 1995; Sofikitis et al., 1995; Yamamoto et al., 1998) have reported an association between smoking and reduction in semen quality using the
standard visual assessment method. However, others (Godfrey, 1981; Rodriguez-Rigau et al., 1982; Shaarawy and Mahmoud, 1982; Hoidas et al., 1985; Vogt et al., 1986) did not observe such an association. While it is true that categorical measures of smoking status have less statistical power than continuous measures of smoking dose, we did not find any association between semen parameters and smoking dose evaluated as a function of cotinine concentration. It is recognized that our sample size may not allow detection of small differences in the usual semen parameters. In addition, our results do not exclude possible effects of smoking on testicular function (assessed by the human chorionic gonadotrophin stimulation test) and sperm fertilizing potential, as was recently observed in humans (Sofikitis et al., 1995) and a rat model (Yamamoto et al., 1998). Such effects might not be apparent in healthy men, but may further deteriorate testicular function in subfertile men.

Analysing for correlations between cadmium exposure through smoking and semen parameters did not reveal any significant result. On the whole, and looking back at the literature, the case for cadmium as a toxic metal for the human testis and sperm function is not strong, at least in the range of environmental exposure most often encountered. While toxicity was first reported back in 1956, the available information is mostly anecdotal in nature or based on animal models (Parizek and Zahor, 1956; Oldereid et al., 1993). For example, when administered to rats at a dose of 20 μmol/kg, cadmium causes interstitial oedema and reduces vascular supply, resulting in testicular tissue hypoxia; this triggers a cascade of events leading to necrosis and sterility (Janecki et al., 1992). Cadmium can also induce apoptosis of rat germinal cells in vitro (Xu et al., 1996). In humans, cadmium concentrations in blood or seminal plasma were found to be inversely correlated with sperm count and parameters of motility (Noack-Füller et al., 1993; Xu et al., 1993). More recently, however, no correlation could be established between cadmium concentration in seminal plasma and the usual semen parameters in fertile men and patients of an infertility clinic (Keck et al., 1995).

Contrary to our expectations, we did not observe any difference in the percentage of sperm DNA fragmentation between the three study groups of healthy volunteers. The study subjects (n = 97) were recruited and stratified with respect to smoking status: 28 smokers, and 69 non-smokers serving as the control group. Multiple studies have measured the integrity of sperm DNA with acridine orange (Angelopoulos et al., 1998; Duran et al., 1998; Spanò et al., 1998) or blue aniline (Morel et al., 1998). Acridine orange fluoresces when interacting with single-strand (ss) DNA or RNA, while increased aniline blue staining of spermatozoa reflects the persistence of histones, suggesting greater DNA instability. Since a large amount of DNA is required for the acridine orange or blue aniline staining methods to detect DNA fragmentation, it has proved difficult to investigate this phenomenon with limited quantities of tissues, and DNA fragmentation usually occurs in discrete cell subpopulations (Tilly and Hsueh, 1993). On the other hand, DNA fragmentation occurs not only in programmed cell death, but also in other forms of cell injury/death (Gold et al., 1994; Majno and Jorris, 1995). For this reason, the present study exploited the specificity of TdT to incorporate biotinylated deoxyuridine into the 3’-OH ends of DNA. In addition, the signal was amplified by streptavidin–FITC conjugate. The signal intensity increased with a rising number of DNA breaks. Spermatozoa with normal DNA, therefore, exhibit only background fluorescence, while those with fragmented DNA (multiple chromatin 3’-OH ends) fluoresce brightly, enabling identification and sorting by flow cytometry.

Our results contradict those published previously (Sun et al., 1997), which detailed an increase in sperm DNA fragmentation in infertile smokers compared with infertile non-smokers. Such apparently conflicting data could be reconciled on the ground that the two studies bear on different populations: normal men versus infertile men. It is possible that the high levels of DNA fragmentation in the spermatozoa of infertile smokers could be associated with other undetermined factors. The relationship between sperm DNA fragmentation and male infertility or semen quality is an unresolved issue. One group (Hughes et al., 1996) found no difference in baseline sperm DNA fragmentation in asthenozoospermic subjects compared with normozoospermic donors. Others (Sun et al., 1997) reported a negative correlation between DNA fragmentation and semen quality reflected by sperm motility, morphology and counts. Recently, it was noted (Aravindan et al., 1997) that there was...
a correlation between sperm DNA fragmentation and the susceptibility of spermatozoa to low pH-induced DNA denaturation, which was previously shown to be negatively correlated with the fertilizing potential of the spermatozoa (Evenson et al., 1980).

The aetiology of sperm DNA fragmentation is not clear. Oxygenated free radicals, such as superoxide anion and hydroxyl radical, can lead to sperm DNA fragmentation (de Lamirande et al., 1997; Kodoma et al., 1997; Aitken et al., 1998) and secondarily to pathological conditions such as varicocele and pyospermia (Lenzè et al., 1994). Toxic environmental substances can also cause sperm DNA fragmentation. However, we failed to demonstrate an association between sperm DNA fragmentation and smoking in a healthy population. A decrease of fecundity has been noted in epidemiological studies comparing smoking and non-smoking couples (Laurent et al., 1992). The male contribution to this defect is far from evident because a number of investigations, including ours, failed to find a correlation between smoking and semen parameters. However, it has been proposed that DNA fragmentation may affect the final events of fertilization such as failure of pronuclear formation (Lopes et al., 1998).

In conclusion, we found no evidence for an association between smoking and sperm DNA fragmentation in healthy volunteers. However, sperm DNA fragmentation could be affected by many other environmental factors as well as confounding factors. Further studies are particularly needed to establish the role of heavy metals in testicular germinal and somatic cell functions in humans, to determine sites of these interactions, and to demonstrate the relative affinities and concentrations of metals to design preventive measures for males exposed to toxic heavy metals such as cadmium.

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