Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study

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Objective: To investigate levels of seminal oxidative stress (OS) and sperm quality in a group of infertile men with a history of cigarette smoking.

Design: A prospective clinical study.

Setting: Male infertility clinic, Urological Institute, the Cleveland Clinic Foundation, Cleveland, Ohio.

Patient(s): Infertile men who smoked cigarettes (n = 20), infertile men who were nonsmokers (n = 32), and healthy nonsmoking donors (n = 13).

Intervention(s): Genital examination, standard semen analysis, sperm DNA damage.

Main Outcome Measure(s): Levels of seminal reactive oxygen species (ROS) and total antioxidant capacity (TAC) measured by a chemiluminescence assay and seminal OS assessed by calculating a ROS-TAC score. Sperm DNA damage was measured by sperm chromatin structure assay.

Result(s): Smoking was associated with a 48% increase in seminal leukocyte concentrations (P < .0001), a 107% increase in ROS levels (P < .001), and a 10-point decrease in ROS-TAC scores (P < .003). Differences in standard sperm variables and DNA damage indices between the infertile smokers and infertile nonsmokers were not statistically significant.

Conclusion(s): Infertile men who smoke cigarettes have higher levels of seminal OS than infertile nonsmokers. Given the potential adverse effects of seminal OS on fertility, physicians should advise infertile men who smoke cigarettes to quit. (Fertil Steril 2002;78:491–9. ©2002 by American Society for Reproductive Medicine.)

Key Words: Cigarette smoking, nuclear DNA, male infertility, semen analysis, spermatozoa

Although cigarette smoking is a widely recognized health hazard and a major cause of mortality, people continue to consume cigarettes on a regular basis. Approximately one-third of the world’s population 15 years or older smokes cigarettes daily (i.e., active smokers) (1). Needless to say, a number of non-smokers are also negatively affected when they inhale side-stream smoke from burning cigarettes. Epidemiological studies in women of reproductive age have shown that cigarette smoking has a dose-related effect that can delay time to conception by 2 months (2) and advance the start of menopause by 2 years (3). The literature also suggests that women who smoke have a small but clinically significant increase in the risk of spontaneous abortion (4, 5) and that smoking reduces fecundity among those undergoing assisted conception (6). The fact that fecundity returns to normal levels when women stop smoking provides perhaps the most compelling evidence for a causal relationship between female smoking and reduced fertility (7).

Given the large number of men worldwide who smoke, and the fact that cigarette smoke is a known somatic cell mutagen and carcinogen, there is a major concern that smoking may adversely affect male reproductive health (8). However, for ethical reasons, it is not possible to test that concern in a randomized controlled trial (6). Moreover, smoke constituents have never been evaluated for their toxicity or effects on human spermatozoa (9). Therefore, the impact of cigarette smoking on male fertility remains a highly controversial issue.

On the one hand, a number of studies have
shown that smoking has a detrimental effect on sperm quality, most significantly sperm concentration, motility, and morphology (10–14). In addition, cigarette smoking has been correlated with poor sperm function in sperm penetration assays (11, 15). Furthermore, paternal smoking has been associated with a significant increase in the percentage of spermatozoa with DNA damage (11, 16–18) and a higher risk of birth defects and childhood cancers in the offspring (19–21). On the other hand, a handful of studies have found no association between smoking and sperm quality (22, 23), sperm function (24), or sperm nuclear DNA damage (9). Such contradictory data could be due in part to the fact that the studies were conducted on two different populations: normal, healthy men and infertile men. Another important source of conflict among studies may be the difficulty in adjusting for confounding factors such as exposure to other toxins, socioeconomic status, and abnormalities of genital examination.

In an attempt to overcome the aforementioned potential sources of bias, the participants of this study were selected from a group of men who had no history of recreational drug use or alcohol drinking within the previous year. Men were excluded if they had a history of exposure to gonadotoxins such as chemotherapy, radiotherapy, or pesticides. The objective of our study was to examine levels of seminal oxidative stress (OS), conventional semen parameters, and sperm nuclear DNA damage in a group of infertile men who smoked cigarettes on a regular basis. We compared the results with a group of infertile men with no history of cigarette smoking and with a group of healthy, nonsmoking donors. In addition, we adjusted our statistical analyses for genital examination abnormalities.

**MATERIALS AND METHODS**

The Institutional Review Board of the Cleveland Clinic Foundation approved this study, and we obtained written informed consent from all participants.

**Study Groups**

Men who were evaluated at our fertility clinic between July, 2000 and July, 2001 with a history of infertility of at least 1 year were eligible for this study. Clinical evaluation of all participants included history, genital examination (testis and scrotum), and digital rectal examination. Scrotal Doppler ultrasound was performed, as needed, to exclude subclinical varicocele. History included data on smoking, alcohol, recreational drugs, fever, and exposure to gonadotoxins such as chemotherapy, radiotherapy, or pesticides. Information on smoking habits included the number of cigarettes smoked per day and the number of years of smoking.

Infertility patients who either smoked cigarettes on a regular basis (at least 20 cigarettes per day for at least 1 year before enrollment in the study) or who were nonsmokers (never smoked before) were included in the study. Patients with a normal genital examination and those with a diagnosis of varicocele (as a form of abnormal genital examination) were included in the study. Patients with a history of recreational drug use (i.e., marijuana use and/or narcotic agents) or alcohol consumption (including social drinking) within the past year were excluded. Also, patients were excluded if they had a history of a recent fever or exposure to gonadotoxins such as chemotherapy, radiotherapy, or pesticides. Patients who had abnormalities in their genital examination other than varicoceles, such as cryptorchidism and genital tract infections, were excluded.

Based on history and genital examination, the patients were classified into one of four groups: group 1, smokers with a normal genital examination (n = 12); group 2, nonsmokers with a normal genital examination (n = 21); group 3, smokers with an abnormal genital examination (i.e., varicocele) (n = 8); and group 4, nonsmokers with an abnormal genital examination (i.e., varicocele) (n = 11). A group of healthy nonsmoker sperm donors (n = 13) was included as a control for the study. All donors had a normal genital examination.

**Semen Analysis**

**Sperm Concentration, Motility, and Morphology**

All subjects were required to collect semen specimens by masturbation in a private room near the laboratory after a period of 48–72 hours of sexual abstinence. Following liquefaction, semen analysis was performed using a Microcell counting chamber (Conception Technologies, San Diego, CA) to determine sperm concentration and motility. Smears of the raw semen were stained using the Diff-Quik kit (Baxter Healthcare Corporation, Inc., McGaw Park, IL) for assessment of sperm morphology. The smears were stained, rinsed in distilled water, air dried, and scored using Kruger’s strict criteria (25).

**Quantification of Seminal Leukocytes**

Leukocyte concentrations in semen were quantified by a myeloperoxidase-staining test (26). A 20-μL volume of liquefied semen specimen was placed in a Corning 2.0-mL cryogenic vial (Corning Costar Corp., Cambridge, MA) with 20 μL of phosphate-buffered saline (PBS) (pH 7.0) and 40 μL of benzidine solution. The solutions were mixed and allowed to sit at room temperature for 5 minutes. Peroxidase-positive leukocytes that stained brown were counted by Makler’s counting chamber (Sefi Medical, Haifa, Israel) under the bright-field objective (magnification, ×20). The results after correction for dilution were recorded as ×10^6 peroxidase-positive leukocytes/milliliter of semen.

**Measurement of Seminal Reactive Oxygen Species**

Levels of seminal reactive oxygen species (ROS) were measured by a chemiluminescence assay using luminol (5-
A negative control was prepared by adding 10 μL of luminol to 400 μL of PBS. Measurement was performed using a luminometer (model LKB 953; Wallac Inc., Gaithersburg, MD) in an integrated mode for 15 minutes. The results were expressed as \(\times 10^6\) counted photons per minute (cpm)/20 \(\times 10^6\) sperm/mL.

**Measurement of TAC**

TAC in seminal plasma was measured with an enhanced chemiluminescence assay (28). Frozen samples of seminal plasma were thawed at room temperature and immediately assessed for TAC. Seminal plasma was diluted 1:20 with plasma were added to 700 μL of PBS. Measurement was performed using a luminometer (model LKB 953; Wallac Inc., Gaithersburg, MD) in an integrated mode for 15 minutes. The results were expressed as \(\times 10^6\) counted photons per minute (cpm)/20 \(\times 10^6\) sperm/mL.

**Sperm Chromatin Structure Assay**

The sperm chromatin structure assay (SCSA) measures the susceptibility of sperm nuclear DNA to acid-induced denaturation in situ (30). Raw semen samples, frozen at -196°C, were thawed in a 37°C water bath and immediately diluted with buffer (0.15 M NaCl, 0.01% Triton X-100, pH 7.4) to obtain a sperm concentration of 1 to 2 \(\times 10^6\) sperm/mL. A 200-μL aliquot was removed and mixed with 400 μL of a low-pH detergent solution (0.08 N HCl, 0.15 M NaCl, 0.01% Triton X-100, pH 1.2). After 30 seconds, spermatozoa were stained by adding 1.20 mL acridine orange (AO) staining solution containing 6 μg/mL of AO (chromatographically purified; catalog no. 04539, Polysciences, Warington, PA) per milliliter of buffer (0.037 M citric acid, 0.126 M Na2 HPO4, 0.0011 mM EDTA [disodium], 0.15 M NaCl, pH 6.0). Immediately after staining, the sample was placed into an Ortho Cytofluorograf 30 flow cytometer (Ortho Diagnostic Inc., Westwood, MA) sample chamber and the sample was run for 2.5 minutes to allow for hydrodynamic and stain equilibrium.

Green (515–530 nm) and red (>630 nm) fluorescence, corresponding to amounts of native DNA and denatured DNA, respectively, were collected for each sperm, measured at a rate of approximately 250 cells/second. Computer-generated means ± SD of green and red fluorescence values derived from a population of approximately 5,000 cells were analyzed. Alpha-t (αt), the basis of all SCSA parameters, was calculated as the ratio of red fluorescence to the total of red and green fluorescence of an individual sperm cell. The variable Xαt, represents the mean population of αt. The SD of αt (SDαt) represents the variability of chromatin structure abnormalities within the sperm population. The variable DFI or DNA fragmentation index, previously known as COMPαt, represents the percentage of cells outside the main population of αt, which includes cells with abnormal chromatin structure.

Spermatozoa with abnormal chromatin have a higher Xαt, SDαt, and %DFI than spermatozoa with normal chromatin. The fourth variable, percentage of high DNA stainability (%HDS) fluorescence, identifies cells with immature nuclei by the characteristic pattern of increased green fluorescence.

**Statistical Analysis**

Univariate comparison of continuous variables among the groups was performed with Kruskal-Wallis test. Pairwise comparisons among groups were performed with the Wilcoxon rank-sum test. Three-way analysis of variance (ANOVA) was used to determine the effects of smoking, abnormal genital examination, and infertility status on standard semen variables, sperm DNA damage, and OS indices (ROS, TAC, and ROS-TAC scores). The three-way ANOVA was used to test the effect of smoking after adjusting for two other potential confounding variables: genital examination results and fertility history. All hypothesis testing was two-tailed; \(P<.05\) was considered statistically significant.

As a secondary analysis, the ROS-TAC score was calculated using principal-component analysis as described in an earlier study (29). The ROS-TAC score is derived from levels of ROS in washed semen and TAC in seminal plasma. The resulting score accounts for the variability present in the individual parameters of OS (ROS alone or TAC alone). Individuals with ROS-TAC scores below 30, the lower limit
of normal, are at higher risk for OS. All analyses were calculated with SAS statistical software package version 8.1 (SAS Institute, Cary, NC). Summary statistics are presented as median and interquartile range (IQR; [25th, 75th percentiles]).

### RESULTS

Of the 78 infertile men initially evaluated for this study, 26 (33%) were excluded based on the predetermined criteria. Of the remaining 52 men, 38% (20/52) were smokers, including 12 men presenting with a normal genital examination (group 1) and eight men with a clinical diagnosis of varicocele (group 3). The remaining 62% (32/52) were nonsmokers, including 21 presenting with a normal genital examination (group 2) and 11 with a clinical diagnosis of varicocele (group 4). The sample size of 52 men had 90% power (two-tailed tests) to determine if smoking either increased or decreased sperm motility and DNA damage by 11% and increased or decreased the ROS-TAC score by 6.5 points. The median (IQR) value of the number of cigarettes smoked per day for group 1 was 25 [20, 29] and was comparable with group 3, 20 [20, 28] ($P=.8$). The median (IQR) value of the duration of smoking in group 1 was 12 years [8, 13] and in group 3 was 5 years [3, 11] ($P=.08$).

There was no significant difference in the median (IQR) age of the participants in this study (donors, 34 [32, 40]; group 1, 38 [30, 38]; group 2, 32 [30, 35]; group 3, 31 [30, 38] and group 4, 32 [28, 36]).

### Standard Semen Parameters

Standard semen parameters in donors and the four study groups are shown in Table 1. Percent sperm motility and normal sperm forms in group 1 were significantly lower than normal donors ($P=.009$ and .02, respectively), but were not significantly different from group 2 ($P=.33$ and .56, respectively). Similarly, percent sperm motility and normal sperm forms in group 3 were significantly lower than normal donors ($P=.02$ and .02, respectively) but were not significantly different from group 4 ($P=.68$ and .86, respectively). Sperm concentration was significantly lower in group 3 as compared with the normal donors ($P=.009$) but was not significantly different from group 4 ($P=.88$). Patients in group 1 had significantly higher levels of seminal leukocyte concentrations compared with the patients in group 2 ($P=.01$) and with the normal donors ($P=.006$). Seminal leukocyte concentrations in group 3 were significantly higher than the normal donors ($P=.04$) but were not significantly different from the patients in group 4 ($P=.3$).

After adjusting for the abnormal genital examination (varicocele) and the infertility status, ANOVA showed that smoking in infertile men was associated with a 48% increase in seminal leukocyte concentrations ($P=.0001$). On the other hand, the abnormal genital examination was associated with a 29% decrease in sperm concentration, a correlation that was statistically significant ($P=.005$), while reduction in percent of sperm motility (9%) and normal sperm forms (8%) were not statistically significant ($P=.3$ and .14, respectively). After adjusting for smoking and the abnormal genital examination, infertility status was associated with a 25% decrease in sperm concentration ($P=.03$), a 15% decrease in sperm motility ($P=.01$), and a 17% decrease in normal sperm forms ($P=.05$).

### OS Indices

Table 2 contains the comparisons of ROS, TAC, and ROS-TAC scores among donors, group 1, and group 2. Table 3 contains the comparisons among donors, group 3, and group 4. Patients in group 1 had significantly lower ROS-TAC scores (i.e., higher OS) than the patients in group 2 ($P=.006$) and the healthy donors ($P=.006$). No statistically significant differences were seen in ROS-TAC scores among the patients in groups 3 and 4 ($P=.76$). After adjusting for abnormal genital examination (varicocele) and infertility status, ANOVA showed that smoking was associated with a 107% increase in seminal ROS levels ($P=.001$) and a

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**Table 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Donors (n = 13)</th>
<th>Group 1 (n = 12)</th>
<th>Group 2 (n = 21)</th>
<th>Group 3 (n = 8)</th>
<th>Group 4 (n = 11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration ($\times 10^6$/mL)</td>
<td>58 (40, 101)</td>
<td>35 (17, 106)</td>
<td>27 (18, 66)</td>
<td>13 (12, 34)</td>
<td>16 (10, 26)</td>
<td>.001</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>67 (58, 75)</td>
<td>43 (29, 56)</td>
<td>50 (38, 62)</td>
<td>38 (33, 54)</td>
<td>33 (29, 57)</td>
<td>.0004</td>
</tr>
<tr>
<td>% Morphology by Kruger’s strict criteria</td>
<td>10 (8, 14)</td>
<td>7 (3, 8)</td>
<td>7 (5, 10)</td>
<td>5 (2, 9)</td>
<td>7 (3.5, 7.5)</td>
<td>.02</td>
</tr>
<tr>
<td>Seminal leukocytes ($\times 10^{6}$/mL)</td>
<td>0.1 (0, 0.2)</td>
<td>1.8 (0.6, 4.4)</td>
<td>0.0 (0.0, 0.4)</td>
<td>1.4 (0.0, 6)</td>
<td>0.2 (0.0, 1.2)</td>
<td>.003</td>
</tr>
</tbody>
</table>

*Note: Values are medians and interquartile range (25th, 75th percentiles). Univariate comparison of continuous variables among the groups was performed with Kruskal-Wallis test; $P<.05$ was considered statistically significant.*

**Saleh. Smoking and seminal oxidative stress. Fertil Steril 2002.**
10-point decrease in ROS-TAC scores \( (P = .003) \). The decrease in TAC levels (11%) in association with smoking was not statistically significant \( (P = .33) \).

**Sperm DNA Damage**

Table 4 contains the comparisons of the four SCSA variables among donors, group 1, and group 2. Table 5 contains the comparisons among donors, group 3, and group 4. The three SCSA variables \( (\chi_{\text{os}}, SD_{\text{os}}, \text{ and } \%\text{DFI}) \), which indicate the extent of DNA damage in spermatozoa, were significantly higher both in group 1 \( (P = .04, .02, \text{ and } .02, \text{ respectively}) \) and in group 3 \( (P = .007, .02, \text{ and } .004, \text{ respectively}) \) compared with the normal donors. However, the same variables in group 2 were not significantly different from those of the normal donors \( (P = .06, .24, \text{ and } .08, \text{ respectively}) \). Differences in the \( \chi_{\text{os}}, SD_{\text{os}}, \text{ and } \%\text{DFI} \) values among the infertile smoker and nonsmoker groups were not statistically significant \( (P > .26) \). The \%HDS, which identifies the percentage of cells with immature nuclei, in the normal donors was significantly lower \( (P = .03) \) than in the four infertile groups and was not statistically significantly different in the smokers vs. the nonsmokers.

Increased \%DFI was significantly correlated with the infertility status and varicocele \( (P = .02 \text{ and } .009, \text{ respectively}) \) but was not significantly correlated with smoking.

**DISCUSSION**

A causal relationship between cigarette smoking and impaired reproductive function is highly suspected because smokers inhale a host of toxins such as nicotine, carbon monoxide, cadmium, and other mutagenic compounds (31). The results of this study indicate that cigarette smoking is significantly correlated with increased levels of seminal OS, as evidenced by a significant reduction in ROS-TAC scores. It has been recently reported that ROS-TAC scores decrease as a result of an imbalance between levels of ROS and antioxidants in semen (29). In this study, the significant reduction of ROS-TAC scores associated with smoking can be attributed to the significant increase in seminal ROS levels. The link between cigarette smoking and increased levels of seminal ROS may be, at least in part, related to the significant increase in leukocyte concentrations in the semen.
An additional factor that may explain why the semen of the smokers has increased levels of ROS may be the fact that cigarette smoke itself contains high levels of ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radicals (35, 36). The finding of increased levels of seminal OS in association with cigarette smoking is of significance and may have important implications in the fertilizing potential of infertile men. Spermatozoa are particularly susceptible to damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids (37) and their cytoplasm contains low concentrations of scavenging enzymes (38–41). In addition, the intracellular antioxidant enzymes cannot protect the plasma membrane that surrounds the acrosome and the tail, forcing spermatozoa to supplement their limited intrinsic antioxidant defenses by depending on the protection afforded by the seminal plasma, which bathes these cells (42). The seminal plasma protects spermatozoa from excessive ROS by means of scavenging enzymes.

### Table 4

Comparison of sperm chromatin structure assay parameters among donors, group 1 (smokers with normal genital examination), and group 2 (nonsmokers with normal genital examination).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Donors (n = 13)</th>
<th>Group 1 (n = 12)</th>
<th>Group 2 (n = 21)</th>
<th>Donors vs. group 1</th>
<th>Donors vs. group 2</th>
<th>Group 1 vs. group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xₘt</td>
<td>220 (206, 253)</td>
<td>281 (238, 291)</td>
<td>246 (223, 292)</td>
<td>.04</td>
<td>.06</td>
<td>.5</td>
</tr>
<tr>
<td>SDₘt</td>
<td>170 (138, 183)</td>
<td>208 (182, 217)</td>
<td>172 (159, 220)</td>
<td>.02</td>
<td>.24</td>
<td>.33</td>
</tr>
<tr>
<td>%DFI</td>
<td>14 (10, 22)</td>
<td>26 (17, 28)</td>
<td>19 (13, 28)</td>
<td>.02</td>
<td>.08</td>
<td>.45</td>
</tr>
<tr>
<td>%HDS</td>
<td>6 (3.3, 8.6)</td>
<td>10 (8, 13)</td>
<td>9 (7, 12)</td>
<td>.03</td>
<td>.03</td>
<td>.8</td>
</tr>
</tbody>
</table>

Note: Values are medians (25th, 75th percentiles). Xₘt = mean population of ΔΔ; SDₘt = variability of chromatin structure abnormalities within the sperm population; %DFI = DNA fragmentation index or percentage of cells outside the main population of sperm with abnormal chromatin structure; %HDS = high DNA stainability identifies percentage of cells with immature nuclei by the characteristic pattern of increased green fluorescence. Wilcoxon rank-sum test was used for the analysis; P<.05 was considered statistically significant.

### Table 5

Comparison of sperm chromatin structure assay parameters among donors, group 3 (smokers with varicocele), and group 4 (non-smokers with varicocele).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Donors (n = 13)</th>
<th>Group 3 (n = 8)</th>
<th>Group 4 (n = 11)</th>
<th>Donors vs. group 3</th>
<th>Donors vs. group 4</th>
<th>Group 3 vs. group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xₘt</td>
<td>220 (206, 253)</td>
<td>309 (272, 357)</td>
<td>268 (241, 345)</td>
<td>.007</td>
<td>.04</td>
<td>.57</td>
</tr>
<tr>
<td>SDₘt</td>
<td>170 (138, 183)</td>
<td>234 (214, 253)</td>
<td>194 (154, 243)</td>
<td>.03</td>
<td>.04</td>
<td>.42</td>
</tr>
<tr>
<td>%DFI</td>
<td>14 (10, 22)</td>
<td>38 (27, 41)</td>
<td>31 (20, 35)</td>
<td>.0004</td>
<td>.03</td>
<td>.26</td>
</tr>
<tr>
<td>%HDS</td>
<td>6 (3.3, 8.6)</td>
<td>16 (9, 26)</td>
<td>18 (11, 22)</td>
<td>.01</td>
<td>.001</td>
<td>.1</td>
</tr>
</tbody>
</table>

Note: Values are medians (25th, 75th percentiles). Xₘt = mean population of ΔΔ; SDₘt = variability of chromatin structure abnormalities within the sperm population; %DFI = DNA fragmentation index or percentage of cells outside the main population of sperm with abnormal chromatin structure; %HDS identifies percentage of cells with immature nuclei by the characteristic pattern of increased green fluorescence. Wilcoxon rank-sum test was used for the analysis; P<.05 was considered statistically significant.

of small molecular weight, free-radical scavengers such as ascorbate, α-tocopherol, uric acid, and ROS-metabolizing enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (43).

In this study, the reduction in the levels of seminal TAC that are associated with cigarette smoking was not statistically significant, an observation that causes more emphasis to be put on the role played by ROS in the pathogenesis of seminal OS in smokers. In a study that compared levels of individual seminal antioxidants in male smokers with that of nonsmokers from the general population, smoking was associated with a significant reduction in levels of α-tocopherol but not ascorbate (16). In the same study, a significant correlation was found between cigarette smoking and increased levels of seminal 8-hydroxydeoxyguanosine, a biochemical marker of oxidative damage to sperm DNA. In our study, the SCSA-defined DNA damage markers (i.e., $X_{\text{at}}$, $SD_{\text{at}}$, and $\%DFI_{\text{at}}$) were significantly higher in the infertile smokers with a normal genital examination as compared with the normal healthy donors. However, the differences in sperm DNA damage variables between the infertile nonsmokers with a normal genital examination and the donors were not statistically significant. On the other hand, levels of SCSA-defined sperm DNA damage in the infertile smokers and nonsmokers with a diagnosis of varicocele in our study were significantly higher as compared with the normal donors, a finding that may indicate a possible relationship between varicocele and increased sperm DNA damage.

Although differences in sperm DNA damage between the infertile smokers and nonsmokers did not reach a statistical significance, smoking may be related to the significant differences observed in DNA damage between infertile smokers who had a normal genital examination and the normal donors. This observation is supported by a recent study, conducted on a group of male partners in an IVF program, which indicated that the smokers had a significantly higher percentage of spermatozoa with DNA damage than the nonsmokers (44). However, another study failed to demonstrate an association between cigarette smoking and sperm nuclear DNA damage in a healthy population (9). Although smoking may not be a primary cause for sperm DNA damage in normal subjects, it may be an important factor mediating the DNA damage in spermatozoa with altered or abnormal chromatin structure, which are commonly found in infertile men. In this context, the additional increase of sperm DNA damage in infertile smokers may be caused, at least in part, by the increased levels of seminal OS. This is supported by recent reports showing that OS not only results in damage to the sperm plasma membrane but also to the sperm nuclear DNA by causing high frequencies of single- and double-strand DNA breaks (6, 45, 46).

Other studies also reported that DNA damage is commonly observed in the spermatozoa of infertile patients and may be mediated by high ROS (44, 47). A recent report has indicated that sperm DNA damage values greater than 27% are highly predictive of pregnancy failure after conventional IVF or intracytoplasmic sperm injection (48). In addition, increased damage to sperm DNA has been linked to an increase in early embryo death (49). Furthermore, recent data from smokers suggested a link between oxidative DNA damage and a high incidence of childhood cancer in offspring (16). In an analysis originating from China, paternal smoking was associated with a four-fold overall increased risk of developing childhood cancer (50). Another independent epidemiological study in the United Kingdom concluded that 14% of all childhood cancers could be directly attributed to paternal smoking (51).

In this study, we did not find significant differences in standard sperm parameters (sperm concentration, percent motility, and percent normal forms) between infertile smokers and infertile nonsmokers, and the overall correlation of these parameters with smoking was not statistically significant. This observation is consistent with the conclusion of a recent review article that the association between cigarette smoking and standard sperm parameters is stronger among studies of healthy men from the general population than among men from infertility clinics (8). Before a conclusion can be reached as to the potential negative effects of cigarette smoking in infertile men, it is important to bear in mind certain study limitations. In our study, smoking data were obtained from a questionnaire directed to the subjects to determine the number of cigarettes smoked per day and the duration of smoking in years. This was not validated by any biochemical test such as serum or salivary cotinine levels. Although we included infertile smokers who smoked a minimum of 20 cigarettes per day for at least 1 year, we were unable to examine the relationship of smoking with semen parameters in a dose-dependent fashion because of the subjective nature of the smoking history.

In addition, our attempts to avoid the potential sources of variability such as alcohol, drugs, and abnormalities of genital examination resulted in a reduction of the total number of smokers in the whole study as well as in each well-defined group. As a result, we were unable to stratify these groups based on the number of cigarettes smoked per day and the years of smoking and adjust for the confounding variables during statistical analysis. A large-scale, longitudinal study to examine the effects of smoking among infertile men while they are active smokers and at different time intervals after they quit smoking would better address the issue of a possible causal relationship between cigarette smoking and male infertility. Despite these limitations, some firm relationships are evident from our study, particularly in relation to cigarette smoking and increased levels of seminal OS. Such a relationship may be, at least in part, due to the significant
increase in seminal leukocyte concentrations observed in infertile smokers.

In conclusion, the detrimental effects of smoking, on standard sperm parameters (concentration, motility, and morphology) and levels of sperm DNA damage may be masked because of the infertility status. Given the potential adverse effects of OS on fertility, physicians should advise infertile men who smoke cigarettes to quit. The argument against smoking is true for anyone wishing to conceive but is particularly important for individuals experiencing infertility problems.

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References