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

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Abstract

Objective: Approximately 37% of men of reproductive age smoke cigarettes, with Europe having the highest tobacco use among all the World Health Organization (WHO) regions. Toxins from tobacco smoking can potentially affect sperm development and function, with a negative effect on semen parameters. Given the high prevalence of smoking and recent changes in the WHO laboratory methods for the examination of human semen, the role of this exposure in face of new WHO methods needs to be clarified.

Evidence acquisition: We conducted a systematic review, followed by a meta-analysis, to determine whether cigarette smoking affects human semen parameters. PubMed, Saint Joseph's University Discover, and Google Scholar were used to identify relevant studies published after release of the latest WHO methods for laboratory evaluation of human semen. Participants were from fertility/urologic clinics and andrology laboratories. The outcome measures were semen volume, sperm concentration, motility, and morphology, the parameters usually used in clinical settings to assess fertility.

Evidence synthesis: Twenty studies with 5865 participants were included in the meta-analysis. Exposure to cigarette smoking was associated with reduced sperm count (mean difference [MD]: $-9.72 \times 10^9$/ml; 95% confidence interval [CI], $-13.32$ to $-6.12$), motility (MD: $-3.48\%$; 95% CI, $-5.53$ to $-1.44$), and morphology (MD: $-1.37\%$; 95% CI, $-2.63$ to $-0.11$). Subgroup analyses indicated that effect size was higher in infertile men than in the general population and in moderate/heavy smokers than in mild smokers. The overall effect size on semen volume, sperm count, and motility remained similar when 2010 and earlier WHO manuals were used for semen analysis but was lower with regard to sperm morphology.

Conclusions: Our results suggest that cigarette smoking has an overall negative effect on semen parameters. The latest WHO laboratory methods for the examination of human semen had a minimal impact on the magnitude of effect size, thus confirming the observed negative effect of smoking on conventional semen parameters.

Patient summary: A new systematic review and meta-analysis comprising 5865 men shows that cigarette smoking is associated with reduced sperm count and motility. Deterioration of semen quality is more pronounced in moderate and heavy smokers.

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1. Introduction

Approximately 37% of male adults worldwide use tobacco, mainly cigarettes [1]. Smoking rates have gradually declined in the United States, but Europe still has the highest tobacco use among all the World Health Organization (WHO) regions [1,2]. Tobacco combustion produces approximately 4000 chemical compounds, and smokers inhale a host of toxins including nicotine, carbon monoxide, cadmium, and other mutagenic compounds, all with potential deleterious effects on male germ cells [3]. The toxins originating from cigarette smoke can decrease sperm mitochondrial activity and damage the chromatin structure in human sperm, therefore impairing fertilization capacity both in vivo and in vitro [4,5].

Smoking cigarettes has been associated with a deterioration of sperm quality including motility, concentration, and morphology, which are the parameters most frequently used in clinical settings to assess fertility [6–8]. However, the evidence is not unequivocal, and some studies have found no effect on semen quality [9–11].

In 2010, the WHO established new criteria for the laboratory examination of human semen [12]. The changes specifically included assessments of (1) volume by weight rather than graduated pipette; (2) motility by two categories, namely progressive and nonprogressive, in contrast with four categories in previous versions; and (3) morphology by strict criteria (Tygerberg) as opposed to the WHO criteria in previous manuals. New reference values for semen characteristics were also proposed that were reconsidered as normal based on the 2010 WHO reference values were reclassified as normal based on the 2010 WHO reference values [13]. Therefore, varying results may be expected with regard to semen analysis when one manual is followed versus another, with obvious implications for the evidence is not unequivocal, and some studies have found no effect on semen quality [9–11].

In a recent study evaluating the impact of these changes, semen characteristics of approximately 15% of patients considered abnormal according to the 1999 WHO reference values were reclassified as normal based on the 2010 WHO reference values [14]. Therefore, varying results may be expected with regard to semen analysis when one manual is followed versus another, with obvious implications for the evidence is not unequivocal, and some studies have found no effect on semen quality [9–11].

We conducted a systematic search using PubMed, Saint Joseph’s University Discover (SJUD), and Google Scholar to identify all relevant studies published from 2010 to August 2015. For SJUD, which includes 189 databases (http://www.sju.edu/int/resources/libraries/drexel/), and PubMed, the Medical Subject Headings search terms used were "smoking" OR cigarette AND semen OR sperm* OR "fertil*" in any language. The following selections were made for the selected articles: “Only studies in humans” and “date range from 2010 onwards.” Articl types selected were clinical study, comparative study, journal article, observational study, randomized controlled trial, review, and systematic review. Trial registers searched were Current Controlled Trials (http://www.controlled-trials.com), ClinicalTrials.gov (http://clinicaltrials.gov), and the World Health Organization International Trials Registry Platform search portal (http://www.who.int/trialsearch). The gray literature was searched using Google Scholar.

The titles and abstracts retrieved were initially screened. Full texts of selected abstracts matching inclusion criteria were obtained. Review articles and reference lists were hand-searched. Studies were analyzed for inclusion independently by two of the authors, and any discrepancies were resolved by discussion.

2. Evidence acquisition

2.1. Search strategy

We conducted a systematic search using PubMed, Saint Joseph’s University Discover (SJUD), and Google Scholar to identify all relevant studies published from 2010 to August 2015. For SJUD, which includes 189 databases (http://www.sju.edu/int/resources/libraries/drexel/), and PubMed, the Medical Subject Headings search terms used were "smoking" OR cigarette AND semen OR sperm* OR "fertil*" in any language. The following selections were made for the selected articles: “Only studies in humans” and “date range from 2010 onwards.” Articl types selected were clinical study, comparative study, journal article, observational study, randomized controlled trial, review, and systematic review. Trial registers searched were Current Controlled Trials (http://www.controlled-trials.com), ClinicalTrials.gov (http://clinicaltrials.gov), and the World Health Organization International Trials Registry Platform search portal (http://www.who.int/trialsearch). The gray literature was searched using Google Scholar.

The titles and abstracts retrieved were initially screened. Full texts of selected abstracts matching inclusion criteria were obtained. Review articles and reference lists were hand-searched. Studies were analyzed for inclusion independently by two of the authors, and any discrepancies were resolved by discussion.

2.2. Selection of studies and validity assessment

Articles were included only if full texts were available, enrolled human participants, did not use workplace passive cigarette smoking, and were not review articles. Smokers were defined as those smoking only cigarettes, whereas nonsmokers were those men who did not smoke at all at the time of each particular study. Studies focusing on the effect of smoking on semen parameters in men with existing specific conditions were excluded (Supplementary Table 1). Authors of unpublished or incomplete data sets were not contacted to request their data for this meta-analysis.

Participants were males aged ≥13 yr regardless of population size and origin. We specified the primary outcome measures a priori as semen volume, sperm count, motility, and morphology because these parameters are the most commonly used measures in the investigation of male fertility. Some studies provided data on all of these measures and others on just some of them. One exposure (smoking) was compared at once (no multivariate analysis). The following characteristics were assessed for each study: study population (infertile vs general population), cigarette consumption per day, and data collection methods (eg, semen analysis according to the edition of the WHO manual).

We adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement to report the results [14]. The initial search yielded 15 119 articles. Of those, 145 abstracts were selected, and 124 full-text articles were obtained. From these, 104 studies were excluded with reasons (Supplement 1, item 5). Twenty studies fulfilled all criteria and were included in the meta-analysis. To determine validity, each included study was assessed according to the criteria for nonrandomized studies to assess the risk of bias [15] (Supplement 1 and Supplementary Table 2).
3.2. Data abstraction

Each semen parameter was evaluated separately and independently. First, we analyzed the studies for smoking and sperm quality comparing smokers with nonsmokers for infertile and healthy men combined. Then we compared infertile smokers versus matched smokers from the general population. We also conducted a subgroup analysis based on the WHO method for the examination of human semen (2010 edition vs previous editions). Lastly, we analyzed results based on the consumption of cigarettes per day. We classified the smokers as mild (1–10 cigarettes), moderate (10–20 cigarettes), or heavy (>20 cigarettes) based on the report about smoking intensity in the United States and the level of cigarette consumption necessary for nicotine regulation [16,17].

3.4. Analysis

Statistical analysis was undertaken using RevMan v.5.0 software (Cochrane Collaboration, Oxford, UK) and Metafor package for R (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org) [18]. Pooled mean differences (MDs) between comparison groups were calculated to determine the effect size. Both fixed effects models (FEMs) and random effects models (REMs) were fitted to assess the model types that were most suited to the data. Heterogeneity was evaluated using the Q test and the I² statistic [19]. Statistical significance was set at a p value <0.05.

We used the REM when heterogeneity was high (I² >50%) [19]. Subgroup analyses were carried out to identify potential sources of the heterogeneity. Sensitivity analyses and assessment of publication bias were conducted to assess the leverage of individual studies on the results [20] (Supplement 1).

3. Evidence synthesis

3.1. Description of studies

Supplementary Figure 1 provides a diagram of the review process. The characteristics of the included studies are provided in Table 1 [8,21–39]. Participants were from infertility and urologic centers as well as andrology laboratories. All semen analyses followed the WHO guidelines.

3.2. Quantitative analysis

Overall, 20 studies were identified, and these included data on 5865 participants. The number of studies included in each meta-analysis varied according to the sperm parameters reported: 13 provided data on semen volume, 20 provided data on sperm count, 15 provided data on motility, and 10 provided data on morphology. Because sperm parameters are continuous data, the MD and associated confidence intervals (CIs) between comparison groups were calculated to determine the effect size of smoking on individual semen parameters.

Table 2 shows the MD on individual sperm parameters and results of heterogeneity tests between smokers and nonsmokers for infertile and healthy men combined and by subgroups based on study participant selection (general population and infertile men). Homogeneity was observed only for the association between smoking and sperm morphology among studies comparing moderate and heavy smokers with nonsmokers (I² <50%). Studies on the remaining parameters varied significantly (p <0.01, I² >50%). Despite including only studies published after the release of the 2010 WHO manual, only seven studies specifically utilized this edition for semen analyses. The remaining studies utilized previous versions, namely the 1999 (4th ed.) and 1992 (3rd ed.).

3.2.1. Semen volume

Mean semen volume ranged from 2.2 to 3.7 ml in smokers and from 2.2 to 3.7 ml in nonsmokers. Overall, semen volume was not significantly affected by smoking (REM MD: −0.16; 95% CI, −0.33 to 0.01).

We conducted two subgroup analyses to examine the potential causes of the heterogeneity. Performing analyses separately according to the WHO editions reduced the heterogeneity estimates, and the MDs became significant in both subgroups, albeit not statistically different between the subgroups (Fig. 1). Likewise, heterogeneity estimates were reduced when participants were selected from the general population (I² = 0%). There was also an increase in the overall effect size in studies where participants had been selected from the general population (MD: −0.21 ml; 95% CI, −0.34 to −0.09; p <0.001) compared with those including only infertile men (MD: −0.15 ml; 95% CI, −0.36 to 0.07) (Supplementary Fig. 2 and Table 2). Sensitivity analyses demonstrated that the observed pooled effect size was not materially affected by the removal of any of the studies (Supplementary Fig. 3). These results suggest that most of the differences between studies is explained by the method of semen analysis and the type of study participants.

3.2.2. Sperm count

Mean sperm count ranged from 33.78 to 113.2×10⁶/ml in smokers and 42.03 to 132.5×10⁶/ml in nonsmokers. The results indicated that the count was lower in smokers than in nonsmokers. The pooled MD by REM was −8.92×10⁶/ml (95% CI, −12.40 to −5.44; p <0.001) (Table 2). Sensitivity analyses indicate that removing the paper by Joo et al [23] slightly increased the MD to −10.0×10⁶/ml, whereas removing the study by Mitra et al [21] decreased the MD to −6.9×10⁶/ml (Supplementary Fig. 4). When any of the other studies were removed, the observed pooled effect size was not affected.

To analyze the potential causes of the heterogeneity, two subgroup analyses were conducted. Heterogeneity remained high (I² >50%) for the 1999 and earlier WHO editions but decreased for the 2010 WHO edition (I² = 53%). In both separate analyses, smoking had a negative effect on count (p <0.001), but the effect size was not different between WHO editions (Fig. 2).

In contrast, heterogeneity estimates were not materially affected by performing subgroup analyses by type of
Table 1 – Characteristics of included studies reporting an association between cigarette smoking exposure and sperm quality

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Study participants</th>
<th>Comparison groups</th>
<th>Semen parameters reported</th>
<th>Main outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collodel et al [29]</td>
<td>Retrospective cohort</td>
<td>271 infertile men and 25 controls with proven fertility</td>
<td>118 smokers, 153 nonsmokers</td>
<td>Count, motility, morphology</td>
<td>Lower count in heavy smokers compared with mild and nonsmoker</td>
</tr>
<tr>
<td>Liu et al [33]</td>
<td>Prospective cohort</td>
<td>147 infertile men</td>
<td>79 nonsmokers, 68 smokers</td>
<td>Count, motility, morphology</td>
<td>Inseminated smokers had lower morphology and higher DNA fragmentation than other groups. Heavy smokers had lower count, motility, and morphology than moderate and mild smokers</td>
</tr>
<tr>
<td>El-Melegy et al [22]</td>
<td>Case control</td>
<td>130 fertile and infertile subjects</td>
<td></td>
<td>Volume, count, motility, morphology</td>
<td></td>
</tr>
<tr>
<td>Al-Matubsi et al [8]</td>
<td>Cross-sectional</td>
<td>204 randomly selected adults</td>
<td>111 smokers, 93 nonsmokers</td>
<td>Volume, count, motility</td>
<td>Reduced count and motility in smokers, but no difference in volume. Higher testosterone and LH levels, but no difference in SHBG and FSH levels in smokers versus nonsmokers</td>
</tr>
<tr>
<td>Aghamehammadi and Azfari [39]</td>
<td>Cross-sectional</td>
<td>280 infertile men</td>
<td>144 nonsmokers, 40 smokers</td>
<td>Volume, count, motility, morphology</td>
<td>No difference in semen parameters between smokers and nonsmokers</td>
</tr>
<tr>
<td>Joo et al [23]</td>
<td>Prospective cohort</td>
<td>62 healthy donors</td>
<td>13 nonsmokers, 22 smokers, 27 heavy smokers</td>
<td>Count, motility, morphology</td>
<td>Sperm count decreased in heavy smokers</td>
</tr>
<tr>
<td>Mitra et al [21]</td>
<td>Cross-sectional</td>
<td>304 infertile men</td>
<td>178 smokers, 126 nonsmokers</td>
<td>Volume, count, motility, morphology</td>
<td>Lower sperm motility, reduced morphology, lower DNA integrity, higher FSH and LH levels, and decreased testosterone levels in smokers compared with nonsmokers</td>
</tr>
<tr>
<td>Davar et al [30]</td>
<td>Prospective cohort</td>
<td>151 infertile men</td>
<td>98 nonsmokers, 53 smokers</td>
<td>Count, motility, morphology</td>
<td>No difference in semen parameters between groups. No correlation between semen parameters and cigarette consumption</td>
</tr>
<tr>
<td>Gaisamudre et al [31]</td>
<td>Prospective cohort</td>
<td>100 infertile men</td>
<td>50 nonsmokers, 50 smokers</td>
<td>Count</td>
<td>No difference in semen parameters between groups. Inverse correlation between cigarette consumption and sperm count</td>
</tr>
<tr>
<td>Caserta et al [28]</td>
<td>Cross-sectional</td>
<td>648 infertile men</td>
<td>200 smokers, 448 nonsmokers</td>
<td>Count, motility, morphology</td>
<td>Lower count and motility in smokers than nonsmokers. No correlation between sperm parameters and smoking intensity</td>
</tr>
<tr>
<td>Meri et al [26]</td>
<td>Retrospective cohort</td>
<td>960 infertile men</td>
<td>564 nonsmokers, 266 heavy smokers, 130 non-heavy smokers</td>
<td>Volume, count, motility, morphology</td>
<td>Reduced motility and morphology in smokers compared with nonsmokers. Lower count, motility, and morphology in heavy smokers compared with non-heavy smokers</td>
</tr>
<tr>
<td>Anifandis et al [24]</td>
<td>Cross-sectional</td>
<td>207 infertile patients</td>
<td>98 nonsmokers, 76 moderate smokers, 33 heavy smokers</td>
<td>Volume, count, motility</td>
<td>Dose–response association between smoking and semen volume, and sperm DNA fragmentation. No significant difference on count and motility among groups</td>
</tr>
<tr>
<td>Zhang et al [34]</td>
<td>Retrospective cohort</td>
<td>1512 infertile men</td>
<td>775 nonsmokers, 180 mild smokers, 327 moderate smokers, 230 heavy smokers</td>
<td>Volume, count, motility, morphology</td>
<td>Lower volume, progressive motility, viability, and morphology, and higher number of leukocytes in smokers than nonsmokers. Dose–response correlation between morphology and amount of smoking</td>
</tr>
<tr>
<td>Yu et al [27]</td>
<td>Cross-sectional</td>
<td>322 infertile patients</td>
<td>147 heavy smokers, 175 nonsmokers</td>
<td>Volume, count, motility, morphology</td>
<td>Decreased morphology in heavy smokers, but no difference in progressive motility and vitality between groups. Sperm histone replacement abnormalities were correlated with sperm motility, viability, count, and cotinine levels</td>
</tr>
</tbody>
</table>
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Study participants</th>
<th>Comparison groups</th>
<th>Semen parameters reported</th>
<th>Main outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamad et al [32]</td>
<td>Cross-sectional</td>
<td>54 infertile men</td>
<td>19 nonsmokers</td>
<td>Volume, count, motility, morphology</td>
<td>Lower volume, count, motility, and morphology in smokers than nonsmokers</td>
</tr>
<tr>
<td>Jeng et al [35]</td>
<td>Cross-sectional</td>
<td>192 healthy men</td>
<td>89 nonsmokers</td>
<td>Volume, count, motility, morphology</td>
<td>Lower morphology and testosterone levels in smokers compared with nonsmokers</td>
</tr>
<tr>
<td>Kumar et al [38]</td>
<td>Cross-sectional</td>
<td>240 male partners</td>
<td>102 nonsmokers</td>
<td>Count, motility, morphology</td>
<td>No difference in semen parameters between smokers and nonsmokers</td>
</tr>
<tr>
<td>Moretti et al [36]</td>
<td>Cross-sectional</td>
<td>110 men</td>
<td>71 nonsmokers</td>
<td>Volume, count, motility, morphology</td>
<td>No difference in semen parameters between smokers and nonsmokers</td>
</tr>
<tr>
<td>Al-Turki [37]</td>
<td>Retrospective</td>
<td>258 infertile men</td>
<td>168 nonsmokers</td>
<td>Volume, count, motility, morphology</td>
<td>Lower volume, count, and motility in smokers than nonsmokers</td>
</tr>
</tbody>
</table>

FSH = follicle-stimulating hormone; LH = luteinizing hormone; MDA = malondialdehyde; SHBG = sex hormone-binding globulin.

Table 2 – Results of the meta-analysis of the studies for smoking and sperm quality comparing smokers with nonsmokers for infertile and healthy men combined (overall) and by subgroups based on study participant selection (general population and infertile men)

<table>
<thead>
<tr>
<th>Sperm parameter</th>
<th>Study participants</th>
<th>IV</th>
<th>95% CI</th>
<th>Q(p)</th>
<th>I², %</th>
<th>Test for overall effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count</td>
<td></td>
<td>-8.92</td>
<td>-12.40 to -5.44</td>
<td>&lt;0.001</td>
<td>90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>General population</td>
<td></td>
<td>-0.22</td>
<td>-8.36 to 7.92</td>
<td>&lt;0.001</td>
<td>86</td>
<td>0.96</td>
</tr>
<tr>
<td>Infertile men</td>
<td></td>
<td>-11.29</td>
<td>-15.13 to -7.44</td>
<td>&lt;0.001</td>
<td>84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sperm motility</td>
<td></td>
<td>-3.48</td>
<td>-5.53 to -1.44</td>
<td>&lt;0.001</td>
<td>78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>General population</td>
<td></td>
<td>-4.78</td>
<td>-9.29 to -0.27</td>
<td>&lt;0.001</td>
<td>86</td>
<td>0.04</td>
</tr>
<tr>
<td>Infertile men</td>
<td></td>
<td>-2.58</td>
<td>-4.10 to -1.05</td>
<td>0.01</td>
<td>38</td>
<td>0.009</td>
</tr>
<tr>
<td>Semen volume</td>
<td></td>
<td>-0.16</td>
<td>-0.33 to 0.01</td>
<td>&lt;0.001</td>
<td>85</td>
<td>0.07</td>
</tr>
<tr>
<td>General population</td>
<td></td>
<td>-0.21</td>
<td>-0.34 to -0.09</td>
<td>0.37</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infertile men</td>
<td></td>
<td>-0.15</td>
<td>-0.36 to 0.07</td>
<td>&lt;0.001</td>
<td>84</td>
<td>0.19</td>
</tr>
<tr>
<td>Sperm morphology</td>
<td></td>
<td>-1.37</td>
<td>-2.63 to -0.11</td>
<td>&lt;0.001</td>
<td>87</td>
<td>0.03</td>
</tr>
<tr>
<td>General population</td>
<td></td>
<td>-1.70</td>
<td>-5.26 to 1.86</td>
<td>&lt;0.001</td>
<td>95</td>
<td>0.35</td>
</tr>
<tr>
<td>Infertile men</td>
<td></td>
<td>-1.22</td>
<td>-2.62 to 0.18</td>
<td>&lt;0.001</td>
<td>81</td>
<td>0.09</td>
</tr>
</tbody>
</table>

CI = confidence interval; IV = inverse variance; Q(p) = Q test (probability).

Fig. 1 – Forest plot showing the effect of smoking on semen volume, including subgroup analyses according to different World Health Organization (WHO) criteria.

CI = confidence interval; IV = inverse 1
participants. However, the mean effect size was markedly reduced when participants were selected from the general population (REM MD: \(-0.22 \times 10^6/ml; 95\% \text{ CI} = -8.36\) to \(-7.92\)) compared with infertile men (REM MD: \(-11.29 \times 10^6/ml; 95\% \text{ CI} = -15.13\) to \(-7.44\); \(p < 0.0001\) (Supplementary Fig. 5). This indicates that some of the differences between studies might be explained by the participant types (Table 2) and is consistent in suggesting a negative association between smoking and sperm count. However, further studies are warranted to elucidate the size of this association in the general population of smokers.

### 3.2.3. Sperm motility

Motility ranged from 16.6\% to 72.3\% in smokers and from 21.7\% to 74.3\% in nonsmokers. Overall, smoking was a risk factor for reduced motility. The pooled MD by REM was 3.48\% (95\% CI, 5.53 to 1.44; \(p < 0.001\)) (Table 2).

Sensitivity analyses indicated that removal of the study by Al-Matubsi et al [8] slightly decreased the MD to 2.9\% (Supplementary Fig. 6). Removal of any of the other studies had no effect on the observed pooled effect size.

Subgroup analyses conducted according to WHO editions and type of participants revealed that the overall effect size remained similar in both subgroups. However, heterogeneity decreased for the 2010 WHO subgroup (\(I^2 = 38\%\)). The pooled MD for participants selected from general population was 4.78\% (95\% CI, 9.29 to 0.27; \(p = 0.04\)) compared with 2.58\% (95\% CI, 4.10 to 1.05; \(p = 0.0009\)) in infertile men (Supplementary Fig. 7 and Table 2). These results are therefore consistent in suggesting a negative association between smoking and sperm motility and indicate that the heterogeneity in the data can be explained by study participant type and the method of semen analysis.

### 3.2.4. Sperm morphology

Overall, smoking was a risk factor for impaired morphology. The pooled MD by REM was \(-1.37\% (95\% \text{ CI} = -2.63\) to \(-0.11; \(p = 0.03\)) (Table 2). Sensitivity analyses indicated that the observed pooled effect size was not affected by removal of any of the studies (Supplementary Fig. 8).

Heterogeneity was dramatically reduced (\(I^2 = 0\%\)) for the 2010 WHO subgroup, whereas it remained high for 1999 and the earlier WHO subgroup (\(I^2 = 89\%\)). The overall effect of smoking on sperm morphology depended on the WHO edition that was used. The pooled MD for 1999 and earlier editions was \(-2.58\% (95\% \text{ CI} = -4.10\) to \(-1.05; \(p = 0.0009\)) in infertile men (Supplementary Fig. 7 and Table 2). These results suggest that the method for semen analysis affected the magnitude
of effect size (Fig. 4). In contrast, heterogeneity estimates were not materially affected by performing analyses separately according to study participant type. In both subgroups, smoking was not significantly associated with a negative effect on morphology. The pooled MD by REM for the general population subgroup was $1.70\%$ (95% CI, 5.26 to 1.86) compared with $1.22\%$ (95% CI, 2.62 to 0.18; $p = 0.09$) in infertile men (Supplementary Fig. 9 and Table 2).

The overall effect size estimated by the analysis of all the studies may therefore be conservative due to the influence of confounding factors such as semen analysis method and study participant type.

3.3. Subgroup analysis for the effect of cigarette consumption on sperm quality

3.3.1. Semen volume

Three studies [22–24] evaluated the effects of cigarette consumption on semen volume. Pooled results indicated that volume was lower in moderate smokers (REM MD: $0.32\; ml$; 95% CI, $-0.62$ to $0.02$; $p = 0.04$) and heavy smokers (REM MD: $-0.77\; ml$; 95% CI, $-0.96$ to $-0.58$; $p < 0.001$) than in nonsmokers. Mild smokers were shown to have higher volumes than moderate and heavy smokers ($p = 0.001$) (Supplementary Fig. 10).

3.3.2. Sperm count

Four studies [21–24] compared the effects of cigarette consumption on sperm count. The pooled results showed that counts were significantly lower in moderate smokers (REM MD: $-9.93\%$; 95% CI, $-18.04$ to $-1.82$; $p = 0.02$) and heavy smokers (REM MD: $-28.06\%$; 95% CI, $-42.27$ to $-8.86$; $p = 0.004$) than in nonsmokers. The effect size was more pronounced in heavy smokers than in moderate smokers ($p = 0.0003$) (Supplementary Fig. 11).

3.3.3. Sperm motility

Three studies [22–24] reported the effects of cigarette consumption on sperm motility. The pooled results indicated that motility was decreased by moderate smoking (REM MD: $3.98\%$; 95% CI, $6.84$ to $1.11$; $p < 0.006$) and heavy smoking (REM MD: $4.62\%$; 95% CI, $11.08$ to $1.84$), albeit not significantly different in the latter. Sperm motility was higher in mild smokers than in moderate and heavy smokers (REM MD: $4.14\%$; 95% CI, $1.03$–$7.25$; $p < 0.01$) (Supplementary Fig. 12).

3.3.4. Sperm morphology

Two studies [22,23] reported the effects of cigarette consumption on sperm morphology. The pooled results showed that morphology was decreased by moderate smoking (FEM MD: $-0.9\%$; 95% CI, $-1.68$ to $-0.12$; $p = 0.02$), moderate smoking (MD: $-2.47\%$; 95% CI, $-3.31$ to $-1.64$; $p < 0.001$), and heavy smoking (MD: $-4.24\%$; 95% CI, $-5.02$ to $-3.46$; $p < 0.001$). The higher the cigarette consumption, the higher the magnitude of the effect size ($p < 0.0001$) (Supplementary Fig. 13).
3.4. Discussion

With changes in the reference ranges and laboratory methods for the evaluation of human semen, the relationship between cigarette smoking and semen quality needs to be clarified [12,40]. To our knowledge, this meta-analysis is the first to summarize the evidence currently available on the association between cigarette smoking and sperm quality after the publication of the latest WHO laboratory manual for the examination of human semen. We included studies evaluating the effect of cigarette smoking on semen volume, sperm count, motility, and morphology, the semen parameters most commonly used to assess male fertility potential.

Our results suggest that cigarette smoking has an overall negative effect on conventional semen parameters. Cigarette smoking was associated with reduced sperm count, sperm motility, and sperm morphology, but the effects on semen volume were equivocal. These effects were overall more pronounced in infertile men than in the general population, and deterioration of semen quality was particularly associated with moderate and heavy smoking. The consistency in the direction of overall effects estimated for all outcomes adds confidence to our findings. However, utilization of the 2010 WHO method decreased the effect size for sperm morphology, but the results were not significantly different for volume, count, and motility compared with previous WHO editions.

In 2010, the WHO introduced the first semen criteria based on a population-based study of fertile men [40]. The reference values were markedly lower than those thought to be compatible with normal male fertility [13]. Subsequently, the WHO issued its latest laboratory manual (5th ed.) for the examination of human semen with important changes in the methods for conducting such analyses compared with previous editions, as mentioned earlier [12,41,42]. We therefore reassessed the effect of cigarette smoking on conventional semen parameters in view of the changes introduced by the WHO manual. This is important because WHO manuals are widely used by laboratories worldwide, and smokers with abnormal semen analysis results based on older WHO criteria might be considered normal according to the new criteria. Hence the negative association between smoking and sperm quality, as suggested by earlier studies and meta-analyses [6–11,43,44], might be perceived as nonsignificant or nonexistent, which could influence counseling and management given by health care providers and decision makers.

Our study aggregated the published evidence on the effect of cigarette smoking on semen parameters in a limited time period (2010–2015) to reflect best the changes introduced by the WHO. Given that 13 of the 20 studies published after 2010 had not followed the latest WHO manual for the laboratory examination of human semen, we conducted a subgroup analysis taking into account the method of semen analysis (new or old WHO criteria) to determine whether or not the method for conducting these analyses would interfere with the magnitude of the effect size. Our results are nonetheless reassuring of an overall negative effect of smoking on sperm parameters in the studies that applied the 2010 WHO methods. The overall effect size on semen volume, sperm count, and motility remained similar when 2010 and earlier WHO manuals were used, but it differed with regard to sperm morphology. Assessment of morphology by strict criteria represented a major change in the 2010 WHO laboratory manual [12]. A possible reason for the discrepancy in the WHO criteria used
The mechanisms through which smoking affects semen parameters are not fully understood, but chemical compounds produced by cigarettes were shown to have deleterious effects on the development of male germ cells [3]. Nicotine was reported to have a negative influence on sperm morphology and sperm count; seminal cotinine had a negative effect on sperm motility [46]. Nevertheless, studies included in our meta-analysis were mostly observational, which prevents us from making inferences on cause-and-effect mechanisms.

Our results indicate that the negative effect of cigarette smoking was more pronounced in infertile smokers than in their counterparts from the general population. Because semen parameters are usually compromised in infertile men, we speculate that the spermatozoa of infertile men are more vulnerable to the negative effects of the inhaled toxins. Whereas levels of cytokines and radical oxygen species are often elevated in infertile men, the protective mechanisms against oxidative stress are less likely to be disrupted in the male general population [47]. Notwithstanding, the association between oxidative stress markers, semen quality, and smoking status in infertile and healthy men needs further investigation.

Cigarette consumption also influenced the magnitude of effect size. We found that heavy and moderate smokers had worse semen quality than mild smokers and nonsmokers. Others have corroborated our results by reporting an inverse correlation between cigarette consumption and semen parameters [48]. Although smoking status was essentially self-reported in the studies included in our meta-analysis, accuracy between smoking status reported and biochemical elements in the blood is high [49]. On the contrary, the effect of smoking duration and smoking cessation on semen parameters remains unclear even after our meta-analysis. Earlier studies published before the release of the current WHO manual for the evaluation of human semen had suggested that smoking cessation was associated with an improvement in semen parameters [50,9]. However, none of the studies included in our review evaluated the impact of smoking duration/cessation on the semen characteristics.

The results of this study have implications for men of reproductive age, health care providers, and decision makers alike. Since smoking is a modifiable lifestyle factor that is particularly prevalent among such men [1–3], health programs focusing on smoking cessation are expected to have a positive impact on semen quality and consequently male fertility. The clinical implication of smoking on fecundity rates remains inconclusive because conventional semen analysis is limited in its ability to predict fertilization capacity and fecundity according to its results [51]. Although a few observational and epidemiological studies have suggested an association between conventional semen parameters and time to pregnancy [52–54], routine semen analysis does not evaluate sperm dysfunctions such as immature chromatin or fragmented DNA [55]. Of note, pregnancy as an outcome measure was not assessed in any of the studies included in our review.

This study has some limitations. Heterogeneity was high in most of the meta-analyses conducted, which may be explained by the relative small number of participants in each study and other confounders. For instance, not all risk factors such as participant age, use of medication, and obesity, which might have affected semen quality, were consistently reported. The population of infertile men and smoking status also varied across studies. Although randomization is not an issue with observational studies, selection of controls may lead to bias. Another limitation refers to the quality of included studies that also varied. Smaller trials are usually analyzed with less methodological rigor than studies involving larger cohorts, and an asymmetrical funnel plot (Supplement 1) suggests that selective reporting may have led to an overestimation of effect sizes in small trials. Bias might have also been introduced because data not published as full articles were excluded from our meta-analysis, and a few studies without data to calculate the mean and standard deviation were also excluded.

Nevertheless, our meta-analysis included >5000 participants, and the methods used were applied rigorously to explain the source of heterogeneity. The pooled MDs on individual semen parameters indicated consistency of the results between the subgroup types. Sensitivity analyses also demonstrated minimal differences when individual studies were excluded, which suggests our results to be conservative.

4. Conclusions

Cigarette smoking was found to be a significant risk factor for decreased semen parameters in adult men. The WHO laboratory manual used for the examination of human semen had a negligible impact on the observed pooled effect size, except for sperm morphology. Given that most studies published after 2010 utilized previous manuals for semen analysis and considering that WHO methods will remain a reliable source for the laboratory evaluation of human semen, further research is needed to fully understand the impact of modified methods on the association between smoking and sperm quality. Studies focusing on subcellular sperm damage such as sperm DNA integrity and oxidative stress markers, which are not assessed during conventional semen analyses, are also required. This would improve the precision of the estimated effect sizes and allow better judgment of the likely clinical importance of the findings.

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Study concept and design: Agarwal.
Acquisition of data: Sharma, Harlev.
Analysis and interpretation of data: Sharma, Esteves, Harlev.
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Appendix A. Supplementary data

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References


