RESEARCH PAPER

# Comparison of Semen Parameters in Smoking and Non-Smoking Men Attending Infertility Clinic: A Cross-Sectional Study from a Tertiary Care Centre

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**ABSTRACT**

**Background:**

Infertility affects a substantial proportion of couples, with approximately half of all cases being caused by male factors. Smoking, a prevalent and preventable habit, is associated with oxidative stress and impaired spermatogenesis. Semen analysis is a widely used first-line technique for assessing male fertility. This study aims to analyze semen samples between smokers and non-smokers and to compare semen parameters between these two groups.

**Materials and methods:**

This observational cross-sectional study included 120 men attending the infertility clinic at a tertiary care centre between September 2025 and January 2026. Of these, 60 were smokers, and 60 were non-smokers. Semen samples were analyzed for semen volume, pH, time to liquefaction, sperm count and concentration, motility, morphology, viability, and leukocyte count in accordance with WHO guidelines (2021). Statistical analysis was performed using independent t-tests and chi-square tests, with p-values $< 0.05$ considered statistically significant.

**Results:**

Participants were aged 20–45 years. Smokers showed significantly reduced semen volume ($1.4 \pm 0.5$ mL vs. $2.1 \pm 0.7$ mL), sperm count ($50.9 \pm 47.3$ vs. $81.9 \pm 48.6 \times 10^6$/ejaculate), reduced sperm concentration ($27.8 \pm 22.6$ vs. $49.2 \pm 28.2 \times 10^6$/mL), reduced progressive motility ($26.2 \pm 16.9\%$ vs. $55.2 \pm 15.4\%$), reduced viability ($30.9 \pm 19.7$ vs. $58.3 \pm 10.9\%$), compared to non-smokers ($p < 0.001$). Abnormal semen findings were observed in 83.3% of smokers compared to 5.0% of non-smokers with RR = 16.7 (95% CI: 5.49–50.3; $p < 0.001$). Smokers were 16.7 times more likely to exhibit abnormal semen parameters compared to non-smokers.

**Conclusion:**

Following the revised WHO 2021 guidelines, this study demonstrates a significantly higher incidence of combined semen abnormalities, with decreased semen quantity and quality, among smokers.

**Keywords:** Infertility, Semen analysis, Smokers, Non-smokers, Sperm, Semen parameters.

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**Conflict of interest:** None

## INTRODUCTION

Infertility is the inability to conceive after one year of regular, unprotected intercourse and affects nearly 10 –15 percent of couples around the world, and male factors cause about one-third of these cases(1). New assisted reproductive technologies, such as blastocyst transfer, embryo biopsy, and intracytoplasmic sperm injection, have greatly improved results in reproductive medicine(2). In recent years, increasing attention has been directed towards declining sperm counts globally, a trend that may be linked to several lifestyle factors, including obesity and exposure to environmental toxins and radiation(3). Male reproductive function relies on the hypothalamic-pituitary-gonadal axis. For healthy sperm production, both in quantity and quality, the body needs sufficient levels of gonadotropins and testosterone(4). Male infertility represents a worldwide health concern that affects individuals physically, psychologically, and socially(5).

Smoking and semen quality:

Cigarette smoking is one of the most prevalent modifiable risk factors known to negatively impact semen quality and quantity. Toxic constituents in smoke from tobacco smoking - nicotine and carbon monoxide - induce oxidative stress, DNA damage, and inflammation(6). These substances can contribute to degeneration and shrinkage of the seminiferous tubules and testicular tissue(7).

Smoking not only lowers male fertility but also raises the risk of sperm aneuploidy(8), causes genetic mutations, leads to DNA fragmentation, and can induce apoptosis of spermatogenic cells(9). Given the high prevalence of smoking among men and the presence of multiple mutagenic and carcinogenic compounds in cigarette smoke, its potential impact on male reproductive health is a growing concern. Several studies have documented that semen quality is adversely affected by chronic and excessive smoking(6). Smoking cigarettes is linked to higher rates of male infertility and lower sperm quality(10).

Semen analysis remains the first-line investigation for the evaluation of male infertility, providing information on both the quantitative and qualitative aspects of testicular functions(2). Although semen analysis cannot always reliably distinguish fertile from infertile men except in cases of azoospermia, it remains a key diagnostic tool.

Although several research studies have explored the relationship of cigarette smoking with semen quality, data referring to the WHO 2021 reference standards is comparatively scarce among India's infertility clinic populations. Furthermore, limited studies have been conducted to analyze the association between combined semen abnormalities and smoking intensity. The current study was conducted to address these knowledge gaps.

## AIM AND OBJECTIVES

1. To compare the semen parameters, including sperm count, concentration, motility, and morphology, among smokers and non-smokers.
2. To assess the prevalence of abnormal semen parameters among smokers and non-smokers and to evaluate the impact of smoking on semen quality.

## METHODS

This observational study took place in the Department of Pathology at Chettinad Hospital and Research Institute between September 2025 and January 2026.

The sample size was determined using a two-group means comparison formula, with a 95% confidence level ($Z\alpha/2 = 1.96$) and an 80% statistical power ($Z\beta = 0.84$). To determine the pooled variance, the variances from both groups were averaged: $s_p^2 = (s_1^2 + s_2^2) / 2$. The minimum required sample size was estimated to be 120.

**Inclusion criteria:** The study included 120 male participants, aged between 20–45 years, who were referred from an infertility clinic for semen analysis and who were willing to provide consent and participate in this study.

**Exclusion criteria:** Patients with a history of cryptorchidism, biopsy-confirmed testicular atrophy, systemic or chronic illnesses such as diabetes mellitus or tuberculosis, varicocele, history of drug or alcohol abuse, or prior testicular injury were excluded.

Detailed information was collected for the study, including age, marital status, and cigarette smoking, along with information on the duration and frequency of smoking, using a structured questionnaire as per the study proforma.

Semen samples were obtained after 2 to 5 days of sexual abstinence by masturbation, using clean, wide-mouthed containers. The samples were collected and processed within 1 hour and analyzed in accordance with the World Health Organization (2021) guidelines(3). All analyses were performed

by a single trained professional to ensure consistency.

**Physical examination of semen:** Semen samples were examined for color, viscosity, volume, odor, and pH following complete liquefaction as per the World Health Organization guidelines.

**Microscopic Evaluation**: The semen samples were gently mixed to distribute spermatozoa and minimize variability in concentration and motility. Wet-mounts were prepared on a clean glass slide with a coverslip and examined after stabilization of sperm movement. A fresh preparation was made if the drift persisted for more than 1 minute. Semen analysis was performed after complete liquefaction, and motility was assessed in multiple microscopic fields located at least 5 mm from the coverslip margins. Semen samples were evaluated by examining at least 5 microscopic fields in accordance with the WHO 2021 recommendations. Based on the sperm movement, spermatozoa were grouped as progressively motile, non-progressively motile, or non-motile. Sperm count was calculated using a hemocytometer, and the dilution was selected based on an initial assessment of the sperm concentration. The microscopic examination was initially performed at low magnification to assess sperm distribution, mucus strands, and any aggregation/agglutination, followed by higher magnification for detailed assessment of motility. Smears were prepared using the feathering method, air-dried, and fixed for 5 to 15 minutes in a solution of equal parts 95% ethanol and ether. Sperm morphology was evaluated on Papanicolaou-stained smears, and sperm vitality was assessed using the Eosin–Nigrosin staining technique by examining at least 200 spermatozoa under oil immersion. All these parameters were compared between the smokers and non-smokers.

**Ethical considerations:** Approval was obtained from the Institutional Human Ethics Committee prior to the commencement of the study (IHEC-I/4292/25). Participants provided written informed consent, and confidentiality of personal details was strictly maintained.

**STATISTICAL ANALYSIS**

Descriptive analysis reported means and standard deviations for quantitative variables, as well as frequencies and proportions for categorical variables. Normality was assessed using histograms, Q-Q plots, and the Shapiro-Wilk test. A p-value above 0.05 indicated normality.

After descriptive analysis, independent t-tests were used to compare the means of normally distributed quantitative parameters between groups. For categorical outcomes, the Chi-square or Fisher's Exact test (if sample size <20 or cell count <5) was used. Statistical significance was $p < 0.05$. The data were analyzed using SPSS v22**.**

**RESULTS**

A total of 120 semen samples were analyzed, among which 60 were from smokers and 60 from non-smokers, all aged 20-45 years, with most individuals in the 26–35-year range, as shown in Table 1. The mean age was 34.2 ± 5.1 years for smokers, and 31.7 ± 4.9 years for non-smokers.

In this study, smokers had a statistically significantly lower mean semen volume than non-smokers (1.4 ± 0.5 mL vs. 2.1 ± 0.7 mL; $p < 0.001$). However, semen pH did not differ significantly between smokers and non-smokers ($p = 0.085$). The liquefaction time was prolonged (54.9 ± 18.2 minutes) among the smokers. Sperm count and concentration showed a statistically significant decline in smokers (50.9 ± 47.3 $\times 10^6$/ejaculate and 27.8 ± 22.6 $\times 10^6$/mL, respectively) compared to non-smokers (81.9 ± 48.6 $\times 10^6$/ejaculate and 49.2 ± 28.2 $\times 10^6$/mL, respectively), both at $p < 0.001$. Sperm motility, both progressive (26.2 ± 16.9 %) and non-progressive (6.4 ± 3.7%), was significantly reduced among the smokers ($p < 0.001$ and $p = 0.013$, respectively), accompanied by an increased percentage of immotile spermatozoa (66.8 ± 17.3 % vs. 35.6 ± 15.3%), $p < 0.001$. Normal sperm morphology was statistically significant among the smoker group (51.1 ± 19.4 %) compared with non-smokers (63.4 ± 11.1%) ($p < 0.001$). Viability was significantly reduced among smokers (30.85 ± 19.67%) relative to non-smokers (58.3 ± 10.9%), and WBC count was increased significantly among the smokers, 1.2 ± 0.7 $\times 10^6$/mL compared to non-smokers, 0.9 ± 0.4 $\times 10^6$/mL with a $p = 0.013$ as shown in Table 2 and Figure 1.

Table 3 and Figure 2, show the differences among the smokers and non-smokers in semen abnormalities. Out of 60 smokers, 20 (33.3%) had oligoasthenozoospermia, 18 (30.0%) had asthenozoospermia, 10 (16.7%) had normozoospermia, 4 (6.7%) had oligozoospermia and 8 (13.3%) had azoospermia. Among the 60 non-smokers, 57 (95.0%) had normozoospermia, while asthenozoospermia, oligozoospermia, and oligoasthenozoospermia were each reported in 1 case (1.7%). This difference was statistically

significant ($p < 0.001$), suggesting that smokers are more likely to have abnormal semen parameters.

A strong association was observed between smoking and abnormal semen findings. Among the 60 smokers, 83.3% had abnormal semen parameters compared with only 5.0% of non-smokers. The relative risk of abnormal findings among smokers was 16.7 (95% CI: 5.49 –50.3), indicating that smokers were sixteen times more likely to show abnormalities. The odds ratio further confirmed the strength of association, with a value of 95.0 (95% CI: 24.8-363.9). The chi-square test showed a statistically significant relationship ($\chi^2 = 74.649$, $p < 0.001$), as shown in Table 4.

Among 60 smokers, 14 men who smoked fewer than five cigarettes a day predominantly had normal semen quality, with fewer cases of asthenozoospermia and oligoasthenozoospermia. In contrast, among the 46 men who smoked more than five cigarettes a day, most had abnormal semen parameters, especially oligoasthenozoospermia and asthenozoospermia. Only 2.2% of this group had normal semen quality, as shown in Table 5.

**Table 1- Age-wise distribution of semen quality (N=120)**

| Semen quality | Age group (years) | | | | |
|---|---|---|---|---|---|
| | 20 - 25 | 26 - 30 | 31 - 35 | 36 - 40 | 41 - 45 |
| Normozoospermia | 7 | 23 | 19 | 17 | 1 |
| Oligozoospermia | 1 | 1 | 1 | 2 | 0 |
| Asthenozoospermia | 0 | 6 | 8 | 3 | 2 |
| Oligoasthenozoospermia | 0 | 5 | 5 | 6 | 5 |
| Azoospermia | 0 | 1 | 3 | 3 | 1 |

**Table 2: Comparison of various semen parameters among smokers and non-smokers (N=120)**

| Variable | Smoking habits | | | | |
|---|---|---|---|---|---|
| | Yes (N=60) | | No (N=60) | | P value |
| | Mean | SD | Mean | SD | |
| Volume (mL) | 1.4 | 0.5 | 2.1 | 0.7 | < 0.001 |
| pH | 7.8 | 0.3 | 7.9 | 0.2 | 0.085 |
| Liquefaction Time (minutes) | 54.9 | 18.2 | 20.8 | 6.4 | < 0.001 |
| Sperm Count (×10⁶/ejaculate) | 50.9 | 47.3 | 81.9 | 48.6 | < 0.001 |
| Sperm Concentration (×10⁶/mL) | 27.8 | 22.6 | 49.2 | 28.2 | < 0.001 |
| Motility – Progressive (%) | 26.2 | 16.9 | 55.2 | 15.4 | < 0.001 |
| Motility – Non Progressive (%) | 6.4 | 3.7 | 9.2 | 7.3 | 0.013 |
| Motility – Immotile (%) | 66.8 | 17.3 | 35.6 | 15.3 | < 0.001 |
| Morphology – Normal (%) | 51.1 | 19.4 | 63.4 | 11.1 | < 0.001 |
| Morphology Abnormal (%) | 49.1 | 19.5 | 36.6 | 11.1 | < 0.001 |
| Viability (%) | 30.9 | 19.7 | 58.3 | 10.9 | < 0.001 |
| WBC (×10⁶/mL) | 1.2 | 0.7 | 0.9 | 0.4 | 0.013 |

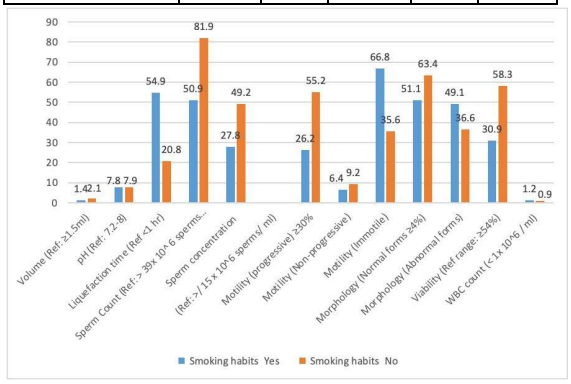


**Figure 1: Representation of various semen parameters among smokers and non-smokers**

**Table 3: Comparison of semen abnormalities between smoking habits (N=120)**

| | Yes (N=60) | No (N=60) | Chi square | P value |
|---|---|---|---|---|
| **Asthenozoospermia** | 18 (30.0 %) | 1 (1.7 %) | 75.171 | < 0.001 |
| **Oligozoospermia** | 4 (6.7 %) | 1 (1.7 %) | | |
| **Normozoospermia** | 10 (16.7 %) | 57 (95.0 %) | | |
| **Oligoasthenozoospermia** | 20 (33.3 %) | 1 (1.7 %) | | |
| **Azoospermia** | 8 (13.3 %) | 0 (0.0 %) | | |

**Table 4: Comparison of abnormal semen findings among smokers and non-smokers**

| Impression | Smoking Habits | | Chi square | P value |
|---|---|---|---|---|
| | Yes (N=60) | No (N=60) | | |
| Normal | 10 (16.7%) | 57 (95.0%) | 74.649 | < 0.001 |
| Abnormal | 50 (83.3%) | 3 (5.0%) | | |

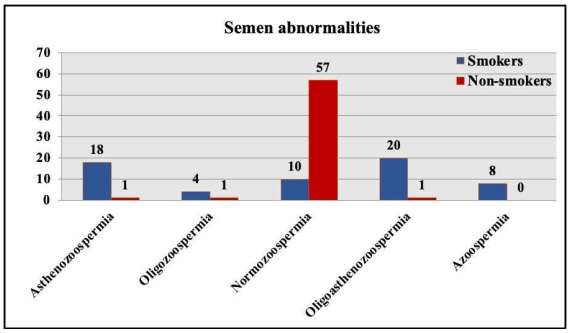


**Figure 2: Representation of semen abnormalities between smoking habits**

**Table 5: Distribution of semen quality according to cigarette smoking intensity**

| Number of cigarettes/ day | Semen quality | | | | |
|---|---|---|---|---|---|
| | Normozoospermia | Asthenozoospermia | Oligozoospermia | Oligoasthenozoospermia | Azoospermia |
| < 5/ day (N=14) | 9 (64.3%) | 4 (28.6%) | 0 (0%) | 1 (7.1%) | 0 (0%) |
| >5/ day (N=46) | 1 (2.2%) | 14 (30.4%) | 4 (8.7%) | 19 (41.3%) | 8 (17.4%) |

## DISCUSSION

Infertility is the inability to conceive even after one year of regular unprotected intercourse. It affects approximately one in seven couples worldwide (11). Male infertility remains a significant global health concern with multiple etiological factors such as genetic abnormalities, hormonal disturbances, and disorders of the reproductive tract. Increasing evidence indicates that lifestyle habits, especially cigarette smoking, significantly affect male reproductive health(12).

The negative impact of smoking on male reproductive function is more prominent in comparison with alcohol or caffeine consumption(13). Smoking is known to negatively affect semen quality, although the specific mechanisms are not completely understood(5). Carcinogenic and mutagenic compounds found in tobacco smoke include cadmium, benzopyrene, nitrosamines, and polonium, which may adversely affect sperm function. Nicotine and its metabolite cotinine detected in seminal plasma indicate that tobacco-derived toxins can cross the blood–testis barrier and directly affect spermatogenesis(14).

Smoking reduces zinc levels in seminal plasma, disrupting antioxidant defenses and potentially decreasing sperm count. Zinc depletion may lead to seminiferous tubule atrophy and impaired spermatogenesis(15). Cigarette smoking has been shown to alter $Ca^{2+}$-ATPase activity in spermatozoa and lead to sperm dysfunction. The toxic effects of smoking on semen parameters are probably attributed to toxic compounds in tobacco smoke that compromise male germ cell integrity and

maturation(16). Assessment of sperm morphology provides a glimpse beyond mere reproductive potential into the functional status of the testes and epididymis(3).

Existing studies indicate that tobacco exposure enhances the generation of reactive oxygen species within the testes, causing oxidative stress. Excessive reactive oxygen species disrupt spermatogenesis, resulting in reduced sperm production, impaired motility, and morphology(13). Spermatogenesis is considered an early marker of overall health and is particularly sensitive to environmental and lifestyle influences. Disruptions in this process may reflect broader systemic disturbances and potential long-term health risks, including metabolic and cardiovascular disorders. It has become widely clear that an increasing number of men are seeking preconception evaluation and counseling (17).

In this present study, according to the WHO 2021 criteria, we found that a considerable proportion of smokers attending our infertility clinic had multiple semen defects, especially oligoasthenozoospermia. We also observed that semen quality declined as smoking increased.

Smokers had a statistically significant lower mean semen volume (1.4 ± 0.5 mL) than non-smokers (2.1 ± 0.7 mL, $p < 0.001$). Similarly, Gupta et al. in their study reported that smoking was associated with deterioration of semen quality, including semen volume(18). These findings support the present study results and are in line with those of previous studies, indicating that smoking negatively affects seminal parameters. Reduced semen volume may reflect accessory gland dysfunction, especially decreased secretion from the seminal vesicles and prostate(19).

Liquefaction time was prolonged in smokers compared with non-smokers (54.9 ± 18.2 vs 20.8 ± 6.4 minutes; $p < 0.001$). The findings were compared with those of Kanetkar et al.(20), who observed that smokers demonstrated significantly prolonged liquefaction time, consistent with the present study.

In this study, smokers showed significantly lower sperm count and concentration ($p < 0.001$), consistent with Lingappa et al.(21), who also reported a statistically significant reduction in sperm count and concentration among the smokers (p-value < 0.001). Ramlau-Hansen et al.(22) found that cigarette smokers had lower sperm counts, less semen volume, and fewer motile sperm than men who did not smoke.

In our study, both progressive and non-progressive motility were significantly lower among smokers ($p < 0.001$ and $p = 0.013$, respectively), along with an increased number of immotile spermatozoa ($p < 0.001$). These results align with Ramon et al.(23), who found that men who smoked tended to have lower sperm movement, with lower total and progressive motility and a greater proportion of immotile sperm ($p < 0.05$). They also noted a reduction in normal sperm morphology among smokers, suggesting that smoking negatively affects overall semen quality. Zhang et al.(24) found that cigarette smoking led to a significant drop in concentration and motility of sperm, as well as changes in sperm shape and higher seminal leukocyte levels. The present study also showed a reduction in sperm count and motility among the smokers compared to non-smokers.

In our study, both groups had morphologically normal spermatozoa above WHO reference limits, but smokers showed a significantly lower proportion of normal sperm. Ramon et al. (23) demonstrated that smokers are at increased risk of oligozoospermia, teratozoospermia, and asthenozoospermia compared with non-smokers. Our results align with these findings, although we did not observe any cases of teratozoospermia.

Alghamdi et al.(25) found that smokers have lower sperm concentration, reduced motility, and poorer morphology than non-smokers. Our results confirm these findings and additionally show that smokers have decreased semen volume and sperm count.

This study highlights that smokers had a relative risk of 16.7 of abnormal semen characteristics compared to non-smokers. These results suggest that smoking may impair spermatogenesis as well as overall sperm quality. Stemming from tobacco smoke, harmful substances, such as nicotine and oxidative compounds, induce oxidative stress and lower sperm motility and morphology. The relatively low occurrence of abnormal semen parameters of non-smokers also confirms smoking as a significant and preventable risk factor for male infertility.

Previous studies had mixed research results regarding the effects of smoking on semen characteristics. Taszarek et al.(26) and Hassa et al.(27) found no difference in sperm concentration or morphology between smokers and non-smokers; however, they observed a significant decrease in sperm motility among smokers. The study by Trummer and his colleagues(28) also indicated no major differences in sperm count, motility, or

morphology between the smokers and non-smokers, but smokers had more round cells and leukocytes in their sperm. This variation is suggested to cause increased production of reactive oxygen species, which could lead to infertility.

**Table 6: Comparison of Current Study Findings with Previous Studies on Smoking and Semen Parameters**

| Studies | Key Findings among Smokers | Comparison with Present Study |
|---|---|---|
| Gupta et al. (18) | Semen volume, sperm concentration, and morphology are reduced among the smokers. No significant differences in motility were observed. | The results demonstrate decrease in semen volume, sperm concentration, morphology, and motility. |
| Kanetkar et al. (20) | Liquefaction time is longer than normal. | There was also a similar delay in the liquefaction of semen. |
| Lingappa et al. (21) | There is a significant drop in both sperm count and concentration. | Smokers consistently had lower sperm count and concentration. |
| Ramlau-Hansen et al. (22) | Semen volume, sperm count, and motility are all lower than normal. | These results demonstrate reduction in semen volume, sperm count, and motility. |
| Ramon et al. (23) | There is a higher risk of oligozoospermia and teratozoospermia, and motility is also reduced. | There is strong agreement with the observation of multiple semen defects. |
| Zhang et al. (24) | Concentration and motility are reduced, and morphology is abnormal. | A comparable deterioration was noted in key semen parameters. |
| Alghamdi et al. (25) | Volume, concentration, motility, and morphology are all reduced. | These results match the current findings. |
| Taszarek et al. (26) | No significant difference in sperm concentration or morphology, but motility was significantly reduced. | The results are partially similar because reduced motility was also observed, but more parameters were affected in this study. |
| Hassan et al. (27) | No significant change in concentration or morphology, though motility declined significantly. | These findings are partially consistent, due to reduced motility, but the present study identified a wider range of abnormalities. |
| Trummer et al. (28) | There were no significant differences found in sperm count, motility, or morphology, but there was an increase in round cells and leukocytes. | The results differ in major semen parameters. |

**STRENGTHS OF THE STUDY**

- The semen analysis was performed following the World Health Organization 2021 reference standards to ensure consistent and reliable results.
- The study measured several semen parameters, including semen volume, time of liquefaction, sperm concentration, count, motility, and morphology.
- Relative risk analysis evaluated the chance of abnormal semen features in smokers versus non-smokers. The study also showed that semen quality worsened with

increased smoking exposure, indicating a possible dose-related impact on male reproductive health.

**LIMITATIONS OF THE STUDY**

This was a single-centre study conducted at a tertiary infertility clinic, so the findings may not be generalizable to the broader population. Although we excluded participants with alcohol abuse and major systemic illnesses, we did not evaluate other potential confounding factors, including BMI, occupational exposure, environmental toxins, or detailed patterns of alcohol use. Hormonal assays, sperm DNA fragmentation index, and oxidative stress markers were not studied, so we have limited insight into the biological mechanisms involved.

**CONCLUSION**

When compared with non-smokers, overall smokers demonstrated significantly reduced semen volume, sperm concentration, motility, and a lower proportion of morphologically normal spermatozoa. The findings indicate that even low levels of cigarette consumption may be associated with adverse semen parameters and highlight smoking as a significant modifiable risk factor.

Since smokers are more likely to have abnormal semen parameters, it is important to include smoking cessation as part of infertility treatment and counseling to help people quit smoking, which should be included in fertility care. Further studies are recommended to examine whether smoking-related semen alterations are reversible and to assess their long-term effects on fertility.

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