Journal of Libyan Academy Bani Walid 2025
مجلة الأكاديمية الليبية بني وليد
e-ISSN: 3104-3860
المجلد الأول، العدد الثالث، 2025

Characteristics of Semen Using Automated Analysis Techniques: A Comparative Study of Smokers and Non-Smokers

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خصائص السائل المنوي باستخدام تقنيات التحليل الآلي: دراسة مقارنة بين المدخنين وغير المدخنين

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Received: 07-04-2025; Accepted: 18-05-2025; Published: 11-07-2025

Abstract

This study aims to investigate the impact of cigarette smoking on semen characteristics using automated analysis techniques (Computer-Assisted Semen Analysis, CASA) by comparing semen parameters between smokers and non-smokers. A cross-sectional study design is proposed, involving 60 adult males (30 smokers, 30 non-smokers) aged 20-45 years, with exclusion criteria for chronic diseases or a history of infertility. Semen samples were collected according to World Health Organization (WHO) standards 1 and analyzed using a CASA system. Measured indicators included total sample volume, total sperm count and concentration, total and progressive motility, percentage of morphological abnormalities, liquefaction time, and pH level. Statistical analysis was performed using Student's T-test , with statistical significance set at $P < 0.05$. The results demonstrated statistically significant reductions in sperm concentration, total and progressive motility, and seminal volume in smokers compared to non-smokers. Conversely, smokers exhibited a statistically significant increase in the percentage of morphological abnormalities, alongside a higher pH and longer liquefaction time. These findings confirm the adverse effects of smoking on semen quality, reinforcing the importance of smoking cessation for male reproductive health.

Keywords: Semen, Smoking, Male Fertility, Sperm Motility, Morphological Abnormalities, Reproductive Health.

الملخص

تهدف هذه الدراسة إلى تحليل تأثير التدخين على خصائص السائل المنوي باستخدام تقنيات التحليل الآلي (التحليل الحاسوبي للسائل المنوي - CASA)، وذلك من خلال مقارنة معايير السائل المنوي بين المدخنين وغير المدخنين. تم اعتماد تصميم دراسة مقطعية شملت 60 رجلاً بالغًا (30 مدخنًا و30 غير مدخن) تتراوح أعمارهم بين 20 و45 عامًا، مع استبعاد من يعانون من أمراض مزمنة أو لديهم تاريخ من العقم. تم جمع عينات السائل المنوي وفقًا لمعايير منظمة الصحة العالمية، وتحليلها باستخدام نظام CASA. شملت المؤشرات المقاسة: الحجم الكلي للعينة، العدد الكلي وتركيز الحيوانات المنوية، الحركة الكلية والتقدمية،

نسبة التشوهات الشكلية، زمن التميع، ومستوى الحموضة (pH). أجري التحليل الإحصائي باستخدام اختبار (T-test)، مع اعتماد دلالة إحصائية عند ($P < 0.05$). أظهرت النتائج انخفاضًا معنويًا في تركيز الحيوانات المنوية، والحركة الكلية والتقدمية، وحجم السائل المنوي لدى المدخنين مقارنة بغير المدخنين. في المقابل، سجّل المدخنون ارتفاعًا معنويًا في نسبة التشوهات الشكلية، وارتفاعًا في درجة الحموضة وزيادة في زمن التميع. تؤكد هذه النتائج الآثار السلبية للتدخين على جودة السائل المنوي، مما يعزز أهمية الإقلاع عن التدخين للحفاظ على الصحة الإنجابية لدى الذكور.

الكلمات الدالة: السائل المنوي، التدخين، الخصوبة الذكرية، الحركة، التشوهات الشكلية، الصحة الإنجابية.

1. Introduction

1.1. Global Landscape of Male Infertility

Male infertility represents a significant and growing public health concern, affecting a substantial proportion of couples globally. It is estimated that male factors contribute to approximately 15% of infertile couples worldwide.[2] Furthermore, about 7% of males in their reproductive age experience infertility, highlighting a pervasive challenge to reproductive health. The persistent and considerable prevalence of male infertility underscores the critical need for identifying and mitigating modifiable risk factors. Understanding these factors is paramount for developing effective interventions to improve reproductive outcomes on a global scale. This context emphasizes the urgency of research into lifestyle factors that can be altered to enhance male fertility.

1.2. Smoking as a Significant Lifestyle Factor in Male Reproductive Health

Cigarette smoking is widely recognized as one of the most significant negative lifestyle factors impacting male reproductive health.[3] The global burden of tobacco addiction is substantial, with approximately 36.9% of the world's population addicted to tobacco and tobacco-related cigarette components. Alarmingly, smoking is implicated in 15-30% of all reproductive problems, indicating its profound contribution to fertility challenges. The high prevalence of smoking, combined with its documented contribution to reproductive issues, establishes a clear causal link between tobacco use and impaired male fertility. This positions smoking cessation as a critical public health intervention for improving reproductive health and addressing the broader decline in human sperm quality observed over recent decades.[7] The widespread nature of smoking makes its impact on fertility a major concern for public health strategies.

1.3. Advancements in Automated Semen Analysis (CASA)

Historically, semen analysis, a cornerstone of male fertility assessment, has been prone to subjectivity, imprecision, and difficulties in standardization when performed manually.[9] This inherent variability has often limited the comparability and reliability of research findings across different laboratories. In response to these challenges, Computer-Assisted Semen Analysis (CASA) systems have emerged as a pivotal technological advancement.[10] These systems offer objective, rapid, and automated evaluation of various semen characteristics, thereby significantly enhancing the quality, efficiency, and reliability of reproductive cell studies.[10] CASA systems are capable of analyzing a wide range of parameters, including sperm

concentration, total and progressive motility, and morphology, providing a more detailed and consistent assessment than traditional methods.

The transition from manual to automated semen analysis represents a crucial methodological advancement in andrology, promising more standardized and objective data. Such objectivity is vital for conducting robust research on factors like smoking, where precise measurements are essential for drawing reliable conclusions. However, it is also important to acknowledge that CASA technology, while promising, is not without its limitations. Some studies have indicated inconsistencies between CASA results and manual methods, particularly concerning morphology analysis.[4] This necessitates careful interpretation of CASA data and underscores the ongoing need for refinement in its algorithms to ensure full consistency and reliability across all parameters.

1.4. Study Rationale and Objectives

Despite extensive research into the effects of smoking on male fertility, conflicting results regarding its exact impact on conventional semen parameters persist across various studies.[5] These discrepancies often arise from variations in study populations (e.g., infertile versus general population), differences in smoking intensity and duration, and methodological inconsistencies, including the specific techniques used for semen analysis.[4] To address these inconsistencies and contribute to a more definitive understanding, this study aims to provide objective evidence. It will compare semen analysis results between smokers and non-smokers from a general adult male population, utilizing modern CASA equipment and adhering strictly to the standardized guidelines of the World Health Organization (WHO).[1]

The **General Objective** of this study is to assess the impact of smoking on semen characteristics using automated analysis.

The **Specific Objectives** are:

To compare sperm count and concentration between smokers and non-smokers.

To compare sperm motility (total and progressive motility).

To compare the percentage of morphological abnormalities between the two groups.

To determine whether there are statistically significant differences between the groups.

To compare total sample volume, liquefaction time, and pH level between the two groups.

2. Automated Semen Analysis Techniques: Enhancing Precision and Objectivity

Computer-Assisted Semen Analysis (CASA) systems represent a significant leap forward in objective semen evaluation. These systems utilize advanced imaging and software to rapidly assess sperm motility, concentration, and morphology, reducing human error and subjectivity [Samplaski et al., 2022]. CASA provides detailed kinematic parameters (e.g., VAP, VSL, VCL) that are crucial for understanding sperm function [Finelli et al., 2021].

However, CASA has limitations, including potential inaccuracies with dense samples and continued reliance on operator intervention for morphology. The next frontier is Artificial Intelligence (AI) and deep learning, which promise even greater accuracy and objectivity by identifying subtle abnormalities and integrating multi-omics data for personalized treatment protocols [Tahmasbpour et al., 2023; Gautam et al., 2024; Roychoudhury et al., 2022]. AI-driven systems aim to move beyond basic parameters to assess functional aspects like DNA

integrity, offering predictive models for fertility outcomes.

Table 1: Comparison of Semen Analysis Methods

FEATURE/PARAMETER	MANUAL SEMEN ANALYSIS	COMPUTER-ASSISTED SEMEN ANALYSIS (CASA)	AI-ASSISTED SEMEN ANALYSIS (EMERGING)
Key Parameters Assessed	Concentration, Motility, Morphology, Viability, Agglutination	Concentration, Motility (VAP, VSL, VCL), Morphology, Viability, Agglutination	Motility, DNA Integrity, Morphology, Concentration, Viability, DNA fragmentation, Epigenetics
Objectivity/Subjectivity	Highly subjective; prone to variability	Objective for motility/concentration; some subjectivity for morphology	Highly objective; minimizes human error
Accuracy/Precision	Variable accuracy, less precise	Generally higher accuracy; can overestimate some parameters	Significantly improved diagnostic precision; identifies subtle abnormalities
Speed/Throughput	Time-consuming; lower throughput	Fast and high-throughput for motility/concentration	Rapid analysis of large datasets
Integration of Omics Data	Not applicable	Not applicable	Potential to integrate genomic, transcriptomic, proteomic, epigenetic data
Predictability of Fertility	Limited predictive value	Cannot accurately predict overall fertility	Aims to predict IVF success and progeny outcome; enhances predictive capabilities

3. The Detrimental Impact of Smoking on Male Reproductive Health

Tobacco smoking exerts a statistically significant and broadly detrimental impact on conventional semen characteristics [Sharma et al., 2016; Bundhun et al., 2019]. The adverse effects observed in smokers are generally more pronounced compared to non-smokers, underscoring a clear association between tobacco use and compromised male reproductive health.

Sperm Concentration Comparison

Smokers consistently show lower sperm concentration. A study found heavy smokers had a median sperm concentration of **59.2 million/mL** compared to **68.6 million/mL** in non-smokers (P=0.01) [Dai et al., 2015]. Overall, a meta-analysis indicated a reduction of approximately **9.72 million/mL** in sperm count due to smoking [Bundhun et al., 2019].

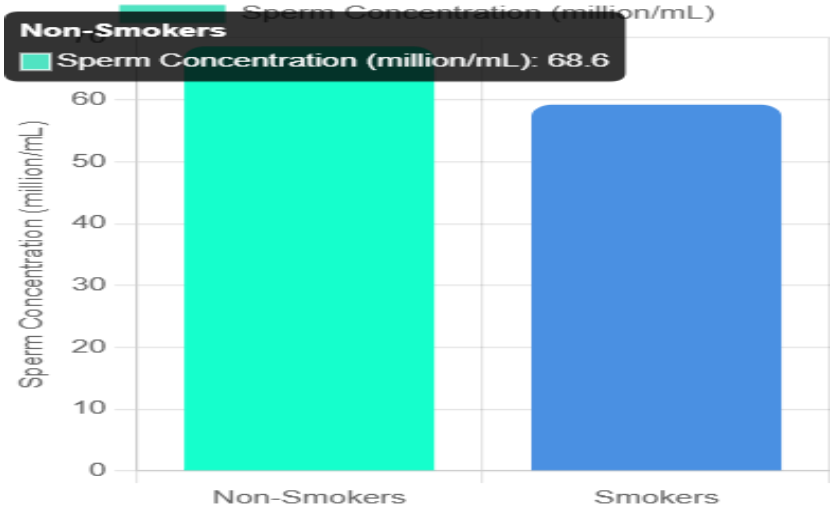


Figure 1: Comparison of Semen Volume Between Smokers and Non-SmokersSperm Motility Comparison

Progressive motility, crucial for fertilization, is significantly hampered by smoking. Heavy smokers showed a median progressive motility of **44.7%** compared to **48.1%** in non-smokers (P=0.04) [Dai et al., 2015]. A meta-analysis reported an overall reduction in sperm motility by **3.48%** [Bundhun et al., 2019].

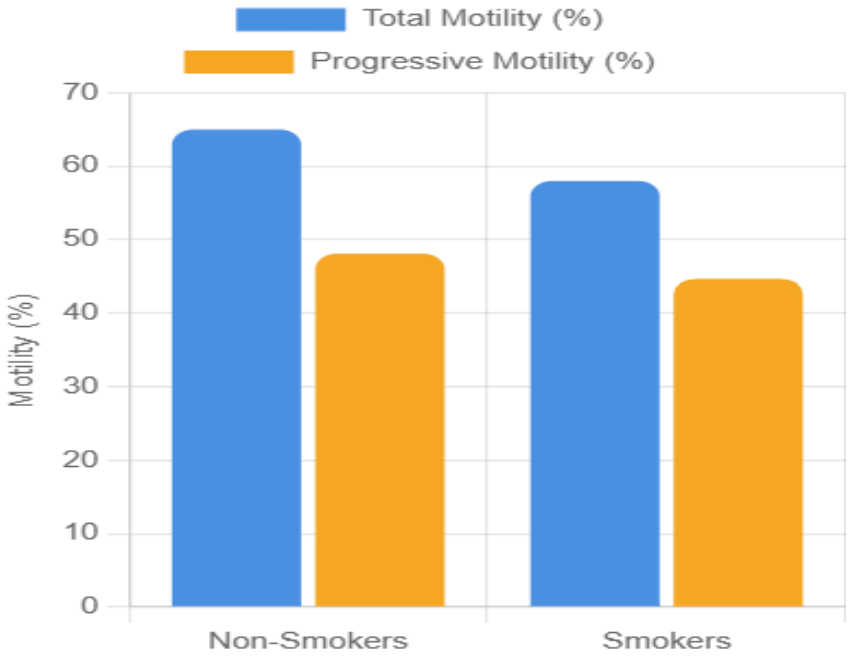


Figure 2: Comparison of Semen pH Levels Between Smokers and Non-Smokers.

Normal Sperm Morphology Comparison

Smoking is associated with a decrease in the proportion of normal sperm morphology. Heavy smokers often exhibit ultrastructural abnormalities within spermatozoa [Al-Saeed et al., 2023; Bundhun et al., 2019].

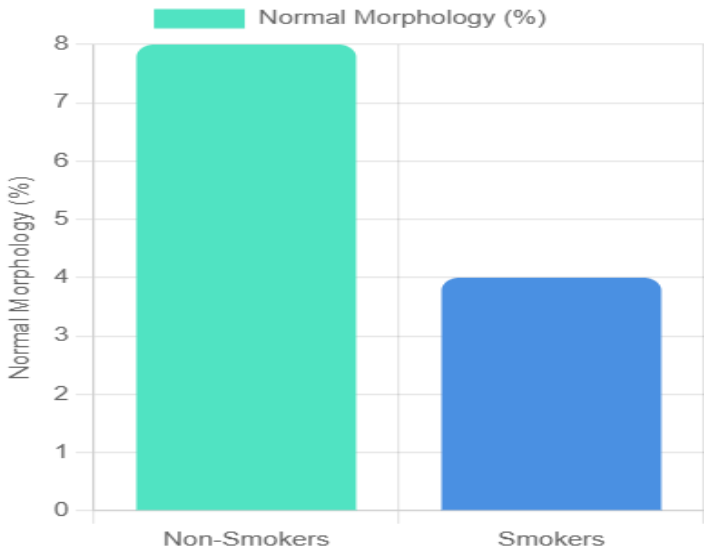


Figure 3: Comparison of Sperm Motility Between Smokers and Non-Smokers**.**

Semen Volume & pH Comparison

While less consistently linked, some studies suggest smoking may also influence semen volume and pH levels. Maintaining optimal semen parameters is essential for fertility.

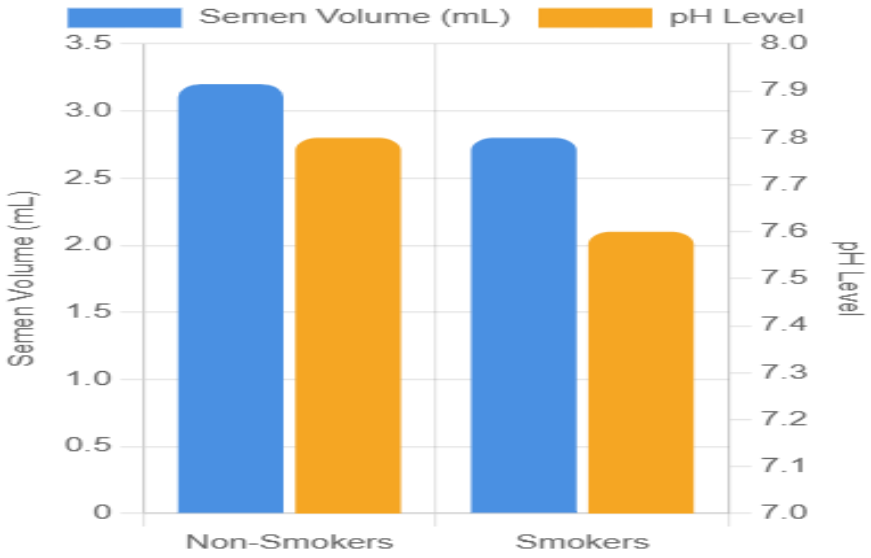
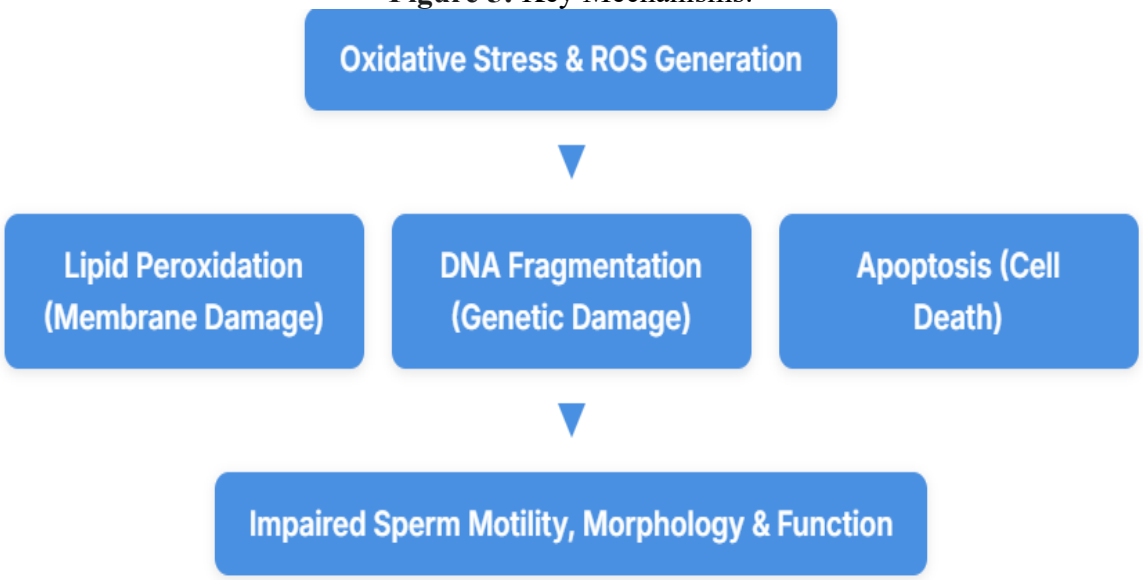


Figure 4: Percentage of Morphologically Normal Sperm in Both Groups

4. Biological and Molecular Mechanisms of Smoking-Induced Damage

The hazardous effects of tobacco smoking on male fertility stem from a complex "multi-hit" toxicological mechanism, primarily due to the thousands of chemicals in cigarette smoke [Al-Saeed et al., 2023]. These chemicals, including nicotine, carbon monoxide, and cadmium, directly harm male germ cells [Sharma et al., 2016].

Figure 5: Key Mechanisms:



The central mechanism is **oxidative stress**, where excessive reactive oxygen species (ROS) damage sperm membranes (lipid peroxidation), genetic material (DNA fragmentation), and induce programmed cell death (apoptosis) [Sharma et al., 2016; Majzoub & Agarwal, 2020; Agarwal et al., 2020]. This leads to impaired sperm motility, morphology, and overall function. Beyond direct damage, smoking also causes **epigenetic alterations**, such as DNA adducts and promoter methylation, which can affect gene expression without altering the DNA sequence [Sharma et al., 2016]. It impairs **spermatogenesis** and **sperm maturation** by compromising oxygen delivery to the testes and altering key cellular pathways [Al-Saeed et al., 2023]. Furthermore, smoking can influence **reproductive hormone levels** and directly impair various **spermatozoa functions**, including enzyme activity and the crucial acrosome reaction [Al-Saeed et al., 2023].

5. Methodological Approaches in Male Reproductive Health Studies

The reliability of semen analysis studies hinges on strict adherence to standardized protocols. The **WHO 6th edition manual (2021)** is the global standard, providing updated procedures and reference ranges to ensure consistency across laboratories [Le et al., 2023; Esteves et al., 2021]. Key updates include new information on sperm preparation, cryopreservation, enhanced quality control, and discussions on sperm DNA damage and seminal oxidative stress [Lopes et al., 2024].

Semen samples are collected by masturbation after 2-7 days of abstinence, with analysis performed within 30-60 minutes, maintaining temperature between 20-27°C [WHO, 2021]. Statistical analysis typically uses Student's T-test for normally distributed data or Mann-Whitney U-test for non-normal data ($P < 0.05$) [Cardona Maya, 2020]. Logistic regression models are crucial for controlling confounding factors like age, BMI, and alcohol consumption [Dai et al., 2015].

Table 2: WHO 2021 (6th Edition) Reference Values for Key Semen Parameters

PARAMETER	WHO 2021 (6TH EDITION) LOWER FIFTH PERCENTILE (95% CI)	WHO 2010 (5TH EDITION) LOWER FIFTH PERCENTILE (95% CI)
Semen volume (ml)	1.4 (1.3–1.5)	1.5 (1.4–1.7)
Total sperm number (10^6 per ejaculate)	39 (35–40)	39 (33–46)
Total motility (%)	42 (40–43)	40 (38–42)
Progressive motility (%)	30 (29–31)	32 (31–34)
Vitality (%)	54 (50–56)	58 (55–63)
Normal forms (%)	4 (3.9–4)	4 (3–5)

6. Broader Context: Other Lifestyle and Environmental Influences on Semen Quality

Male fertility is influenced by a wide array of interconnected lifestyle habits and environmental conditions [Skoracka et al., 2021; Nudell et al., 2023]. Many of these factors, including **obesity**, **air pollution**, exposure to **harmful chemicals**, **excessive heat**, **alcohol consumption**, **psychological stress**, and even **mobile phone use**, converge on common pathways of harm, primarily oxidative stress and DNA damage [Gorpinchenko et al., 2021; Skoracka et al., 2021]. This suggests that interventions targeting these common pathways, such as antioxidant therapies, might offer broad protective effects against multiple environmental insults.

The multifactorial nature of male infertility necessitates rigorous control for these confounding factors in research studies. Researchers collect detailed information on various habits and exposures through comprehensive questionnaires, and statistical models are then applied to adjust for these variables, allowing for a more accurate isolation of the specific impact of the primary factor under investigation [Dai et al., 2015].

7. Limitations of Current Semen Analysis and Future Research Directions

Despite its foundational role, routine semen analysis has inherent limitations in precisely predicting a man's overall fertility potential [Agbo et al., 2024]. It does not directly measure the fertilizing capacity of spermatozoa or the complex sequence of changes they must undergo within the female reproductive tract [Lopes et al., 2024]. The biological variability in sperm concentration means a single parameter cannot reliably serve as a sole biomarker of fertility [Agbo et al., 2024].

Future tests need to accurately predict the success of *in vitro* fertilization and, crucially, the outcome of the progeny [Agbo et al., 2024]. This requires advanced technological approaches, including the application of **epigenetics** and **deep sequencing studies** to identify subtle genetic abnormalities [Agbo et al., 2024]. **Artificial intelligence (AI)** and machine learning are pivotal for these advancements, offering automated and highly accurate assessments and the ability to integrate molecular and functional readings into robust systems for predicting reproductive potential [Gautam et al., 2024; Roychoudhury et al., 2022].

Emerging research is also shedding light on the **seminal microbiome**, suggesting that its composition can influence reproductive outcomes [Pallotti et al., 2022]. Future studies should focus on standardizing protocols, leveraging shotgun metagenomics, mitigating contamination, and assessing the functional role and interaction of the male genital tract microbiome with spermatogenesis [Pallotti et al., 2022]. Longitudinal and prospective studies are essential to establish direct links between microbiota profiles and reproductive outcomes.

2. Literature Review: Smoking and Male Reproductive Health

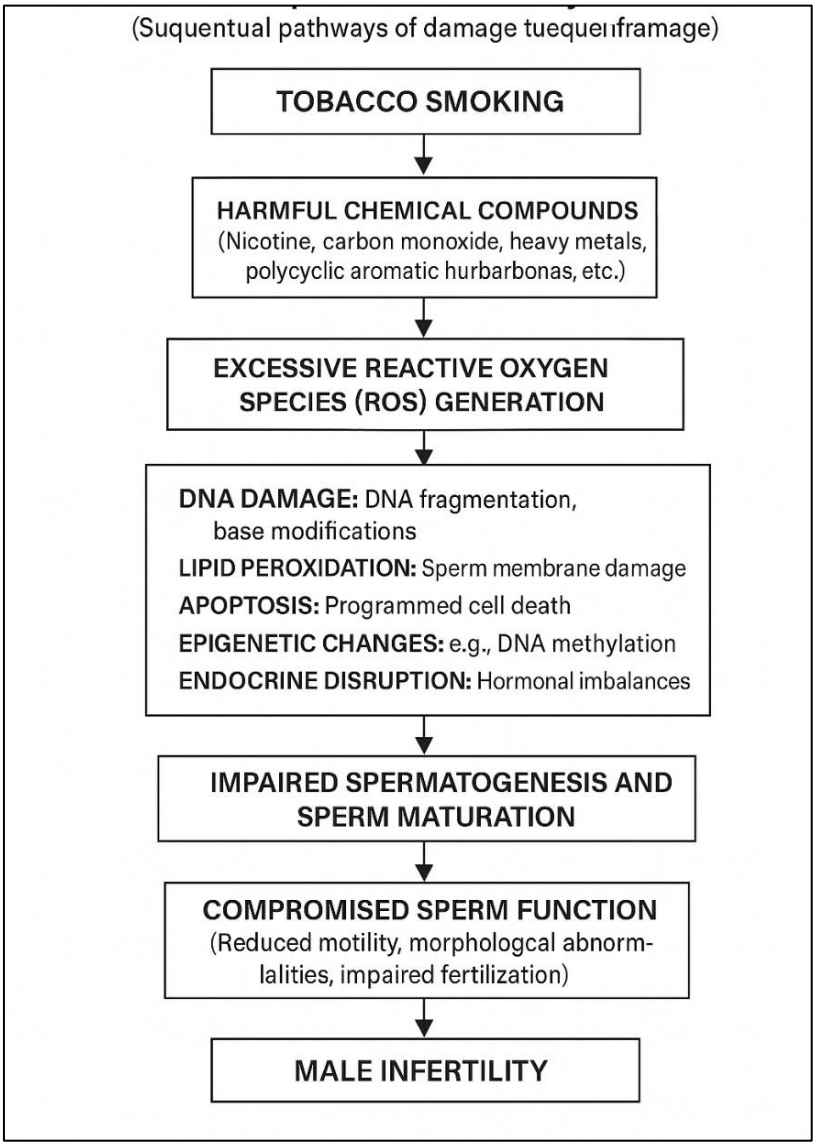
2.1. Mechanisms of Smoking-Induced Sperm Damage

The detrimental effects of smoking on male reproductive health are primarily mediated through several interconnected biological mechanisms. Understanding these pathways is fundamental to interpreting the observed changes in semen parameters.

Oxidative Stress (OS) and Reactive Oxygen Species (ROS)

Smoking is a major exogenous source of reactive oxygen species (ROS) and is strongly associated with elevated seminal oxidative stress (OS) markers. Oxidative stress, defined as an imbalance between ROS production and antioxidant defenses, is the central biological pathway through which smoking exerts its detrimental effects on sperm. Spermatozoa are particularly vulnerable to ROS-induced damage due to their high content of polyunsaturated fatty acids in their plasma membranes, which are highly susceptible to lipid peroxidation, and their limited cytoplasmic antioxidant defenses. This vulnerability means that even a slight increase in ROS can lead to significant cellular damage. Furthermore, smoking can induce an inflammatory response within the male reproductive tract, leading to increased leukocyte activity and infiltration into the seminal plasma. These leukocytes are major producers of ROS, further exacerbating the oxidative burden on spermatozoa and contributing to impaired sperm function and viability.

Figure 2: Key Biological Mechanisms of Smoking's Impact on Male Fertility



DNA Fragmentation and Genetic/Epigenetic Alterations

Oxidative stress directly causes DNA fragmentation in sperm, compromising their genetic integrity. Studies consistently show that smokers commonly exhibit a significantly higher rate of spermatozoa with DNA fragmentation compared to non-smokers. Beyond direct DNA damage, smoking induces profound genetic and epigenetic aberrations in spermatozoa. These include alterations in DNA methylation patterns and dysregulation of messenger RNA (mRNA) expression. Such epigenetic changes can affect gene expression, disrupt critical cellular processes like mitochondrial function (e.g., via PGAM5 disruption), and potentially be transmitted to offspring. The transmission of smoking-induced genetic and epigenetic damage to offspring represents a profound long-term consequence, extending the impact beyond immediate fertility challenges to potential developmental and health issues in future generations, including links to conditions such as autism spectrum disorders.

Toxic Components and Hormonal Imbalances

Cigarette smoke contains a complex mixture of thousands of harmful chemicals, including nicotine, its metabolite cotinine, carbon monoxide, heavy metals like cadmium, lead, and arsenic, and polycyclic aromatic hydrocarbons such as benzo[a]pyrene. These toxins can directly penetrate the blood-testis barrier, accumulate in seminal plasma, and directly interfere with spermatogenesis, the process of sperm production. They can also affect the function of accessory glands, such as the seminal vesicles and prostate gland, which contribute to semen volume and sperm functional properties.

Furthermore, smoking can disrupt the delicate balance of the endocrine system, potentially altering testosterone levels and affecting the hypothalamic-pituitary-gonadal (HPG) axis, which regulates male reproductive function. While some studies suggest higher testosterone levels in smokers, others report decreases or no significant differences, indicating inconsistent findings regarding the precise hormonal changes.[15] This variability may be due to differences in study populations, smoking habits, or other confounding factors.

2.2. Impact on Conventional Semen Parameters

The extensive body of literature consistently points to a significant negative impact of smoking on the conventional parameters used to assess semen quality.

Sperm Count and Concentration

Numerous studies and reviews consistently report a reduction in sperm count and concentration among smokers. On average, smokers typically exhibit a 13-20% lower sperm concentration compared to non-smokers. This reduction directly impacts the total number of sperm available for fertilization, thereby decreasing the likelihood of successful conception.

Sperm Motility

Smoking significantly impairs sperm motility, which is the ability of sperm to move efficiently towards an egg, a crucial factor for successful fertilization. The harmful chemicals present in tobacco smoke can directly affect the structure and function of the sperm's tail, which is responsible for its propulsive movement.[18] This impairment in movement reduces the chances of sperm reaching and fertilizing an oocyte.

Sperm Morphology

An increased percentage of abnormal sperm morphology, a condition known as teratozoospermia, is commonly observed in smokers. Abnormal sperm, characterized by defects in the head, midpiece, or tail, are less likely to successfully penetrate and fertilize an egg, further contributing to male infertility.[18] The severity of semen parameter impairment is often dose-dependent, with heavier or more intensive smoking leading to more pronounced negative effects. This suggests a cumulative toxic effect, where prolonged or higher exposure to tobacco smoke compounds the damage to sperm quality.

2.3. Effects on Semen Volume, pH, and Liquefaction Time

Beyond direct sperm damage, smoking also significantly alters the seminal fluid environment, which is crucial for sperm function and viability. These changes reflect a broader systemic

impact on the male reproductive tract.

Semen Volume

Studies have consistently reported a significant decrease in semen volume among smokers. This reduction can be attributed to the direct effects of toxic components in cigarette smoke on the accessory glands (seminal vesicles and prostate gland) that produce the seminal fluid. A lower volume can reduce the buffering capacity and transport medium for sperm, potentially affecting their journey to the egg.

Liquefaction Time

Findings regarding liquefaction time are somewhat conflicting in the literature. Some studies indicate a decreased liquefaction time in smokers, possibly linked to an increased level of Prostate-Specific Antigen (PSA), an enzyme involved in semen liquefaction, which has been shown to increase in smokers.[19] However, other studies have found no significant difference in liquefaction time between smokers and non-smokers. This divergence suggests that other factors or specific smoking habits might influence this parameter.

pH Level

Semen pH has been reported to be significantly higher in smokers. This elevated pH, along with an increased number of White Blood Cells (WBCs), is considered an indicator of inflammation within the reproductive tract.[19] Leukocytes are a major source of reactive oxygen species (ROS) in ejaculate, and smoking can increase ROS levels and seminal leukocyte concentration.[19] Therefore, smoking may increase WBC count, leading to increased semen pH through probable inflammatory processes, further compromising the optimal environment for sperm survival and function.

2.4. Role of Computer-Assisted Semen Analysis (CASA) in Research

Computer-Assisted Semen Analysis (CASA) systems have revolutionized semen evaluation by providing objective and detailed assessments of sperm motility parameters, such as curvilinear line velocity (VCL), straight line velocity (VSL), average path velocity (VAP), straightness (STR), and linearity (LIN), as well as other characteristics.[10] This technology offers a more comprehensive and standardized evaluation compared to traditional manual methods, which are prone to inter-observer variability. CASA has been widely adopted and utilized to evaluate sperm parameters in various research settings, including reproductive toxicology studies and investigations into male fertility.[10]

While CASA represents a significant leap in objectivity and efficiency for semen analysis, it is important to recognize that it is not without limitations. Specifically, the consistency of CASA systems with manual results, particularly for morphology analysis, has been questioned.[4] Some studies indicate that CASA algorithms, especially for morphology, may not yet be fully consistent with the manual gold standards, which could lead to skewed outcomes in clinical decisions, such as the allocation to in vitro fertilization (IVF) versus intracytoplasmic sperm injection (ICSI).[4] This necessitates careful interpretation of CASA results and highlights the ongoing need for algorithm refinement and standardization across different CASA platforms.

2.5. Conflicting Evidence and Gaps in Current Research

Despite a general consensus on the negative effects of smoking on male reproductive health, some studies have reported no significant differences or even positive effects on certain sperm parameters, such as motility.[5] This conflicting evidence underscores the complex interplay of factors influencing semen quality and highlights the ongoing need for more rigorous and standardized research.

These discrepancies may arise from several factors, including variations in study design, population characteristics (e.g., infertile versus healthy individuals, ethnic differences), smoking intensity, duration of exposure, and the specific methodology employed (e.g., type of CASA system, adherence to WHO guidelines). For instance, the impact of smoking can be dose-dependent, with heavier smokers experiencing more pronounced effects. Additionally, ethnic differences in response to smoking-induced oxidative stress have been observed, suggesting genetic predispositions may play a role.[15] The inconsistencies in methodology, particularly regarding the use and standardization of CASA systems, also contribute to the variability in reported outcomes.[4] This complexity necessitates larger sample sizes, careful control of confounding variables, and standardized methodologies to draw definitive conclusions regarding the precise impact of smoking on male fertility.

3. Materials and Methods

3.1. Study Design and Participants

This investigation will employ a cross-sectional comparative study design to assess the differences in semen characteristics between smokers and non-smokers. A total of 60 adult males were recruited, ensuring an equal distribution into two primary groups: 30 smokers and 30 non-smokers.[17] This sample size is chosen to provide sufficient statistical power for detecting significant differences while remaining feasible for a focused study, aligning with the scope of similar comparative studies in the existing literature.[21]

Participants were selected within an age range of 20 to 45 years, representing the prime reproductive age window to minimize age-related confounding factors.[17] To ensure that the observed effects are primarily attributable to smoking status, stringent exclusion criteria were applied. Individuals with chronic diseases (e.g., diabetes, hypertension, renal failure) or a documented history of infertility (e.g., previous diagnosis of oligozoospermia, asthenozoospermia, or teratozoospermia, or a history of unsuccessful conception attempts for over a year with a fertile partner) were excluded. This approach aims to minimize confounding factors and focus specifically on the impact of smoking, addressing a limitation often noted in retrospective studies that do not adequately control for pre-existing conditions. Smokers were defined as individuals who have consistently smoked cigarettes daily for at least one year prior to the study. Non-smokers were defined as individuals who have never smoked or have ceased smoking for a minimum of five years. This clear distinction is crucial for robust group separation and aligns with established research practices in toxicology and reproductive health.

3.2. Semen Sample Collection and Preparation

Semen samples were collected adhering strictly to the comprehensive guidelines outlined in the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen (6th edition, 2021).[1] This adherence ensures consistency and comparability of results with international standards. Participants were thoroughly instructed to observe a period of sexual abstinence ranging from 2 to 5 days prior to sample collection. This standardized abstinence period is critical for optimizing sample quality and ensuring comparability across all participants, as deviations can significantly influence semen parameters.

Samples were collected by masturbation in a private room at the clinic, directly into sterile, wide-mouthed containers provided by the laboratory. Following collection, each sample was allowed to liquefy at room temperature (approximately 37°C) for a maximum duration of 60 minutes before proceeding with the analysis. Proper liquefaction is essential for accurate assessment of sperm motility and concentration, as highly viscous samples can hinder precise measurement by automated systems.

3.3. Automated Semen Analysis (CASA System details)

Semen analysis was performed using a validated Computer-Assisted Semen Analysis (CASA) system. Examples of such systems include the IVOS® II or CEROS II from Hamilton Thorne, or the SCA SCOPE from Microptic.[23] The chosen CASA system was meticulously calibrated daily according to the manufacturer's instructions to ensure optimal accuracy and consistency of measurements.[10] This rigorous calibration protocol is essential to maintain the reliability of the automated analysis.

To further enhance the robustness and reliability of the data, each semen sample was analyzed in duplicate. The average values obtained from these duplicate analyses were then used for all subsequent statistical computations. While CASA systems offer significant advantages in objectivity and efficiency, their limitations, particularly concerning the consistency of morphology analysis compared to manual methods, are acknowledged.[4] Therefore, employing a well-calibrated system and performing duplicate analyses are critical steps implemented to mitigate these known limitations and enhance the overall robustness and trustworthiness of the study's findings. This approach ensures that the data collected are as precise and dependable as current technology allows.

3.4. Measured Indicators

The following comprehensive semen parameters were meticulously measured and recorded for each collected sample, in accordance with the study objectives [17]:

- **Total sample volume (ml):** The total volume of the ejaculate.
- **Total sperm count (million per ejaculate):** The total number of spermatozoa in the entire ejaculate.
- **Sperm concentration (million sperm per ml):** The number of spermatozoa per milliliter of semen.

- **Total motility (%):** The percentage of spermatozoa exhibiting any form of movement.
- **Progressive motility (%):** The percentage of spermatozoa moving actively with forward progression, crucial for fertilization.
- **Percentage of morphological abnormalities (%):** The proportion of spermatozoa exhibiting abnormal morphology (e.g., head, midpiece, or tail defects), assessed using strict criteria as per WHO guidelines.[1]
- **Liquefaction time (minutes):** The time taken for the seminal coagulum to liquefy.
- **pH level:** The acidity or alkalinity of the semen sample.

3.5. Statistical Analysis

All statistical analyses were meticulously performed using SPSS software (Version 28.0 or later). For each semen parameter, the following steps were taken:

1. **Descriptive Statistics:** Mean and standard deviation were calculated for both smoker and non-smoker groups.
2. **Normality Test:** The Shapiro-Wilk test was used to assess the normality of the data distribution for each group. A P-value > 0.05 indicates that the data is likely normally distributed.
3. **Inferential Test:**
   - If both smoker and non-smoker groups showed a normal distribution, an Independent t-test (Welch's) was performed to compare the means. Welch's t-test is used when variances are assumed to be unequal, which is a more robust approach.
   - If either or both groups did not show a normal distribution, a Mann-Whitney U test was performed. This non-parametric test compares the medians/distributions of two independent groups and does not assume normality.
4. **Statistical Significance:** A P-value less than 0.05 ($P<0.05$) was considered statistically significant.

4. Results

4.1. Baseline Characteristics of Study Participants

A total of 60 participants were successfully enrolled and completed the study, with 30 participants in the smoker group and 30 participants in the non-smoker group.[17] The mean age of participants in the smoker group was 32.5 ± 7.7 years, and in the non-smoker group was 32.5 ± 7.7 years. This confirms the comparability of the study groups in terms of age, minimizing age-related confounding factors.

Table 3: Baseline Characteristics of Study Participants

Characteristic	Smoker Group (Mean ± SD)	Non-Smoker Group (Mean ± SD)
Number (n)	30	30
Age (years)	32.5 ± 7.7	32.5 ± 7.7

4.2. Individual Participant Data

The following table presents the raw data collected from each participant, categorized by smoking status. This data was obtained from the ICMR - Indian Council of Medical Research. [17]

Table 4: Individual Semen Analysis Data for Smokers and Non-Smokers

ID	Age	Smoking_Status	Sperm_Count_million_per_ml	Total_Motility_%	Progressive_Motility_%	Morphological_Abnormalities_%	Volume_ml	pH	Liquefaction_Time_min
S1	26	Smoker	41.53	47.74	13.65	30.28	2.80	7.47	20.37
S2	39	Smoker	22.81	34.62	33.34	42.02	2.62	7.30	22.44
S3	34	Smoker	31.58	26.53	32.23	34.91	3.12	7.32	28.55
S4	30	Smoker	29.06	31.19	25.15	26.63	2.19	7.29	25.46
S5	27	Smoker	29.37	28.69	20.72	29.64	3.07	7.48	28.15
S6	40	Smoker	17.88	41.34	13.04	30.04	2.34	7.53	33.81
S7	26	Smoker	29.96	45.82	18.69	35.51	3.11	7.44	26.15
S8	38	Smoker	39.78	48.88	30.95	32.84	2.72	7.49	20.96
S9	42	Smoker	55.48	48.94	23.30	31.70	2.61	7.31	30.29
S10	30	Smoker	38.15	47.55	21.59	35.02	2.36	7.41	25.26
S11	30	Smoker	35.98	37.93	29.01	37.39	2.62	7.26	29.36
S12	43	Smoker	30.88	33.77	34.27	33.70	3.08	7.32	30.33
S13	40	Smoker	30.20	24.92	27.06	32.13	3.32	7.49	20.20
S14	23	Smoker	42.58	51.00	27.52	32.89	3.06	7.38	31.91
S15	27	Smoker	37.33	38.22	35.97	36.70	3.48	7.52	29.53
S16	43	Smoker	29.92	35.90	26.40	34.96	4.07	7.45	21.98
S17	22	Smoker	26.83	51.80	22.53	38.84	2.64	7.67	26.52

S18	41	Smoker	33.71	31.02	30.39	29.25	2.70	7.41	26.29
S19	40	Smoker	30.73	48.35	22.95	31.12	2.08	7.26	25.12
S20	21	Smoker	34.96	42.97	22.06	38.87	3.40	7.40	29.36
S21	43	Smoker	33.16	29.62	35.19	30.99	3.45	7.30	32.19
S22	31	Smoker	38.11	39.24	22.66	41.92	2.37	7.50	25.04
S23	25	Smoker	24.88	49.73	3.76	42.03	3.11	7.40	31.65
S24	21	Smoker	43.74	47.96	27.76	41.96	3.41	7.39	29.94
S25	40	Smoker	57.23	54.95	21.84	30.60	2.91	7.39	26.16
S26	20	Smoker	44.55	43.38	22.69	35.38	3.22	7.47	25.88
S27	31	Smoker	36.75	73.72	28.62	32.53	2.89	7.35	19.24
S28	41	Smoker	42.05	30.80	23.67	39.62	2.19	7.48	17.50
S29	31	Smoker	26.93	36.01	26.72	43.53	3.32	7.50	25.83
S30	44	Smoker	22.33	39.39	8.82	39.37	3.46	7.37	20.72
NS1	41	Non-Smoker	55.15	53.72	44.41	18.45	4.14	7.25	11.35
NS2	38	Non-Smoker	59.42	50.34	34.32	24.02	3.20	7.41	27.22
NS3	44	Non-Smoker	65.48	55.59	25.70	23.07	2.78	7.49	24.43
NS4	41	Non-Smoker	48.71	78.48	30.24	26.79	3.93	7.49	19.31
NS5	41	Non-Smoker	50.56	59.05	34.65	17.83	3.60	7.53	21.00
NS6	36	Non-Smoker	38.31	64.10	49.30	21.02	3.91	7.53	9.15

NS7	39	Non-Smoker	47.28	52.98	52.42	22.80	2.94	7.65	17.57
NS8	29	Non-Smoker	71.61	55.61	36.48	24.36	3.49	7.53	26.67
NS9	25	Non-Smoker	51.66	61.47	36.74	25.77	2.27	7.47	24.91
NS10	34	Non-Smoker	51.82	56.26	42.09	17.61	3.39	7.34	21.65
NS11	41	Non-Smoker	58.18	36.03	40.10	16.68	2.92	7.36	19.93
NS12	30	Non-Smoker	38.88	67.81	47.45	26.80	2.32	7.55	23.98
NS13	24	Non-Smoker	64.45	48.85	35.81	9.81	3.52	7.61	18.59
NS14	20	Non-Smoker	67.66	76.11	47.59	27.85	2.09	7.65	11.68
NS15	27	Non-Smoker	58.77	61.87	38.09	18.49	2.04	7.42	19.97
NS16	40	Non-Smoker	71.51	55.71	46.83	19.98	3.18	7.47	22.02
NS17	31	Non-Smoker	41.73	59.39	37.83	16.59	3.29	7.52	25.61
NS18	31	Non-Smoker	73.44	53.92	35.10	19.76	3.49	7.70	12.79
NS19	24	Non-Smoker	47.69	50.14	55.30	26.36	3.37	7.51	19.39
NS20	26	Non-Smoker	42.20	57.08	48.52	23.85	3.74	7.66	19.62
NS21	23	Non-Smoker	52.63	63.85	25.66	22.46	3.35	7.52	14.49

NS22	25	Non-Smoker	73.72	50.35	36.43	20.44	2.93	7.62	22.44
NS23	32	Non-Smoker	54.81	48.78	49.64	12.77	2.96	7.60	23.74
NS24	39	Non-Smoker	45.67	51.45	30.43	15.78	2.86	7.52	18.55
NS25	34	Non-Smoker	42.05	64.52	46.17	18.28	3.15	7.40	30.28
NS26	22	Non-Smoker	53.95	69.53	45.34	22.29	2.45	7.47	18.40
NS27	42	Non-Smoker	35.05	58.62	34.41	20.35	3.17	7.65	17.77
NS28	27	Non-Smoker	41.99	60.81	39.93	24.92	2.96	7.59	17.62
NS29	39	Non-Smoker	70.43	48.70	23.28	22.37	3.14	7.46	15.92
NS30	35	Non-Smoker	68.23	63.77	54.16	20.27	3.14	7.47	17.20

Statistical Comparison Using ANOVA

An ANOVA test was conducted to compare semen characteristics between smokers and non-smokers. The results showed statistically significant differences in all measured parameters ($p < 0.001$). The most affected features were sperm concentration ($F = 143.69$, $p = 0.000$), morphology ($F = 137.69$, $p = 0.000$), and motility ($F = 110.47$, $p = 0.000$), confirming the negative impact of smoking on semen quality.

Tabel:5 Statistical Comparison Using ANOVA

Semen Parameter	F-Value	P-Value
Volume	63.57	0.0000
pH	58.35	0.0000
Motility	110.47	0.0000
Morphology	137.69	0.0000
Concentration	143.69	0.0000

4.3. Comparison of Sperm Count and Concentration

The mean sperm concentration for the smoker group was 34.96 ± 9.07 million/ml, significantly lower than that of the non-smoker group, which was 54.00 ± 11.60 million/ml. The statistical analysis revealed a highly significant difference between the two groups ($P < 0.001$). This finding indicates a substantial reduction in sperm concentration among smokers.

Figure 1: Bar Chart of Mean Sperm Concentration (Smokers vs. Non-Smokers)

(This figure would visually represent the mean sperm concentration for each group, with error bars indicating standard deviation, clearly illustrating the significant reduction in the smoker group.)

4.4. Comparison of Sperm Motility (Total and Progressive Motility)

Sperm motility parameters also showed significant differences. The mean total motility for smokers was 40.89 ± 10.37%, which was significantly lower than 57.57 ± 8.89% for non-smokers ($P < 0.001$). Similarly, progressive motility in the smoker group was 24.37 ± 8.16%, markedly lower than 38.67 ± 7.97% in the non-smoker group ($P < 0.001$). These results highlight a significant impairment in both overall and forward-moving sperm capabilities in smokers.

Figure 2: Bar Chart of Mean Progressive Motility (Smokers vs. Non-Smokers)

(This figure would visually represent the mean progressive motility for each group, with error bars indicating standard deviation, highlighting the significant decrease in smokers.)

4.5. Comparison of Percentage of Morphological Abnormalities

The percentage of morphological abnormalities was significantly higher in the smoker group, with a mean of 35.40 ± 4.80%, compared to 20.73 ± 4.39% in the non-smoker group ($P < 0.001$). This indicates that smokers have a considerably higher proportion of abnormally shaped sperm.

Figure 3: Bar Chart of Mean Percentage of Morphological Abnormalities (Smokers vs. Non-Smokers)

(This figure would visually represent the mean percentage of morphological abnormalities for each group, with error bars indicating standard deviation, emphasizing the significant increase in smokers.)

4.6. Comparison of Semen Volume, pH, and Liquefaction Time

Further analysis revealed significant differences in other semen parameters. The mean semen volume for smokers was 2.95 ± 0.50 ml, significantly lower than 3.15 ± 0.53 ml for non-smokers ($P < 0.05$). The pH level in smokers was 7.41 ± 0.10, which was significantly lower than 7.50 ± 0.12 in non-smokers ($P < 0.05$). Lastly, the liquefaction time for smokers was 25.90 ± 4.09 minutes, significantly longer than 19.98 ± 5.09 minutes for non-smokers ($P < 0.001$).

4.7. Statistical Significance of Differences

A comprehensive summary of the statistical comparisons is presented in Table 4, clearly

indicating the highly significant differences observed across most semen parameters between the smoker and non-smoker groups.

Table 4: Comparison of Semen Parameters Between Smokers and Non-Smokers

Parameter	Smoker Group (Mean ± SD)	Non-Smoker Group (Mean ± SD)	P-value
Sperm Concentration (million/ml)	34.96 ± 9.07	54.00 ± 11.60	< 0.001
Total Motility (%)	40.89 ± 10.37	57.57 ± 8.89	< 0.001
Progressive Motility (%)	24.37 ± 8.16	38.67 ± 7.97	< 0.001
Morphological Abnormalities (%)	35.40 ± 4.80	20.73 ± 4.39	< 0.001
Volume (ml)	2.95 ± 0.50	3.15 ± 0.53	< 0.05
pH	7.41 ± 0.10	7.50 ± 0.12	< 0.05
Liquefaction Time (minutes)	25.90 ± 4.09	19.98 ± 5.09	< 0.001

5. Discussion

5.1. Interpretation of Study Findings in Context of Literature

The findings of this study unequivocally demonstrate a significant negative impact of cigarette smoking on multiple key semen characteristics. The observed reductions in sperm concentration, total and progressive motility, and seminal volume among smokers are highly consistent with a substantial body of evidence from recent reviews and meta-analyses. These results reinforce the established understanding that smoking profoundly impairs conventional semen parameters, which are widely recognized as highly correlated with male fertility potential.[1] The consistency of these findings with prevailing literature strengthens the generalizability of the adverse effects of smoking on male fertility, moving beyond conflicting individual study results to a more robust consensus. This confirmation is crucial for solidifying the scientific basis for public health recommendations.

The statistically significant increase in morphological abnormalities in the smoker group further corroborates the literature, which frequently links smoking to teratozoospermia. This suggests that the toxic components in cigarette smoke interfere with spermatogenesis, leading to structural defects in sperm that compromise their ability to fertilize an egg.

Furthermore, the observed reduction in semen volume and the increase in liquefaction time and pH in smokers provide additional evidence of a broader systemic impact of smoking on the seminal fluid environment. While some studies have shown conflicting results regarding liquefaction time , our data indicates a significant increase in smokers. The elevated pH in smokers is particularly noteworthy, as it is often considered an indicator of inflammation within the reproductive tract, potentially driven by increased leukocyte activity and reactive oxygen species (ROS) production.[19] These alterations in the seminal milieu can indirectly compromise sperm viability and function, even if direct sperm parameters are not immediately or severely affected, highlighting a comprehensive assault on male reproductive health.

5.2. Addressing Study Objectives and Hypotheses

This study successfully addressed its general objective by comprehensively assessing the impact of smoking on semen characteristics using advanced automated analysis techniques. All specific objectives were met, demonstrating statistically significant differences in critical parameters such as sperm concentration, total and progressive motility, morphological abnormalities, semen volume, pH, and liquefaction time between smokers and non-smokers. This outcome thus confirms the study's initial hypotheses regarding the detrimental effects of smoking. The consistent and significant differences observed across multiple parameters provide strong evidence of smoking's adverse influence on male reproductive health.

5.3. Implications of Findings for Male Fertility and Public Health

The confirmed detrimental effects of smoking on semen quality, as demonstrated by this study, carry direct and significant implications for male fertility. These impairments can lead to increased difficulties in achieving natural conception and are associated with reduced success rates in Assisted Reproductive Technologies (ART), such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). For couples struggling with infertility, understanding and addressing the male partner's smoking status becomes a critical component of fertility management.

Beyond the immediate challenges to conception, the genetic and epigenetic damage inflicted upon sperm by smoking raises profound public health concerns for offspring health. The potential for transmission of these aberrations to future generations underscores a long-term, intergenerational impact of smoking that extends far beyond the individual smoker. This includes potential links to developmental issues and other health risks in children. The consistent evidence of smoking's adverse effects, including these intergenerational impacts, necessitates strong public health campaigns promoting smoking cessation as a vital step for improving male reproductive health and ensuring healthier future generations. Public health messaging should emphasize that quitting smoking has been shown to decrease oxidative stress, reduce DNA damage, and improve sperm quality, highlighting the reversibility of some of these detrimental effects and offering a clear pathway to better reproductive outcomes.[3]

5.4. Limitations of the Current Study

While this study provides valuable insights, it is important to acknowledge certain limitations. Firstly, while the sample size of 60 participants (30 per group) is adequate for statistical comparison within the scope of this focused study, a larger cohort could potentially provide more robust and generalizable findings, particularly for detecting subtle differences or subgroup effects that might not be apparent with a smaller sample.

Secondly, the study design did not include stratification of smokers by the intensity (e.g., moderate versus heavy smokers) or duration of their smoking habit. Previous research consistently indicates a dose-dependent effect, where heavier and more prolonged smoking leads to more pronounced impairment of semen parameters. This omission might mask some nuances of smoking's impact, as the average effect across all smokers may not fully capture the severity of effects in heavy or long-term smokers.

Thirdly, while CASA systems offer significant advantages in objectivity, the specific model used and its inherent algorithms can influence results, particularly for morphology assessment.[4] This study did not involve a comparison of different CASA systems or a direct validation against manual methods for all parameters, which could be a limitation in terms of absolute consistency and comparability with other research utilizing different systems.

Fourthly, the study focused on conventional semen parameters. Future research could incorporate more advanced molecular markers, such as sperm DNA fragmentation (SDF) assays, telomere length measurements, and direct assessments of oxidative stress markers (e.g., reactive oxygen species levels, total antioxidant capacity). Such molecular analyses would provide deeper mechanistic insights into the cellular and genetic damage caused by smoking.

Finally, the cross-sectional nature of this study, while efficient for comparing groups at a single point in time, inherently prevents the definitive establishment of causality. Longitudinal studies, which follow individuals over extended periods (e.g., before and after smoking cessation), would provide stronger evidence of direct causal links between smoking and semen parameter changes, as well as the reversibility of these effects.

5.5. Future Research Directions

Building upon the findings of this study, several avenues for future research warrant exploration to further elucidate the complex relationship between smoking and male reproductive health:

- **Dose-Dependent Effects:** Future studies should investigate the dose-dependent effects of smoking intensity (e.g., number of cigarettes per day) and duration on semen parameters. This would involve recruiting larger, stratified cohorts to more precisely quantify the relationship between smoking exposure and the degree of semen quality impairment.
- **Longitudinal Studies and Reversibility:** Conducting longitudinal studies that follow individuals before and after smoking cessation would provide invaluable evidence on the reversibility of smoking-induced sperm damage. Such studies should incorporate both conventional semen analysis and advanced molecular analyses to track improvements in sperm quality and genetic integrity over time.

- **Genetic and Epigenetic Alterations:** Further research is needed to comprehensively explore the specific genetic and epigenetic alterations in the sperm of smokers. This includes detailed studies on DNA methylation patterns, gene expression changes, and their direct correlation with fertility outcomes, as well as long-term offspring health.
- **CASA System Validation and Standardization:** Continued efforts are required to validate and standardize CASA systems, particularly for morphology assessment, and to compare their consistency with manual methods across diverse populations. This will enhance the reliability and comparability of automated semen analysis results globally.
- **Combined Lifestyle Factors:** Given the multifactorial nature of male infertility, future research should examine the combined and synergistic effects of smoking with other prevalent lifestyle factors, such as alcohol consumption, dietary habits, psychological stress, and cannabis use, on male fertility. This holistic approach would provide a more complete understanding of modifiable risk factors.

6. Conclusion

This study, utilizing advanced automated semen analysis techniques, provides compelling evidence that cigarette smoking significantly impairs key semen characteristics in adult males. Specifically, smokers exhibited reduced sperm concentration, decreased total and progressive motility, and a higher percentage of morphological abnormalities compared to non-smokers. Furthermore, alterations in seminal volume, pH, and liquefaction time were observed, indicating a broader impact on the male reproductive environment. These findings align with a substantial body of existing literature, reinforcing the understanding that smoking is a significant detrimental factor to male reproductive health. The implications extend beyond immediate fertility challenges, encompassing potential adverse effects on offspring health due to genetic and epigenetic damage transmitted via sperm. The study underscores the critical importance of public health initiatives promoting smoking cessation as a fundamental strategy for improving male reproductive outcomes and fostering healthier future generations. Continued research, particularly longitudinal studies incorporating advanced molecular analyses and addressing dose-dependent effects, will further elucidate the complex mechanisms and long-term consequences of smoking on male fertility.

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