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From Smoke to Sperm: Evaluating the Synergistic Effects of Smoking Cessation, L-Carnitine, and B-Complex Supplementation on Male Fertility

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ABSTRACT

Objective: Smoking is a major risk to the health, yet its detrimental impact on male fertility status is often underreported clinically. This study aimed to evaluate the effects of smoking cessation on seminal parameters in infertile male smokers and compare the results with those who continued smoking. **Methods:** A double-center, follow-up cohort study was conducted in Mosul, Iraq between January 2025 and April 2025. A total of 67 infertile male smokers aged 18–55 years were enrolled and divided into two groups: the smoking cessation group (STSMO, n=35) and the smoking continuation group (CTSMO, n=32). Both groups received B-complex and L-Carnitine supplementation. Semen and hormonal analyses were performed at baseline and after three months. **Results:** The STSMO group demonstrated significant improvements in sperm count (18.99 ± 11.41 to 30.31 ± 15.08 million/ml, $p < 0.0001$), progressive motility (10.57 ± 4.661% to 19.57 ± 8.607%, $p < 0.0001$), sluggish motility (14 ± 6.278% to 18.43 ± 6.942%, $p = 0.0025$), and viability (44.29 ± 12.38% to 56.71 ± 13.56%, $p < 0.0001$). In contrast, the CTSMO group showed no significant changes in these parameters. **Conclusion:** Smoking cessation significantly improves sperm count, motility, and viability in infertile male smokers, highlighting the detrimental effects of smoking on male fertility. Infertility specialists should counsel patients on quitting smoking as part of fertility treatment.

1. Introduction

Cigarette smoking is a leading cause of preventable morbidity and mortality. It is associated with numerous adverse health effects. Despite the well-documented adverse effects of smoking on general health and fertility, it remains a worldwide issue[1]. According to the World Health Organization (WHO), approximately one-third of the global population over the age of 15 is a smoker. Young adult males in the reproductive period exhibit the highest prevalence of smoking, with 46% of smokers falling within the age range of 20 to 39 years old[2,3].

Male infertility, as defined by the WHO, is the incapacity of a male to impregnate a fertile female after a minimum of one year of consistent unprotected intercourse. The male is exclusively accountable for approximately 20% of infertility instances and is a contributing factor in an additional 30% to 40% of cases.[4].

While most individuals are aware of the impact of smoking on heart and lung health, fewer are aware of its effects on male reproductive health. Numerous investigations have been conducted to ascertain potential correlations between smoking and male infertility, with some revealing conflicting results. Several studies have demonstrated a negative impact of smoking on semen analysis parameters and male infertility[5,6], conversely, other studies have found no such effects, while others have noted positive impacts on sperm motility and reductions in nuclear DNA damage in sperm[7–9] Inconsistent and contradictory findings concerning the effect of smoking on male infertility have been documented[10,11].

The presence of contradictory and confusing findings is expected. Multiple hypotheses may be formulated to elucidate the reasons behind inconsistent outcomes. First of all, a variety of measures were performed to assess the effect of smoking on male fertility, including semen parameters, spermatozoa function, histologic alterations, and more. Using a range of measurements may result in distinct results between studies. Additionally, the mechanisms by which smoking affects male fertility are not well understood[12].

L-carnitine is a naturally occurring amino acid derivative that plays a vital role in energy production and is widely used to improve male fertility. It has been shown to enhance sperm motility, count, and overall quality by reducing oxidative stress and improving mitochondrial function in sperm cells. Additionally, B-complex vitamins are highly beneficial for male fertility as they support hormonal balance, improve energy metabolism, and reduce oxidative damage to sperm. Vitamins such as B9 (folate) and B12 are particularly important for DNA synthesis and the production of healthy sperm, contributing to improved fertility outcomes.[13,14]

The purpose of this study is to compare the various semen parameters of infertile smokers who continue smoking cigarettes to those who cease smoking over a three-month period. The aim is to determine the influence of smoking cessation on the quality of seminal fluid.

2. Materials and methods

Study design, period, and ethical approval

This is a double-center, follow-up cohort study carried out at a private urology clinic in Mosul, Nineveh province, from January 2025 to April 2025. This study aimed to examine the effects of three months of smoking cessation on infertile male smokers and compare the outcomes with those of infertile men who continued smoking. Since spermatogenesis requires three months for completion, a three-month follow-up study is deemed appropriate. The research adheres to the ethical standards established in the Declaration of Helsinki by the World Medical Association, which regulates the ethical practices in studies involving human participants and/or animal specimens. Participants provided written informed consent after receiving detailed information regarding the study's design. Participants were invited to complete a study questionnaire. Consultants subsequently evaluated the participants to ascertain their eligibility according to the inclusion criteria and to eliminate any abnormalities.

Study participants

All individuals who participated in the study were enrolled based on strict selection criteria using a convenience sampling technique. The inclusion criteria were: Males with diagnosed cases of infertility (primary or secondary), heavy smoking (< 20 cigarettes per day for at least two years), with normal hormonal assessments, and aged between 18 and 55 years. Exclusion criteria included patients with abnormal hormonal levels, hyperprolactinemia, varicocele, testicular atrophy, or those over 55 years of age.

A total of 85 participants were enrolled in this 3-month follow-up study. After providing written informed consent, participants were assessed at baseline to collect demographic and clinical data, including age, smoking history, number of cigarettes smoked per day, and hormonal and semen analysis parameters. Participants were then divided into two groups: Smoking cessation group (STSMO) who agreed to quit smoking for the duration of the study and received B-complex (once daily) and L-Carnitine (500 mg twice daily) treatment (35 participants) and the Smoking continuation group (CTSMO) who continued smoking while also receiving B-complex and L-Carnitine treatment (32 participants).

Follow-Up Procedures

Participants were followed up for three months, with assessments conducted at baseline and at the end of the study (3 months). During each follow-up visit, the following data were collected: Semen analysis, Hormonal levels, Smoking status (self-reported) and any adverse events. Of the 85 participants initially enrolled, 67 completed the 3-month follow-up period, resulting in a retention rate of 78%. Reasons for dropout included loss of contact, withdrawal of consent, or non-compliance with the study protocol.

Study procedure, clinical presentation, and biomarker assessment

Semen analysis

Semen samples were collected via masturbation into sterile, wide-mouthed containers in a private collection room at the laboratory or clinic. Samples will be delivered to the laboratory within 30 minutes of collection. Samples will be maintained at body temperature (37°C) during transport to the laboratory to preserve sperm motility and viability.

Semen analysis was performed according to the World Health Organization (WHO) guidelines (2021), with repeated duplicate assessments for each sample to ensure accuracy and reproducibility[15]. Sperm concentration was quantified using both a hemocytometer and a validated automated semen analyzer (SQA-V Gold, Medical Electronic Systems, USA). Sperm motility assessments were performed by two trained laboratory technologists independently, utilizing a phase-contrast microscope (Olympus CX23, Japan) at 400× magnification and categorized into progressive, non-progressive, and immotile spermatozoa. Discrepancies were reconciled by joint review to minimize interobserver variability. At least 200 spermatozoa were assessed under a phase-contrast microscope. Morphology was evaluated using Papanicolaou staining or Diff-Quik staining. A minimum of 200 spermatozoa were analyzed for normal and abnormal forms. Lastly, viability was assessed using eosin-nigrosin staining to differentiate live and dead sperm cells, in which non-viable sperm take up the dye, whereas live sperm remain unstained. Quality control was routinely carried out according to WHO laboratory recommendations[16].

Hormonal analysis

To do the biochemical analysis, five milliliters of blood were drawn from a vein with a single-use syringe. The blood was placed in a plain tube and left to coagulate at room temperature; then, ten minutes of centrifugation at 3,000 revolutions per minute collected the serum. Hormonal analysis was then performed on this serum. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (TEST), and prolactin (PRL) were determined using a VIDAS immunoassay analyzer (bioMérieux, France). The system operates on the

Table 1. Baseline characteristics of the studied groups

Baseline characteristics		
Parameter	**CTSMO (n=32)**	**STSMO (n=35)**
Age (years)	30.5 ± 7.384	28.83 ± 7.209
Duration of infertility (years)	3.344 ± 2.266	3.371 ± 4.312
Number of cigarettes/days	32.5 ± 9.837	33.14 ± 11.57

Data are presented as mean ± SD using one-way ANOVA followed by Tukey's post hoc multiple comparison test. *CTSMO*; Smoking continuation group*, STSMO*; Smoking cessation group.

Enzyme-Linked Fluorescent Assay (ELFA) principle, a two-site immunoassay that uses enzyme-labeled antibodies and fluorescence detection to measure antigen levels accurately. It is highly sensitive and specific, making it suitable for clinical diagnostics. First of all, the sample and the reagent interacted, and then an antigen-antibody reaction occurred, resulting in the formation of a sandwich complex. The washing process was followed, then fluorescent detection occurred, which is directly proportional to the concentration of the antigen in the sample. To ensure reliable hormone measurements, each serum sample was analyzed three times, and the average value was used for statistical analysis. The VIDAS platform is clinically validated for reproductive endocrinology, with daily quality control and routine calibration performed according to the manufacturer's guidelines. Inter-assay and intra-assay variations remained below 10%, ensuring precision and reliability of the results.

Statistical analysis

The data are presented as the mean values with the standard deviations (mean±SD). A paired t-test was implemented to facilitate individual comparisons of the tested data. The statistical variations of the various examined groups were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test to identify any significant variability in the groups' mean. Prior to conducting any statistical analysis, the normality tests (Kolmogorov-Smirnov, Shapiro-Wilk) were used to verify the normal distribution of the enrolled groups. The statistical significance was determined using GraphPad Prism 8.0.1, with a p-value of less than 0.05 considered significant.

3. Results

Baseline characteristics

The baseline characteristics of the study participants at the start of the study in the CTSMO and the STSMO were comparable, ensuring a balanced comparison between the two groups. The mean age of participants in the CTSMO group was 30.5 ± 7.384 years, slightly higher than the STSMO group, which had a mean age of 28.83 ± 7.209 years. The duration of infertility was similar between the two groups, with the CTSMO group reporting an average of 3.344 ± 2.266 years and the STSMO group reporting 3.371 ± 4.312 years. Additionally, the average number of cigarettes smoked per day was comparable, with the CTSMO group smoking 32.5 ± 9.837 cigarettes per day and the STSMO group smoking 33.14 ± 11.57 cigarettes per day.

Hormonal assessment

FSH, LH, total testosterone, and prolactin were assessed baseline period to detect the hormonal status of participants. Hormonal assessment of both the STSMO and the CTSMO showed that all parameters

Table 2. Hormonal assessment of the studied groups

Parameter	CTSMO (n=32)	STSMO (n=35)
FSH (m IU/ml)	4.608 ± 2.875	3.478± 1.753
LH (m IU/ml)	3.616 ± 1.297	3.307 ± 1.284
T. TESTOSTERONE (ng/ml)	4.703 ± 1.626	3.925 ± 1.522
PROLACTIN (ng/ml)	9.192 ± 3.41	9.869 ± 3.741

Data are presented as mean ± SD using one-way ANOVA followed by Tukey's post hoc multiple comparison test. *CTSMO*; Smoking continuation group*, STSMO*; Smoking cessation group, *FSH*; follicle stimulating hormone, *LH*; luteinizing hormone, *T*; Total

Table 3: Seminal analysis of the Smoking Continuation Group (CTSMO)

CTSMO (n=32)			
Parameter	**Before**	**After**	***p* value**
Sperm count (million/ml)	22.66 ± 12.09	24.09± 13.55	0.1534
Sperm motility / progressively motile (%)	14.06 ± 9.791	12.91 ± 6.883	0.7371
Sperm motility / sluggish motile (%)	14.06 ± 6.891	14.69 ± 7.177	0.3244
Viability (%)	47.5 ± 17.55	46.25 ± 15.19	0.8624

Data are presented as mean ± SD using one-way ANOVA followed by Tukey's post hoc multiple comparison test. *CTSMO*; Smoking continuation group.

Table 4. Seminal analysis of the Smoking Cessation Group (STSMO)

STSMO (n=35)			
Parameter	**Before**	**After**	***p* value**
Sperm count (million/ml)	18.99 ± 11.41	30.31± 15.08*	<0.0001
Sperm motility / progressively motile (%)	10.57 ± 4.661	19.57 ± 8.607*	<0.0001
Sperm motility / sluggish motile (%)	14 ± 6.278	18.43 ± 6.942*	0.0025
Viability (%)	44.29 ± 12.38	56.71 ± 13.56*	<0.0001

Data are presented as mean ± SD and are significantly different where indicated using one-way ANOVA followed by Tukey's post hoc multiple comparison test. *STSMO*; Smoking cessation group.

are within the normal range. Table 2 summarizes the hormonal assessment of the study groups.

Seminal analysis

Smoking continuation group (CTSMO).

Table 3 shows the results of the seminal analysis of patients in the CTSMO group. Sperm count, sperm motility / progressively motile, sperm motility / sluggish motile and viability after the end of the study are non-significantly changed compared to that before the start of the study. The sperm count increased slightly from 22.66 ± 12.09 million/ml to 24.09 ± 13.55 million/ml, but this change is not significant (p = 0.1534). Similarly, the percentage of progressively motile sperm decreased slightly from 14.06 ± 9.791 to 12.91 ± 6.883, with no significant difference (p = 0.7371). The percentage of sluggish motile sperm showed a minor increase from 14.06 ± 6.891 to 14.69 ± 7.177, but again, this is not statistically significant (p = 0.3244). Lastly, sperm viability showed a negligible decrease from 47.5 ± 17.55 to 46.25 ± 15.19, with no significant difference observed (p = 0.8624). This indicates that the administration of supplementation including B-complex and L-carnitine treatment without smoking cessation does not improve sperm health and fertility in males with infertility.

Smoking cessation group (STSMO)

Regarding the STSMO group, the results of the seminal analysis are significantly changed after three months when compared to the baseline results. Sperm count showed a substantial increase from 18.99 ± 11.41 million/ml to 30.31 ± 15.08 million/ml, which was highly significant (p < 0.0001). Similarly, the percentage of progressively motile sperm nearly doubled, from 10.57 ± 4.661 to 19.57 ± 8.607, with a highly significant p-value (p < 0.0001). The percentage of sluggish motile sperm also increased significantly, from 14 ± 6.278 to 18.43 ± 6.942 (p = 0.0025). Lastly, the viability of the sperms is also significantly increased in the STSMO group at the end of the study compared to that before the start of the study from 44.29 ± 12.38 to 56.71 ± 13.56 with a p value of <0.0001.

4. Discussion

Smoking is a major risk to the health, yet its detrimental impact on male fertility status is often underreported clinically. Several investigations have reported a detrimental impact of smoking on sperm count, motility, and morphology, alongside an increase in seminal reactive oxygen species (ROS) and/or apoptosis[17,18]. On the other hand, some studies did not record any significant changes in seminal parameters[2,19]. The findings of the present study revealed a significant improvement of seminal parameters for patients involved in the STSMO group. At the same time, there were non-significant changes in these parameters for patients involved in the CTSMO group, which reflected the negative impact of smoking on sperm health.

Patients in the STSMO group showed a highly significant increase in sperm count compared to those in the CTSMO group. This finding coincides with results from several previous studies[20,21]. Smoking cigarettes negatively impacts spermatogenesis either directly or indirectly. One of the direct effects is the high levels of free radicals and other oxidants present in the complex mixture of over 6000 chemical components in cigarette smoke. Lipid peroxidation and free radicals are recognized to participate in the pathophysiology of male infertility, possibly due to DNA damage of sperms[9]. Smoking is believed to increase oxidative stress through a number of mechanisms, including direct damage from radicals[22]. Infertile men who smoke cigarettes have higher levels of seminal oxidative stress than infertile nonsmokers. Due to the high amounts of polyunsaturated fatty acids in their plasma membranes and the low concentrations of ROS-scavenging enzymes in their cyto-

plasm, spermatozoa are especially vulnerable to damage caused by excessive ROS[23].

A literature review suggests another mechanism. Nicotine, the primary alkaloid in tobacco, increases the plasma epinephrine levels, which in turn stimulates the secretion of adrenocorticotropic hormone (ACTH). Adrenal cortical hyperactivity, which induces stress, results in increased catecholamine levels in the body, a recognized vasoconstrictor. Vasoconstriction and nicotine adversely affect Leydig cell function, which constitute the predominant type of testicular interstitial cells. Leydig cells synthesize androgens essential for normal spermatogenesis and influence other androgen-responsive organs beyond the testis, while also regulating the populations of testicular macrophages and lymphocytes[24]. These steroidogenesis alterations ultimately lead to decreased sperm count[25].

Additionally, smoking may elevate serum estradiol levels by increasing blood catecholamine levels, which in turn could promote the conversion of testosterone to estradiol through aromatization. Spermatogenesis may be impaired by estrogen through a variety of mechanisms. It prevents the biosynthesis of testosterone in the testis. By increasing catecholamine activity, it may also have a detrimental impact on sperm count. Elevated catecholamine levels may result in seminiferous tubule ischemia, which may impede spermatogenesis and eventually reduce the count of sperm[26].

Regarding sperm motility, the current study showed a significant increase in sperm motility involving both progressive and sluggish motility, in patients from the STSMO group compared to those in the CTSMO group, reflecting the negative effects of smoking on this parameter. In line with these findings, Gaur et al (2007) also demonstrated the negative impacts of smoking on sperm motility[27]. Moreover, Banerjee A et al (1993) also revealed the detrimental effects of smoking on the percentage of motile sperm and total sperm count ($p < 0.05$)[28].

Along with carbon monoxide and hydrocyanic acid, numerous other highly hazardous substances in cigarette smoke adversely affect spermatozoa. Moreover, due to the anatomical resemblances between cilia and sperm tails regarding their tubular structure and functionality, the ciliary toxins present in cigarette smoke may affect sperm tail motility[29]. Additionally, cigarette smoking might affect the ultrastructure of the flagellum, particularly the axoneme of human spermatozoa, which is crucial for sperm motility. The ultra-structural evaluation of axoneme revealed that 99% of smokers exhibited significant ultra-structural abnormalities. In contrast, only 26% of non-smokers showed such abnormalities. This may be a contributing factor to reduced sperm motility in smokers[30].

On the contrary, Adelusi and colleagues reported markedly elevated sperm motility among infertile male smokers, though they did not explain such discrepancy. That discrepancy underscores the current debate on the influence of smoking on semen parameters and male fertility. This may be attributed to varying techniques among reported studies or the existence of confounding factors that are challenging to identify, such as passive smoking or exposure to environmental pollutants among nonsmokers[8].

Finally, the findings of the present study indicated a substantial improvement in sperm viability among infertile individuals who ceased smoking, whereas there were no significant alterations in the CTSMO group. The mechanism underlying the detrimental impact of smoking on semen viability remains incompletely understood. The direct exposure of spermatozoa to cigarette smoke toxins likely disrupts the fragile balance of ROS. Elevated levels of ROS have been demonstrated to adversely affect the DNA of spermatozoa. Consequently, this adversely impacts the viability and morphology of spermatozoa. However, additional research is necessary for understanding the specific mechanisms by which smoking affects sperm quality and male fertility[31].

One of the limitations of the current study is that it was only carried out in two private clinics, and it also involved a relatively small number of participants. Nevertheless, the study provides a clear

insight into the impact of smoking on the parameters of sperm and male fertility.

5. Conclusion

Smoking has significant detrimental effects on sperm parameters involving sperm count, motility, and viability. Smoking may directly or indirectly affect spermatozoa and fertility. Infertility specialists should inform their patients on the detrimental effects of smoking on fertility and recommend the maintenance of a balanced lifestyle while avoiding smoking to prevent future health complications.

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Competing interest

The authors have no relevant financial or non-financial interests to disclose.

Author's contributions

Khalil A. Hadid: Writing – review & editing; writing—original draft preparation; and conceptualization. Muthanna K. Zaki: Writing – review & editing; writing—original draft preparation; and conceptualization. Fawaz A. Alassaf: Supervision, project administration, formal analysis, and conceptualization. Mohammed N. Abed: Supervision, project administration, formal analysis, and Conceptualization. Bashar M. Al-Hammodi: Clinical investigation and data collection. Zaid Saad Khudhur: Clinical investigation and data collection

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The authors affirm that human research participants provided informed consent for publication of the tables 1,2,3 and 4.

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