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Impact of heavy alcohol consumption and cigarette smoking on sperm DNA integrity

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Abstract

The purposes of the presents study were to investigate the impact of alcohol consumption and cigarette smoking on semen parameters and sperm DNA quality, as well as to determine whether tobacco smoking, or alcohol consumption causes more deterioration of sperm quality. Two hundred and eleven semen samples of men were included in this study. Four groups were studied: heavy smokers ($N = 48$), heavy drinkers ($N = 52$), non-smokers ($n = 70$), and non-drinkers ($n = 41$). Semen parameters were determined according to WHO guidelines, protamine deficiency assessed by chromomycin (CMA3) staining, and sperm DNA fragmentation (sDF) evaluated by TUNEL assay. Sperm parameters were significantly higher in non-smokers versus smokers and in non-drinkers versus drinkers ($p < 0.005$). However, protamine deficiency and sDF were significantly lower in non-smokers versus smokers and in non-drinkers versus drinkers ($p < 0.0001$). No significant difference in the semen analysis parameters was observed between heavy smokers and heavy drinkers (semen volume: 3.20 ± 1.43 vs. 2.81 ± 1.56 ml, semen count: 65.75 ± 31.32 vs. 53.51 ± 32.67 mill/ml, total motility: 24.27 ± 8.18 vs. 23.75 ± 1.75%, sperm vitality: 36.15 ± 18.57 vs. 34.62 ± 16.65%, functional integrity: 41.56 ± 18.57 vs. 45.96 ± 17.98% and the morphologically normal spermatozoa: 28.77 ± 11.82 vs. 27.06 ± 13.13%, respectively). However, protamine deficiency was significantly higher among drinkers than smokers (37.03 ± 9.75 vs. 33.27 ± 8.56%, $p = 0.020$). The sDF was also significantly higher among drinkers than smokers (22.37 ± 7.60 vs. 15.55 ± 3.33%, $p < 0.0001$). Thus, cigarette smoking, and heavy alcohol intake can deteriorate sperm quality. However, alcohol consumption deteriorates sperm maturity and damages DNA integrity at significantly higher rates than cigarette smoking.

KEYWORDS
alcohol consumption, cigarette smoking, male infertility, sperm DNA

1 | INTRODUCTION

Over the years, infertility has become a major global problem. Almost 48.5 million couples throughout the world are facing this issue (Inhorn & Patrizio, 2015; Mascarenhas et al., 2012). Infertility is described as a disease characterized by a failure to conceive after 1 year of regular, unprotected intercourse, and is used interchangeably with the term "subfertility" (Zegers-Hochschild et al., 2017).

Male-related factors contribute to 50% of infertile cases (Agarwal et al., 2015). However, 30% of male infertility is yet to be explained, and is therefore classified as idiopathic (Naz & Kamal, 2017).

More than 600,000 cycles of intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF) are performed each year in North America and Europe alone (Department of Health & Services Centers for Disease Control, 2018; EIM et al., 2017). Unfortunately, the success rate has been found to be very low. In 2015, it was reported that ~33.3% of live births in the United States resulted from assisted reproductive technology (ART) cycles (Department of Health & Services Centers for Disease Control, 2018).

IVF and ICSI have greatly helped subfertile couples conceive, but the success of these technologies depends on the semen parameters and sperm DNA quality (Ribas-Maynou et al., 2021). Several studies revealed a wide variation in the estimation of the occurrence of male infertility (from 5% to 35%), thus showing real differences between populations in terms of the following factors: quality of primary health care, environment, occupation, exposure to toxicants responsible for infertility, age, obesity, climate conditions, educational status, occasional use of or constant exposure to drugs, and genetic and epigenetic factors (Aitken, 2020).

The association of tobacco smoking with male infertility is still under debate. In fact, several studies suggest a strong correlation between smoking and altered semen parameters (NICE, 2013). It has been shown that a moderate exposure to heavy metals found in cigarettes, especially cadmium (Cd) and lead (Pb), affects the male reproductive and endocrine functions by decreasing human semen significantly, thus impairing male fertility (Pant et al., 2014; Sengupta et al., 2017).

Moreover, cigarette smoking can increase inflammatory reactions, resulting in increased levels of leukocytes in the testicles (Jorsaraei et al., 2008; Majo et al., 2001). Some other chemicals that are present in tobacco smoke and cause damage to the cells are tar, nicotine, CO, hydrocarbons, such as polycyclic aromatic hydrocarbons, some radioactive compounds, and toxic heavy metals (Halmenschlager et al., 2009). Fragmentation of the sperm DNA, axonemal damage, and decreased concentrations of sperm cells have also been observed among smokers (Amor et al., 2021; Hamad et al., 2014; Hammadeh et al., 2010).

In addition, a smoking habit in men has been shown to have an adverse effect on pregnancy outcomes among IVF patients (Cinar et al., 2014). An association between cigarette smoking and altered ICSI and IVF outcomes was also reported (Amor, Nyaz, et al., 2019).

In a previous study by Klonoff-Cohen et al. (2001), the number of retrieved oocytes decreased by almost 46% in smokers; the men were active smokers, and the women were passive smokers (Klonoff-Cohen et al., 2001). In addition, a decrease in live birth rates was noticed in 166 couples seeking pregnancy using ART (Fuentes et al., 2010).

Alcohol is also known as a dietary factor that affects fertility. However, the studies on couples undergoing ART or any of the infertility treatments remain controversial (Abadia et al., 2017; Ricci et al., 2017). Various studies showed the deleterious effects of alcohol (Eggert et al., 2004; Tolstrup et al., 2003), whereas others demonstrated no association to fertility (Hatch et al., 2012; Mikkelsen et al., 2016). A study of 8344 healthy male subjects reported that consumption of alcohol in moderate quantities correlated with higher testosterone levels, but semen parameters did not show any change (Jensen, Gottschau, et al., 2014). Therefore, the limits beyond which alcohol starts affecting male reproductive functions are still unknown.

Together, drinking alcohol and smoking may be responsible for causing infertility in men and women (Joo et al., 2012; Martini et al., 2004). However, no specific evidence of the harmful effects of smoking and alcohol consumption on sperm and fertility outcomes has been observed (Gaur et al., 2010; Petraglia et al., 2013).

The contradictions in studies on the consequences of heavy tobacco smoking and alcohol consumption on male fertility encouraged us to explore the correlations between smoking, alcohol intake, and sperm quality, including its DNA, in male patients experiencing fertility problems, and to determine which habit causes more damage to sperm quality.

2 | MATERIALS AND METHODS

2.1 | Study population

Study was performed at the Laboratory facility of Reproductive Medicine, Department of Obstetrics and Gynaecology, at the Saarland University Hospital, Germany. Semen samples were collected from Prince Rashid Ben Al Hassan Military Hospital, located in Irbid city of Jordan. The Medical Services of Human Research Committee approved this study (8/2018) and all participants were given a written consent before they were getting included in this study.

In this study, 211 men with primary infertility were recruited. After excluding female factors causing infertility, only young women (<40 years old) with normal ovulation, menstrual cycles, and uterine cavity were included in this study.

Moreover, the criteria that were used to include the male patients in this study were as follow, patients who had no varicocele of any grade, cryptorchidism, hormonal disorder, chemotherapy treatment and medication, inherited and acquired genetic anomalies such as Klinefelter's syndrome patients or microdeletions at Y-chromosome, and drug abuse. Each patient included in the study had a physical evaluation and examination.

Patients were classified according to the smoking duration and number of cigarettes to heavy-smokers group ($n = 48$) (1 pack/day for 10 years minimum or 2 pack/ day for 5 years minimum), and non-smokers ($n = 70$) who did not smoke.

The measurement used for alcohol intake and consumption was estimated by the units of alcohol consumed: 1 alcohol consumption unit was taken and considered as follow: 100 millilitre (ml) wine, and beer one unit equals to 200 ml, 30 ml of whisky or either vodka.

Accordingly, patients were divided to Heavy alcohol consumers ($n = 52$) which include patients who drinks >7 units/week and non-drinkers ($n = 41$).

2.2 | Collection of the sample's procedure

All Semen samples from the patients were collected in a sterile container, through the process of masturbation. Before analysis, sample was kept 30 min, before the analysis, on the heating stage or incubated at 37°C. Then, macroscopic and microscopic examination were performed according to laboratory manual guidance of the world health organization (WHO) in 2010 (World Health Organization, 2010).

To examine sperm morphology, protamine deficiency and sperm DNA fragmentation, 4 smears were prepared using 20 μl of ejaculate. After the semen has liquefied (>30 min), the specimen was good mixed before pipetting the aliquot onto the slide and then mixed again before preparing the next slide. The Slides air dried, fixed and kept until staining.

Papanicolaou staining was performed to analyse and evaluate the sperm total morphology according to World Health Organization (2010) strict criteria. After Slides preparation, 200 spermatozoa were evaluated for head, midpiece, and flagellum defects using a bright-field microscope (Zeiss) 100× objective lens.

2.3 | Examination of sperm DNA

For the evaluation of the protamine deficiency, chromomycin A3 (CMA3) staining was used (Manicardi et al., 1995), and for the evaluation of sperm DNA fragmentation, the terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) test was used (Borini et al., 2006).

For CMA3 Staining, the slides were first fixed for 1 h with acetic acid-methanol (1:3). Then, to each slide, CMA3 stain solution was added and kept in the dark for 30 min at room temperature (RT). Slides were washed with phosphate buffer saline (PBS) and mounted with cover slides. Then, they were left during the night at 4°C.

For TUNEL assay, in situ cell death detection kit fluorescein (Roche Diagnostics GmbH, Germany) was used.

After staining, fluorescence Microscope (Olympus) was used to evaluate at least 500 spermatozoa for each slide (Amor, Shelko, et al., 2019).

For CMA3 stain, spermatozoa were classified as follow: CMA 3 positive for bright green spermatozoon representing spermatozoa with low protamination and CMA3 negative for dull green spermatozoa representing spermatozoa with normal histones-protamines transition.

Spermatozoa were classified after TUNEL test as follow: TUNEL-positive for green stained spermatozoa and TUNEL-negative for blue stained spermatozoa.

2.4 | Statistical analysis

IBM SPSS (Windows software package version 24.0 SPSS Inc.) was used. The samples were not normally distributed according to skewness test, Kurtosis test, Shapiro test, and Z-value. Therefore, Mann–Whitney *U*-test was used for the comparison of the quantitative parameters between the heavy-smokers and heavy-drinkers group and Spearman test for the analysis of the correlation between the different parameters. The p-value <0.05 was considered as statistically significant and $p < 0.01$ as highly significant.

3 | RESULTS

3.1 | Tobacco smoking and its correlation to sperm parameters and DNA quality

Semen parameters were determined and compared between groups of heavy smokers and non-smokers. The semen parameters were significantly higher in non-smokers than in smokers. The total motility (PR + NP %) in smokers was 24.27 ± 31.32% versus 37.86 ± 14.00% ($p < 0.0001$). The same was observed for sperm vitality (36.2 ± 18.56% vs. 42.9 ± 17.74%; $p = 0.035$), membrane integrity (41.6 ± 18.6% vs. 56.2 ± 18.6%; $p = 0.0001$), and morphologically normal sperm (28.8 ± 11.8% vs. 44.13.85%; $p < 0.0001$) (Table 1).

CMA3+ and sDF were significantly higher in the group of heavy smokers than in the group of non-smokers (33.27 ± 8.56 vs. 26.00 ± 8.28; 15.55 ± 3.33 vs. 8.91 ± 4.14 respectively, $p < 0.0001$) (Table 2).

3.2 | Alcohol consumption and its correlation to sperm parameters and DNA quality

A comparison between the heavy drinkers group ($n = 52$) and the non-drinkers group ($n = 41$) showed that the mean sperm count (53.5 ± 32.7 mill/ml), total motility (23.8 ± 10.8%), sperm vitality (34.6 ± 16.6%), membrane functional integrity (45.9 ± 17.9%), and morphologically normal spermatozoa (27.0 ± 13.13%) were significantly lower in drinkers ($p < 0.001$) than in non-drinkers (73.2 ± 30.5 mill/ml, 35.0 ± 19.2%, 45.2 ± 18.4%, 58.5 ± 18.3%, and 35.9 ± 11.9%, $p < 0.001$, respectively) (Table 3).

Protamine deficiency was significantly lower in the non-drinkers group than in the heavy drinkers group (24.76 ± 7.435 vs. 37.03 ± 9.753, $p < 0.0001$). sDF was also shown to be significantly higher in heavy drinkers than in non-drinkers (22.37 ± 7.60 vs. 11.98 ± 5.17 $p < 0.0001$) (Table 4).

3.3 | Comparison between cigarette smoking and alcohol consumption

As illustrated in Table 5, the semen analysis parameters showed no significant differences between the smoker and drinker groups.

TABLE 1 Comparison of the semen parameters between heavy smokers and non-smokers

Parameters	Heavy smokers ($N = 48$) ($M \pm SD$)	Non-smokers ($N = 70$) ($M \pm SD$)	Significance (p-value)
Age (year)	33.12 ± 8.21	31.89 ± 7.84	0.454
Semen volume (ml)	3.20 ± 1.43	3.79 ± 1.67	0.037*
pH	8.64 ± 0.37	8.65 ± 0.37	0.913
Sperm count (mill/ml)	65.75 ± 31.32	67.18 ± 31.38	0.726
Total motility (PR + NP) (%)	24.27 ± 8.18	37.86 ± 14.00	<0.0001**
Sperm vitality (%)	36.15 ± 18.57	42.86 ± 17.74	0.035*
Functional integrity (%)	41.56 ± 18.57	56.21 ± 18.54	<0.0001**
Normal sperm morphology (%)	28.77 ± 11.82	44.13 ± 13.85	<0.0001**

Abbreviations: *M*, mean; *N*, number; SD, standard deviation.
*p-value is statistically significant at the 0.05 level.
**p-value is statistically high significant at the 0.01 level.

TABLE 2 Comparison of the grade of protamine deficiency in sperm DNA assessed by Chromomycine-A3 (CMA3+) and sperm DNA fragmentation assessed by TUNEL-assay (sDF) between heavy smokers and non-smokers

Parameters	Heavy smokers ($N = 48$) ($M \pm SD$)	Non-smokers ($N = 70$) ($M \pm SD$)	Significance (p-value)
Protamine deficiency (CMA3+) (%)	33.27 ± 8.561	26.00 ± 8.283	<0.0001**
Sperm DNA fragmentation sDF (%)	15.55 ± 3.334	8.91 ± 4.147	<0.0001**

Abbreviations: *M*, mean; *N*, number; SD, standard deviation.
**p-value is statistically high significant at the 0.01 level.

TABLE 3 Comparison of the semen parameters between heavy-drinkers and non-drinkers

Parameters	Heavy drinkers ($N = 52$) ($M \pm SD$)	Non-drinkers ($N = 41$) ($M \pm SD$)	Significance (p-value)
Age (year)	35.19 ± 7.055	36.44 ± 5.840	0.441
Semen volume (ml)	2.817 ± 1.5688	3.171 ± 1.3828	0.118
pH	8.685 ± 0.3770	8.620 ± 0.4094	0.369
Sperm count (mill/ml)	53.519 ± 32.6728	73.244 ± 30.5219	0.002**
Total motility (PR + NP) (%)	23.75 ± 10.750	35.00 ± 19.170	0.001**
Sperm vitality (%)	34.62 ± 16.652	45.24 ± 18.471	0.009**
Functional integrity (%)	45.96 ± 17.988	58.54 ± 18.345	0.001**
Normal sperm morphology (%)	27.06 ± 13.136	35.95 ± 11.969	0.001**

Abbreviations: *M*, mean; *N*, number; SD, standard deviation.
**p-value is statistically high significant at the 0.01 level.

TABLE 4 Comparison of the grade of protamine deficiency in sperm DNA assessed by Chromomycine-A3 (CMA3+) and sperm DNA fragmentation assessed using TUNEL-assay (sDF) between drinkers and non-drinkers

Parameters	Heavy drinkers ($N = 52$) ($M \pm SD$)	Non-drinkers ($N = 41$) ($M \pm SD$)	Significance (p-value)
Protamine deficiency (CMA3+) (%)	37.03 ± 9.753	24.76 ± 7.435	<0.0001**
Sperm DNA fragmentation sDF (%)	22.37 ± 7.602	11.98 ± 5.172	<0.0001**

Abbreviations: *M*, mean; *N*, number; SD, standard deviation.
**p-value is statistically high significant at the 0.01 level.

However, the protamine deficiency was significantly greater among drinkers than smokers (37.03 ± 9.75 vs. 33.27 ± 8.56, $p = 0.02$) (Table 6). The same was true for sDF, which was significantly higher among drinkers than smokers (22.37 ± 7.60 vs. 15.55 ± 3.33, $p < 0.0001$) (Table 6).

By studying the correlation between protamine deficiency and the standard sperm parameters, we found that CMA3+ had a high negative correlation with the sperm count ($r = -0.359$, $p = 0.009$), total motility ($r = -0.442$, $p = 0.001$), sperm vitality ($r = -0.347$, $p = 0.012$) and the morphologically normal sperm ($r = -0.382$,

TABLE 5 Comparison of the semen analysis parameters between heavy smokers and heavy drinkers

Parameters	Heavy smokers ($N = 48$) ($M \pm SD$)	Heavy drinkers ($N = 52$) ($M \pm SD$)	Significance (p-value)
Age (year)	33.12 ± 8.21	35.19 ± 7.05	0.127
Semen volume (ml)	3.20 ± 1.43	2.81 ± 1.56	0.073
pH	8.64 ± 0.37	8.685 ± 0.37	0.479
Sperm count (mill/ml)	65.75 ± 31.32	53.51 ± 32.67	0.056
Total motility (PR + NP) (%)	24.27 ± 8.18	23.75 ± 1.750	0.470
Sperm Vitality (%)	36.15 ± 18.57	34.62 ± 16.65	0.835
Functional integrity (%)	41.56 ± 18.57	45.96 ± 17.98	0.127
Normal sperm morphology (%)	28.77 ± 11.82	27.06 ± 13.13	0.332

Abbreviations: *M*, mean; *N*, number; SD, standard deviation.

TABLE 6 Comparison of protamine deficiency (CMA3 +) and sperm DNA fragmentation (sDF) between heavy smokers' group and heavy drinkers' group

Parameters	Heavy smokers ($N = 48$) ($M \pm SD$)	Heavy drinkers ($N = 52$) ($M \pm SD$)	Significance (p-value)
Protamine deficiency (CMA3+) (%)	33.27 ± 8.56	37.03 ± 9.75	0.020**
Sperm DNA fragmentation sDF (%)	15.55 ± 3.33	22.37 ± 7.60	<0.0001**

Abbreviations: *M*, mean; *N*, number; SD, standard deviation.
***p*-value is statistically high significant at the 0.01 level.

TABLE 7 Correlation between protamine deficiency (CMA3+)) and sperm DNA fragmentation (sDF) (%) with the investigated sperm parameters in heavy drinkers' group ($N = 52$)

		Protamine deficiency (CMA3+) (%)	Sperm DNA fragmentation (sDF) (%)
Age (year)	*r*	0.215	0.026
	p	0.126	0.857
Semen volume (ml)	*r*	−0.111	0.055
	p	0.433	0.701
pH	*r*	0.010	−0.048
	p	0.942	0.738
Sperm count (mill/ml)	*r*	−0.359[a]	−0.178
	p	0.009	0.206
Total motility (PR + NP) (%)	*r*	−0.442[a]	−0.058
	p	0.001	0.681
Sperm vitality (%)	*r*	−0.347[b]	−0.082
	p	0.012	0.564
Functional integrity (%)	*r*	−0.105	0.289[b]
	p	0.459	0.038
Normal sperm morphology (%)	*r*	−0.382[a]	−0.101
	p	0.005	0.477
Protamine deficiency (CMA3+) (%)	*r*	—	0.402[a]
	p	—	0.003

[a]Correlation is high significant at the 0.01 level,
[b]Correlation is significant at the 0.05 level.

$p = 0.005$) (Table 4). On the other hand, sDF correlated positively with sperm functional integrity ($r = 0.289$, $p = 0.038$) and with protamine deficiency ($r = 0.402$, $p = 0.003$) (Table 7).

In heavy smokers' group, CMA3+ correlated positively with age ($r = 0.377$, $p = 0.008$) and negatively with sperm count ($r = -0.289$, $p = 0.046$). In contrast to the heavy drinkers' group, no correlation

TABLE 8 Correlation between the investigated sperm parameters, CMA3+ and sDF in heavy smokers' group ($N = 48$)

		Protamine deficiency (CMA3+) (%)	Sperm DNA fragmentation (sDF) (%)
Age (year)	*r*	0.377[a]	0.117
	p	0.008	0.427
Semen volume (ml)	*r*	0.089	−0.158
	p	0.549	0.284
pH	*r*	−0.007	−0.050
	p	0.963	0.738
Sperm count (mill/ml)	*r*	−0.258	0.113
	p	0.076	0.443
Total motility (PR + NP) (%)	*r*	0.031	0.209
	p	0.835	0.154
Sperm vitality (%)	*r*	−0.289[b]	−0.044
	p	0.046	0.767
Functional integrity (%)	*r*	−0.259	−0.128
	p	0.075	0.386
Normal sperm morphology (%)	*r*	−0.090	−0.069
	p	0.543	0.643
Protamine deficiency (CMA3+) (%)	*r*	—	0.099
	p	—	0.503

[a]Correlation is significant at the 0.01 level,
[b]Correlation is significant at the 0.05 level.

was observed between sDF and any of the studied parameters, including CMA3+ (Table 8).

4 | DISCUSSION

In the last decade specifically, infertility has become a global health problem not only for aged couples, but also for couples in their reproductive years (Fang et al., 2018). Different studies have shown that the number of couples with secondary infertility is increasing compared to that of couples with primary infertility diagnoses (Fang et al., 2018). Male infertility factors are subdivided into extrinsic ones, including environmental and lifestyle factors, and intrinsic ones, like congenital disorders (Wang et al., 2020).

In fact, different studies have shown that semen parameters may be affected by various lifestyles, advancements in technology, environmental pollution (Boeri et al., 2019), alcohol intake, smoking (Amor et al., 2021; Hammadeh et al., 2010; Jensen, Gottschau, et al., 2014), and psychological stress (Barazani et al., 2014; Rakhit et al., 2013; Sullivan & Pfefferbaum, 2014). Obesity and dietary factors are also keys and important factors, as reported earlier (Afeiche et al., 2013; Ying Li et al., 2011; Mendiola et al., 2009).

Therefore, in the first part of the present investigation, we focused on how smoking adversely affects sperm quality (volume, density, functional or membrane integrity, DNA maturation and DNA fragmentation) in men using assisted reproduction technology. The results indicated that the semen parameters were significantly higher in non-smokers than in smokers. Also, semen volume and sperm vitality were significantly higher in the group of non-smokers in comparison to heavy smokers ($p = 0.037$ and 0.035, respectively). The same was noticed for the total motility, the mean percentage of morphologically normal spermatozoa, and membrane integrity ($p < 0.0001$) (Table 1). However, protamine deficiency (CMA3) and DNA fragmentation (TUNEL) were significantly higher in smokers than in non-smokers ($p < 0.0001$) (Table 2).

These findings are in agreement with previous reports from our institutions that had larger sample sizes (Amor et al., 2021; Hammadeh et al., 2010).

It is worth noting that sperm DNA can be also damaged by apoptosis (Asadi et al., 2021), DNA strand breakage, and any defect during the sperm maturation process (Sakkas & Alvarez, 2010; Cho et al., 2017).

Nevertheless, these negative effect of smoking on spermatozoa and the damage to the DNA may be due to excessive reactive oxygen species (ROS) production (Saleh et al., 2003).

ROS and nitrogen production affects the whole process of spermatogenesis (Agarwal & Allamaneni, 2004; Doshi et al., 2012). Exposure to high quantities and levels of ROS can generate various modified forms of DNA bases, causing mutagenicity as well as carcinogenicity (Singh et al., 2011; Soultanakis et al., 2000).

It was demonstrated that smoking increases leukocyte levels in sperm by 48% compared to non-smokers (Saleh et al., 2002). Cigarette smoking also promotes ROS production, as reported earlier in several studies (Hammadeh et al., 2010; Kumar et al., 2015; La

Maestra et al., 2015; Perrin et al., 2011). Smoking has deleterious effects on sperm motility (Harlev et al., 2015; Saleh et al., 2003), morphology (Mostafa, 2010), and sperm DNA (Cui et al., 2016; Opuwari & Henkel, 2016). Therefore, the DNA fragmentation index rises from 14.51% in non-smokers to 37.66% in smokers ($p < 0.001$) (Elshal et al., 2009). Besides, ROS production in smokers deteriorates chromatin condensation and changes the protamine 1 and protamine 2 rations of spermatozoa (Hammadeh et al., 2008).

Moreover, an excessive production of ROS leads to oxidative stress, which in turn affects not only sperm nuclear DNA, but also sperm mitochondrial respiratory activity (Piomboni et al., 2012) and the endocrine function, resulting in several pathologies of the male reproductive system. Thus, it may be leading to male infertility (Cho et al., 2017; Darbandi et al., 2018).

In addition, several studies have reported the consequences of smoking on sperm quality and DNA integrity. The spermatozoa of smokers have higher levels of fragmentation in male subjects in comparison to those of non-smokers (Amor et al., 2021; Aydin et al., 2013; Elshal et al., 2009; Hammadeh et al., 2010). These negative effect of smoking on spermatozoa and the damage to the DNA may be due to excessive ROS production (Saleh et al., 2003) and decreases the antioxidant levels in seminal plasma (Pasqualotto et al., 2008). In addition, Taken et al. (2016) and Ramgir and Abilash (2019) noted that nitrous oxide affected sperm motility (Ramgir & Abilash, 2019; Taken et al., 2016). Decreased levels of GSH in spermatozoa also resulted in the loss of sperm cell integrity (Bhardwaj et al., 2000).

Taken together, the results of the present study suggest a negative biological effect of cigarette smoking on sperm parameters, protamination, and DNA fragmentation.

Another lifestyle factor that causes an increase in ROS generation leading to infertility is heavy alcohol consumption (Das & Vasudevan, 2007; Jensen, Gottschau, et al., 2014; Jensen, Swan, et al., 2014). In addition, alcohol intake and smoking affect and repress Nrf2 expression, which plays a major role in protection against oxidative stress (Elsamanoudy et al., 2017).

The impact of alcohol drinking on sperm, with some parameters remains controversial, with some studies conforming a significant negative impact and others reporting no effect.

In the second part of our study, by comparing the parameters between heavy drinkers ($N = 52$) and non-drinkers ($N = 41$), we found that the sperm count, total motility, sperm vitality, functional integrity, and normal morphology were significantly higher in the non-drinkers' group ($p = 0.002$, 0.001, 0.009, 0.001 and 0.001 respectively) (Table 3). The chromatin deficiency (CMA3+) and sDF levels of the drinker group were significantly higher than those of the non-drinkers group were ($p < 0.0001$) (Table 4).

These finding were also supported by the finding of Hansen et al. (2012), who reported that regular alcohol drinking decreased semen volume and concentration of sperm. In addition, one of the meta-analyses with 57 total studies ($n = 29{,}914$ participants) found a positive association between alcohol intake, semen volume, and the morphology and motility of spermatozoa (Li et al., 2011).

As well, a meta-analysis involving 15 different cross-sectional studies reported that regular alcohol consumption greatly affects sperm parameters (Ricci et al., 2017).

Recently, Boeri et al. (2019) also reported a negative correlation between alcohol drinking and sperm parameters.

A negative association was showed between daily alcohol intake and polycyclic aromatic hydrocarbon-DNA (PAH-DNA), an indicator of sperm genotoxicity, in the spermatozoa of infertile patients (Gaspari et al., 2003).

In addition, PAH-DNA adducts correlated negatively with the mean percentage of spermatozoa with normal morphology and with the abnormalities of the neck of the spermatozoa (Gaspari et al., 2003).

Muthusami and Chinnaswamy (2005) showed that FSH, LH, E2, and testosterone levels decreased.

Furthermore, it has been shown that patients who drink alcohol are more likely to have an increase in live birth risk by 2.28 to 8.32 times (odds ratio: 55.49–45.64). That suggests modifying drinking habits may increase ART outcomes (Klonoff-Cohen, 2005).

Alcohol consumption gives rise to the production of metabolites like acetyl and methyl radicals, which are responsible for ROS generation. Regular alcohol consumption triggers lipid peroxidation and consequently increases ROS production, protein degradation, and DNA fragmentation (Wu & Cederbaum, 2003; Zorn et al., 2003). It also lowers SOD antioxidant activity, along with GSH levels (Doshi et al., 2012).

Various studies have shown that alcohol increases oxidative stress (OS) and consequently causes infertility (Yuksel et al., 2005). However, Loft et al. (2003) demonstrated that the breakage of DNA was not related to the consumption of alcohol (Loft et al., 2003).

According to a study conducted by Gaur et al. (2010), alcohol intake decreases sperm count and concentration (asthenozoospermia) and causes damage to sperm morphology, especially in the sperm head. They concluded that the deterioration of sperm quality is proportional to the quantity of alcohol intake. In addition, moderate or heavy smoking affects sperm motility, which decreases the quality of the sperm (Gaur et al., 2010).

In the present study, sperm parameters were also compared between heavy smokers ($n = 48$) and heavy drinkers ($n = 52$) to determine whether smoking or alcohol consumption causes more deterioration to sperm quality (Table 5). It was found that age, semen volume, sperm count, total motility (PR + NP), sperm vitality, functional integrity, and percentage of morphologically normal spermatozoa were not significantly different.

The findings in this study showed that alcohol intake and tobacco smoking have similarly deleterious effects on the sperm parameters. These results were confirmed by the previous finding of Gaur et al. (2010), who reported that alcohol intake decreases the sperm count and concentration (asthenozoospermia) and causes damage to sperm morphology, especially in the sperm head. Therefore, it appears that the quantity of alcohol intake is proportional with the deterioration of sperm quality. Moderate or heavy smoking also affects sperm motility, which decreases the quality of the sperm as well.

Various studies showed changes only in sperm morphology (Condorelli et al., 2015). However, others showed contradictory results (Hansen et al., 2012; Povey et al., 2012). Furthermore, in this study, the protamine deficiency level (CMA3+) in the heavy drinkers' group was significantly higher ($p = 0.020$) than in the heavy smokers' group. DNA fragmentation level was also significantly higher in the drinkers' group ($p < 0.0001$) (Table 6).

In the alcohol drinker group, a negative correlation was shown between protamine deficiency (CMA3+) and sperm count, total motility, vitality, and morphologically normal spermatozoa ($p < 0.01$) (Table 7). Moreover, sDF correlated positively with functional integrity ($p < 0.038$) and protamine deficiency ($p < 0.003$).

These findings confirm previous several studies showing that alcohol and ethanol consumption negatively affecte sperm parameters, like sperm count, DNA integrity, and sperm maturation (protamination) (Jana et al., 2010; Sansone et al., 2018). In addition, they confirm other studies demonstrating a correlation between DNA strand breaks and abnormal protamination (Amor et al., 2021; Aoki et al., 2006; Carrell et al., 2007; Hammadeh et al., 2010; Martini et al., 2004).

The sDF in the smoker group showed no correlation to any of the semen parameters. However, CMA3+ correlated positively with the age of the patients ($p < 0.008$) and negatively with sperm vitality ($p = 0.046$) (Table 8).

These results were confirmed by previous studies' findings (Gaur et al., 2010; Yafei Li et al., 2009; Muthusami & Chinnaswamy, 2005). Various studies showed changes only in sperm morphology (Condorelli et al., 2015), while others showed contradictory results (Hansen et al., 2012; Povey et al., 2012).

Previous studies indicated that patients should adopt lifestyle modifications and quit smoking (Wright et al., 2014). They should also lose weight through different methods, like diet, education, and exercise (Reis & Dias, 2012), and decrease exposure to harmful toxins like phthalate (Sedha et al., 2015). It was also previously reported that heavy alcohol consumption caused an elevation of scrotal temperature and testis, which increased the risk of infertility (Koch et al., 2004). An increase of temperature in testicular and epididymis leads to the production of morphologically abnormal spermatozoa (Ahmad et al., 2012).

Sengupta et al. (2018) recommended a few lifestyle changes to improve fertility outcomes, including avoiding tobacco smoking, ethanol or alcohol consumption; avoiding the use of other illicit and recreational drugs; avoiding too much psychological stress; losing weight; and reducing caffeine intake (Sengupta et al., 2018).

One unique feature of this study is that it offered separate and combined analyses of the influence of smoking and alcohol intake for quite a good number of infertile patients attending ART therapy.

However, some limitations of the study must be considered. First, there was the relatively smaller number of patients due to the single-centre study design. Second, there was the absence of clinical trials. A final restriction of the present study is that data regarding cigarette smoking and alcohol intake was obtained from a questionnaire subject to measurement error.

5 | CONCLUSION

In conclusion, the findings of the present study suggest that, separately or together, tobacco smoking, and alcohol intake separately or together, negatively affect the sperm parameters, and sperm DNA integrity, but their impact on sperm parameters is still disputed.

More advanced studies at the molecular level, like DNA methylation and gene polymorphism, are necessary to elucidate the harmful effect of tobacco and alcohol on sperm structure and its DNA integrity quality.

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CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data are available upon request from the corresponding author.

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