Indian Journal of Experimental Biology
Vol. 61, April 2023, pp. 290-296
DOI: 10.56042/ijeb.v61i04.169

Impact of smoking and alcohol intake in semen profile among infertile men

Puja Devi Nongthombam[1] & Suttur S Malini[1,2]*

[1]Molecular reproductive and human genetics lab, Department of Studies in Zoology;
[2]Department of Studies in Genetics and Genomics, University of Mysore, Mysore-560006, Karnataka, India

Received 03 June 2022; *revised 02 March 2023*

In the modern stressful lifestyle, habits such as smoking and drinking alcohol are reported to increase the likelihood of fertility issues in adults who are of reproductive age. Moreover, there has been rapid rise in male infertility and 50% of the infertility cases are related to male partners. In this context, here we investigated whether smoking and drinking alcohol have an effect on the semen profile of infertile male patients in terms of seminal oxidative stress and male accessory gland function. A total of 327 men with infertility issue were grouped into four categories according to their smoking and drinking habits viz. smokers, moderate drinkers, heavy drinkers, and abstainers. The participants were investigated for clinical, semen, oxidative stress parameters, and male accessory gland function. The mean of sperms count with normal morphology and the level of NOS scavenging activity decreased in smokers compared to abstainers. Heavy drinkers were found to have the lowest normal morphological sperms in comparison to moderate drinkers and abstainers. Interestingly, moderate drinkers showed lower mean sperm concentration, progressively motile sperm, and NOS scavenging activity. The level of seminal citric acid and semen volume had a substantial positive association, according to Pearson's correlation analysis. The above findings suggest that smoking and excessive drinking lowers sperm with normal morphology, while moderate drinking reduces sperm concentration and progressively motile sperm. Therefore, it will be a better strategy for the infertile couples to modify few life style habits, smoking and drinking in particular, to improve their fertility.

Keywords: Antioxidants, Seminal plasma, Citric acid, Fructose, Oxidative stress

Infertility affects an estimated 60-80 million couples worldwide, with male factors accounting for half of the cases[1]. Over the last 35 years, there has been a gradual decline in male fertility[2]. Male infertility is a multifactorial disease in which unhealthy lifestyle choices, such as cigarette smoking and alcohol intake, may play a role[3]. Tobacco use is on the rise around the world, and tobacco smoking is responsible for more than 60% of noncommunicable diseases[4]. The World Health Organization (WHO) reported that in 2020 roughly 22.3% of global population used tobacco, 36.7% of all men and 7.8% of the world's women[5]. According to the National Family Health Survey (NFHS-5) report 2019-21, roughly 38% and 19% of men above 15 years of age in India use tobacco and alcohol, respectively[6]. Cigarette smoke contains about 7000 chemicals, and it has been shown to have a negative impact on the male reproductive process, from spermatogenesis to fertilisation[7]. Moreover, studies have shown a decline in the quality of sperm among alcoholics, which is dose and duration dependent[8]. Smokers are more likely to be exposed to harmful substances, which increases the levels of oxidative stress in the seminal plasma and affects sperm function and poses a reproductive risk[9].

By interfering with the integrity of sperm DNA and plasma membrane, oxidative stress causes 30-80% of cases of male infertility[10]. Superoxide dismutase (SOD) and nitric oxide synthase (NOS), which have activity identified in seminal plasma, are antioxidant enzymes that help to defend against oxidative stress-induced sperm destruction[11,12]. Even after more than 30 years of research, there are still many conflicting results about the effects of smoking and alcohol intake on sperm quality[4]. Hence, in this study, we tried to determine if smoking and drinking had an impact on the clinical, semen, oxidative stress, and accessory gland function of infertile men.

Methods

Patients

After obtaining approval from the Institutional Human Ethical Committee of the University of Mysore (IHEC-UOM No.143/Ph.D/2016-2017, 23/03/17), the current study was considered. After all of the participants had fully comprehended the study's

*Correspondence:
E-Mail: sutturmalini@gmail.com

information, they were asked to sign a consent form after which samples were collected (blood and semen). A total of 327 participants with primary fertility were recruited for the study from numerous infertility hospitals or IVF clinics in and around Mysore, Karnataka, South India, between June 2017 and February 2018. Infertile men between the ages of 25 and 50 were included, as long as they had no history of medical disorders such as cryptorchism, tubule deficiency, erectile dysfunction, genetic and chromosomal abnormalities, and so on. Data on participant's age, age of onset of puberty, frequency of intercourse, cigarette smoking, alcohol use, medical or surgical history, diet etc. were gathered using a questionnaire. In order to determine the infertile phenotype, semen samples were collected. According to their drinking and smoking behaviours, the participants were grouped into four groups: smokers and abstainers (no smoking or drinking); moderate drinkers, heavy drinkers, and abstainers; subjects who smoked and drank; and abstainer.

Semen analysis

Semen examination was done within 30 min after its collection to avoid the effect of room temperature on semen quality. A minimum of 2-4 days of sexual abstinence was maintained before semen collection. Semen analysis was performed according to the WHO laboratory manual for the examination and processing of human semen, 5th edition[13]. Semen parameters like semen volume, liquefaction time, pH, sperm concentration, total sperm count, sperm motility, and sperm morphology were analyzed. Azoospermic patients (n=64) were excluded for examination of sperm concentration, total sperm count, progressively motile sperm, and sperm with normal morphology. A graduated wide mouth semen jar was used for measuring semen volume. To check the pH of the semen, a drop of semen was spread on the pH paper and the colour was compared with the calibrated pH strip. Sperm concentration, total sperm count, and sperm motility were analyzed using the Makler counting chamber and the RAPID PAP^{TM} A3 Minutes Papanicolaou stain kit (BIO LAB) for examination of sperm morphology and observed under the light microscope.

Evaluation of scavenging activity of antioxidant enzymes in semen

The activities of Superoxide dismutase (SOD) and Nitric oxide synthase (NOS) were evaluated from seminal plasma using protocols developed by Kazari *et al.*[14] and Green *et al.*[15], respectively, to assess oxidative stress parameters.

Accessory gland function test

Following the protocols established by Karvonen & Malm[16] and Polakoski & Zaneveld[17], the amounts of fructose and citric acid were determined. These tests are used to determine how well the seminal vesicles and prostate gland, which are male accessory reproductive glands, are functioning.

Statistical analysis

IBM SPSS Statistics 20 was used to conduct the statistical analysis. Quantitative or numerical variables with non-normal distributions, such as semen parameters (semen volume, sperm concentration, total count, progressively motile sperm, and normal morphology), were described in terms of mean, standard deviation, median, and interquartile range, while qualitative or categorical variables, such as infertile groups, were expressed as frequency and percentage. The subjects' age, puberty age, liquefaction time, pH, fructose and citric acid levels, and SOD and NOS scavenging percentages were all represented in mean and standard deviation. The chi square test, independent samples t-test, and one-way analysis of variance (ANOVA) were used to compare two categorical, numerical variables, as well as categorical and numerical variables. A p-value of less than 0.05 was considered statistically significant.

Results

Infertile groups

The average age of the infertile men considered in the study was 35.10±5.96 years. After examining the semen samples, the infertility conditions were verified as idiopathic infertility (normal semen profile), azoospermia (no sperm in semen), oligospermia (sperm count <15 million/mL), oligoasthenospermia (oligospermia+asthenospermia), asthenospermia (sperm motility i.e. Progressively Motile + Non-progressively Motile <40%), necrospermia (all dead sperms in semen) and oligoasthenoteratospermia (oligospermia+asthenospermia+teratospermia). 263 cases were considered for evaluation of semen parameters like sperm concentration, total sperm count, progressively motile sperm, and sperm with normal morphology, excluding 64 azoospermia cases.

Comparison of seminal characteristics between smokers and abstainers

The number of sperm with normal morphology was substantially higher in abstainers (63.31±15.40) than in smokers (50.91 ± 16.12) as shown in Table 1. The mean concentration of seminal citric acid was greater

Table 1 — Impact of smoking on clinical characteristics, semen parameters, accessory gland function and oxidative stress parameters

Clinical characteristics	Total no.=185		p value
	Smokers (n=44)	Abstainer n=141)	
Age in years (mean±SD)	36.18±7.431	35.43±5.607	0.477
Age of onset of puberty (mean±SD)	16.89±2.08	17.11±1.93	0.519
Idiopathic (n,%)	23 (52.27%)	77(54.60%)	0.786
Azoospermia (n,%)	9 (20.45%)	22 (15.60%)	0.452
Oligospermia (n,%)	0(0%)	6 (4.26%)	0.164
Oligoasthenospermia (n,%)	8 (18.18%)	12 (8.5%)	0.071
Asthenospermia (n,%)	2 (4.55%)	20 (14.18%)	0.085
Necrospermia (n,%)	2 (4.55%)	4 (2.84%)	0.576
Oligo-astheno-teratospermia (n,%)	0 (0%)	0 (0%)	NA
Semen parameters			
Semen vol. (per ejaculate in mL) (mean±SD)	1.64±0.85	1.93±1.15	
Median (Q1, Q3)	1.5 (1,2)	1.5 (1,3)	0.131
Liquefaction time in min (mean±SD)	32.86±10.60	39.14±23.10	0.083
pH (mean±SD)	7.79±0.39	7.82±0.38	0.596
Sperm conc. (million/mL) mean±SD,	47.6±32.15	49.50±35.33	0.775
Median (Q1, Q3)	50 (30,68)	40 (15,85)	
Total sperm count (sperm conc.*ejaculate vol.) (mean±SD),	57.29±80.01	98.17±108.5	0.341
Median (Q1, Q3)	61.2 (28,105)	67.5 (25,122.5)	
Progressively motile sperms (grade A sperms) (mean±SD),	21.26±22.28	34.45±20.33	0.425
Median (Q1, Q3)	10 (0,40)	20 (5,40)	
Sperms with normal morphology (mean±SD),	50.91±16.12	63.31±15.40	<0.001*
Median (Q1, Q3)	49 (39,65)	63 (53,75)	
Accessory gland parameter			
Fructose in µmol/ejaculate (mean±SD)	56.25±63.39	46.38±47.97	0.273
Citric acid in mg/ejaculate (mean±SD)	85.26±84.86	51.16±60.65	0.004*
Oxidative stress parameter			
SOD Scavenging% (mean±SD)	67.23±21.74	71.31±19.53	0.242
NOS Scavenging% (mean±SD)	59.84±29.46	69.98±17.48	0.006*

[n=frequency; %= percentage; Interquartile range Q1= 25th percentiles; Q3= 75th percentiles. Azoospermic patients were excluded from the analysis of semen parameters like sperm concentration, total sperm count, progressively motile sperms, and sperm with normal morphology. A total of 154 subjects (35 smokers and 119 abstainers) were considered for analyzing above sperm parameters after excluding 31 azoospermic patients]

in smokers (85.26±84.86 mg/ejaculate) compared to abstainers (51.16±60.65 mg/ejaculate). Also, compared to abstainers (69.98±17.48%), smokers (59.84±29.46%) showed reduced NOS scavenging levels. Smokers and non-smokers did not differ significantly in terms of mean age, puberty age, infertile groups, semen volume, liquefaction time, pH, sperm concentration, total sperm count, gradually motile sperm, fructose level, and SOD scavenging percentage. Though statistically insignificant, smokers had higher rates of azoospermia, oligospermia, and necrospermia.

Comparison of seminal characteristics between moderate and heavy drinkers with abstainers

Table 2 shows a relation between the early onset of puberty and alcohol consumption. The mean sperm concentration among moderate drinkers (34.59±24.92 million/mL) was lowest when compared to heavy drinkers (37.40±21.77 million/mL) and abstainers (49.50±35.33 million/mL). Mean progressively motile sperm decreased from heavy drinkers (30±18.75) to abstainers (24.45±20.33) and moderate drinkers (15.31±15.29). The average number of sperm with normal morphology was significantly reduced in heavy drinkers (45.14±14.02) followed by moderate drinkers (61.25±17.97) and then abstainers (63.31±15.40). In addition, low NOS scavenging activity was observed among moderate drinkers (58.68±24.59) compared to heavy drinkers (67.95±21.12) and abstainers (69.98±17.48). The other variables did not differ between the groups statistically (P >0.005). Heavy drinkers had the highest rates of azo-ospermia, while moderate drinkers had the highest rates of oligoasthenospermia and asthenospermia.

Comparison of seminal characteristics between smokers and drinkers with abstainers

Comparing participants who smoked and drank simultaneously to abstainers, none of the measures were statistically significant. Also, as demonstrated in Table 3, simultaneous drinkers and smokers had decreased mean morphologically normal sperm and NOS scavenging activity, but this was statistically insignificant.

Correlation analysis

Pearson correlation analysis showed that citric acid had a positive correlation with semen volume (r=0.435) and total sperm count (r=0.361) as shown in the Table 4. Because the remaining r values were <0.29 and almost zero, it may be assumed that there is a weak or no correlation between the parameters.

Discussion

Male infertility is mostly caused on by a decline in sperm and sperm quality, which has been directly or indirectly linked to modern lifestyle choices including smoking and alcohol consumption. To our knowledge,

Table 2 — Impact of alcohol consumption in clinical characteristics, semen parameters, accessory gland function and oxidative stress parameters

Clinical characteristics	Total no. =218			p value
	Drinker (n=77)		Abstainer (n=141)	
	Moderate (n=37)	Heavy (n=40)		
Age in years (mean±SD)	33.97±4.17	34.65±6.38	35.43±5.607	0.323
Age of onset of puberty (mean±SD)	16.68±2.04	16.05±1.30	17.11±1.93	0.006*
Idiopathic (n,%)	17 (45.95%)	22 (55%)	77 (54.61%)	0.623
Azoospermia (n,%)	5 (13.51%)	12 (30%)	22 (15.60%)	0.83
Oligospermia (n,%)	0 (0%)	0 (0%)	6 (4.26%)	0.186
Oligoasthenospermia (n,%)	6 (16.21%)	4 (10%)	12 (8.5%)	0.383
Asthenospermia (n,%)	8 (21.62%)	2 (5%)	20 (14.18%)	0.104
Necrospermia (n,%)	0 (0%)	0 (0%)	4 (2.84%)	0.329
Oligo-astheno-teratospermia (n,%)	1 (2.7%)	0 (0%)	0 (0%)	0.086
Semen parameters				
Semen volume (per ejaculate in mL) (mean±SD),	1.87± 1.27	2.12± 1.7	1.93± 1.15	0.577
Median (Q1, Q3)	1.5 (1,2.5)	2 (1.5,2.5)	1.5 (1,3)	
Liquefaction time in min (mean±SD)	37.97± 16.30	32± 8.07	39.14± 23.10	0.141
pH (mean±SD)	7.83± 0.36	7.75± 0.31	7.82± 0.38	0.499
Sperm conc. (milion/mL) mean±SD	34.59± 24.92	37.40± 21.77	49.50± 35.33	0.027*
Median (Q1, Q3)	25 (15,55)	39 (20,58.75)	40 (15,85)	
Total sperm count (sperm conc.*ejaculate vol.) (mean±SD),	76.89± 113.73	79.31± 51.19	98.17± 108.5	0.466
Median (Q1, Q3)	38.5 (22.5,71.5)	63.5 (30,123.75)	67.5 (25,122.5)	
Progressively motile sperms (grade A sperms) (mean±SD),	15.31± 15.29	30± 18.75	24.45± 20.33	0.011*
Median (Q1, Q3)	10 (5,20)	30 (15,40)	20 (5,40)	
Sperms with normal morphology (mean±SD),	61.25± 17.97	45.14± 14.028	63.31± 15.40	<0.001*
Median (Q1, Q3)	59 (50.50,76)	45 (35.75,54)	63 (53,75)	
Accessory gland parameter				
Fructose in µmol/ejaculate (mean±SD)	49.76± 46.34	57.89± 52.75	46.38± 47.97	0.417
Citric acid in mg/ejaculate (mean±SD)	59.21± 13.25	48.39± 40.56	51.16± 60.65	0.798
Oxidative stress parameter				
SOD (Scavenging%)	62.16±21.93	71.32±23.58	71.31±19.53	0.052
NOS (Scavenging%)	58.68±24.59	67.95±21.12	69.98±17.48	0.008*

[A total of 143 subjects (46 moderate drinker, 15 heavy drinker and 82 abstainers) were considered for analyzing above sperm parameters after excluding 82 azoospermic patients]

Table 3 — Impact of smoking and alcohol consumption in clinical characteristics, semen parameters, accessory gland function and oxidative stress parameters

Clinical characteristics	Total no. =206		p value
	Smoking +Alcohol (n=65)	Abstainer (n=141)	
Age in years (mean±SD)	34.58±6.21	35.43±5.607	0.331
Age of onset of puberty (mean±SD)	16.60±2.33	17.11±1.93	0.104
Idiopathic (n,%)	35 (53.85%)	77 (54.61%)	0.919
Azoospermia (n,%)	16 (24.61%)	22 (15.60%)	0.121
Oligospermia (n,%)	4 (6.15%)	6 (4.26%)	0.556
Oligoasthenospermia (n,%)	4 (6.15%)	12 (8.5%)	0.557
Asthenospermia (n,%)	6 (9.23%)	20 (14.18%)	0.320
Necrospermia (n,%)	0 (0%)	4 (2.84%)	0.170
Oligo-astheno-teratospermia (n,%)	0 (0%)	0 (0%)	NA
Semen parameters			
Semen volume (per ejaculate in mL) (mean±SD),	1.84±1.26	1.93±1.15	0.608
Median (Q1, Q3)	1.5 (1,2.5)	1.5 (1,3)	
Liquefaction time in min Mean±SD	39.92± 26.67	39.14± 23.10	0.832
Ph (mean±SD)	7.83±0.37	7.82±0.38	0.830
Sperm concentration (count/ml) Mean±SD,	50.69± 34.66,	49.50± 35.33	0.843
Median (Q1, Q3)	45 (20,80)	40 (15,85)	
Total sperm count (sperm conc.*ejaculate vol.) (mean±SD),	106.42± 123.37 50	98.17± 108.5 67.5	0.667
Median (Q1, Q3)	(22.5,145)	(25,122.5)	
Progressively motile sperms (grade A sperms) (mean±SD),	25.59± 17.32	24.45± 20.33	0.732
Median (Q1, Q3)	30 (10,40)	20 (5,40)	
Sperms with normal morphology (mean±SD),	56.39± 14.48	63.31± 15.40	0.08
Median (Q1, Q3)	57 (46,67)	63 (53,75)	
Accessory gland parameter			
Fructose in µmol/ejaculate (mean±SD)	47.83± 50.52	46.38± 47.97	0.118
Citric acid in mg/ejaculate (mean±SD)	40.38± 40.05	51.16± 60.65	0.737
Oxidative stress parameter			
Sod (scavenging%)	70.67±23.021	71.31±19.53	0.839
Nos (scavenging%)	57.47±21.03	69.98±17.48	0.37

[A total of 168 subjects (49 smokers+drinkers and 119 abstainers) were considered for analyzing above sperm parameters after excluding 38 azoospermic patients]

no studies have examined if smoking and drinking affect the semen profile in terms of male accessory gland function and seminal oxidative stress. In our study, the semen profiles, accessory function status, and antioxidant activity in the semen of 327 men with primary infertility were assessed. The study's objective was to determine whether any of the variables under investigation were affected by

Table 4 — Pearson correlation analysis of semen parameters with SOD, NOS, Fructose, Citric acid. The Pearson correlation coefficient "r" values are shown in the table

Semen parameter	SOD	NOS	Fructose	Citric acid
Semen volume	0.099	0.094	–0.022	0.435
Liquefaction time	0.138	–0.118	–0.077	–0.119
pH	–0.239	–0.108	–0.091	–0.118
Sperm concentration	0.063	–0.017	0.025	0.084
Total sperm count	0.082	0.002	0.001	0.361
Progressively motile sperm	0.202	0.05	–0.002	0.023
Sperm with normal morphology	–0.012	–0.142	–0.093	–0.008

[Its value ranges from -1 to +1. Negative value shows negative correlation; positive value shows positive correlation, and zero means no correlation]

smoking and alcohol usage leading to male factor infertility.

Many studies have demonstrated that a number of intrinsic and extrinsic factors, including age, psychology, weight, diet, lifestyle including smoking and drinking, environment, etc. are responsible for variations from normal semen characteristics[18]. Reduced sperm count has also been associated with male reproductive system infections and inflammation, such as gonorrhoea and chlamydia[19]. Advanced age and excess weight in men have also been linked to a decline in sperm quality[20].

In the current study, almost 13% of the men recruited were smokers, 24% were drinkers, and 20% were both, which represents 57% of all infertile males with risk factors for lifestyle diseases. The above percentage may have been biased, nevertheless, due to self-reporting of these lifestyle choices in a questionnaire. All of the males selected for this study had well-balanced diets that were primarily made at home and were either vegetarians or non-vegetarians. Since they were trying to conceive even though they weren't successful in getting their partners pregnant, their frequency of sexual activity was quite regular. Patients with additional comorbidity cases, medical or surgical histories, STDs (Sexually Transmitted Infections), etc., were excluded. The main flaw in our study is that additional cofounders such hormone profile, BMI, and exposure to environmental or occupational toxins were not taken into account.

Studies have found that infertile smokers had poorer sperm concentration, motility, viability, morphology, and semen volume[7,8]. In spite of this assertion, some research have found no connection between smoking and low-quality sperm in infertile males[21,22]. Therefore, we evaluated the semen parameters, male accessory gland function and the seminal antioxidant level of smokers and abstainers. Smoking promotes oxidative stress, which decreases the level of antioxidants in the seminal plasma and overwhelms the antioxidant capacity, impairing sperm function[6]. Based on a particular research, smoking affects the seminal components and accessory glands by reducing seminal volume and increasing viscosity, which reduces sperm motility[23]. In line with the findings of Ramgir & Abhilash[24], smokers in our study had a significantly lower mean number of sperm with normal morphology than absolute abstainers. Also, smokers in our study demonstrated significantly lower scavenging activity for the antioxidant enzyme nitric oxide synthase (NOS) than abstainers.

Five years of research revealed that heavy alcohol use leads sperm to transition from teratospermia to azoospermia[25]. Chronic ethanol treatment in rats increased oxidative stress and reduced antioxidant activity[26]. Sperm continue to be damaged by the antioxidant activity residue in the seminal fluid, affecting fertility[27]. Men with secondary infertility who were also heavy drinkers had lower sperm concentration[3]. In contrast to moderate drinkers and abstainers, heavy drinkers in our study had the fewest sperm with normal morphology. It's interesting to note that studies have found no negative effects on sperm quality with light to moderate alcohol use[28,29]. However, we recorded lower sperm concentration, progressively motile sperm and significant reduction in NOS scavenging activity in moderate drinkers. This deviates from a meta-analysis finding by Ricci *et al*.[30] that indicated sperm concentration in occasional drinkers was not significantly affected by alcohol consumption. The significant decline in sperm motility and concentration among moderate drinkers in our study may have been caused by decreased antioxidant activity. The volume of semen and the total number of sperm were found to positively correlate with the concentration of citric acid, based on Pearson correlation analysis. In the end, heavy drinking and smoking caused all characteristics of semen quality to decline having a negative effect on a person's reproductive health[31]. The main findings of our study are that drinking and smoking both have an adverse effect on sperm motility and normal morphology, and that both of these lifestyle factors reduce antioxidant capacity.

Conclusion

In summary, we found that heavy drinkers and smokers had significantly less sperms with normal morphology. Moreover, compared to abstainers, smokers, moderate drinkers, and heavy drinkers have lower NOS antioxidant enzyme scavenging percentages. As a result, it's plausible that drinking alcohol and smoking reduce antioxidant activity, which in turn lowers semen parameters and causes infertility. These lifestyle choices not only endanger one's overall health and well-being, but they also have a compounding effect on other infertility causing variables. Lastly, it is advisable that infertile men limit their exposure to passive smoking or discontinuation such hazardous practises to reverse the impact of these lifestyle factors to improve their chances to conceive naturally.

Acknowledgement

This study was funded by the INSPIRE fellowship program; Department of Science and Technology, Ministry of Science and Technology, Government of India (IF170516). We are indebted to Dr. Navya Shankar of Navya Fertility Centre, Mysore for providing samples required for this study.

Conflict of Interest

Authors declare no competing interests.

References

1 Poongothai JENS, Gopenath TS & Manonayaki S, Genetics of human male infertility. *Singapore Med J*, 50 (2009) 336.
2 Sansone A, Di Dato C, de Angelis C, Menafra D, Pozza C, Pivonello R, Isidori A & Gianfrilli D, Smoke, alcohol and drug addiction and male fertility. *Reprod Biol Endocrinol*, 16 (2018) 1.
3 Bai S, Wan Y, Zong L, Li W, Xu X, Zhao Y, Hu X, Zuo Y, Xu B, Tong X & Guo T, Association of alcohol intake and semen parameters in men with primary and secondary infertility: a cross-sectional study. *Front Physiol*, 11 (2020) 1131.
4 Dai J B, Wang ZX & Qiao ZD, The hazardous effects of tobacco smoking on male fertility. *Asian J Androl*, 17 (2015) 954.
5 World Health Organisation. Tobacco factsheet, World Health Organisation [updated 24 May 2022]
6 International Institute for Population Sciences (IIPS) and ICF. 2021. National Family Health Survey (NFHS-5), 2019-21: India.
7 Mostafa T, Cigarette smoking and male infertility. *J Adv Res*, 1 (2010) 179.
8 Yao DF & Mills JN, Male infertility: lifestyle factors and holistic, complementary, and alternative therapies. *Asian J Androl*, 18 (2016) 410.
9 Durairajanayagam D, Lifestyle causes of male infertility. *Arab J Urol*, 16 (2018) 10.
10 Tremellen K, Oxidative stress and male infertility—a clinical perspective. *Hum Reprod Update*, 14 (2008) 243.
11 Yan L, Liu J, Wu S, Zhang S, Ji G & Gu A, Seminal superoxide dismutase activity and its relationship with semen quality and SOD gene polymorphism. *J Assist Reprod Genet*, 31 (2014) 549.
12 Yan L, Guo W, Wu S, Liu J, Zhang S, Shi L, Ji G & Gu A, Genetic variants in nitric oxide synthase genes and the risk of male infertility in a Chinese population: A case-control study. *PLoS ONE*, 9 (2014) e115190.
13 World Health Organisation, WHO laboratory manual for the Examination and processing of human semen. (World Health Organisation World Health Organisation, Switzerland), 2010.
14 Kazari D, Samanta L & Chainy GBN, A modified spectrophotometric assay for superoxide dismutase using nitrite formation by superoxide radicals. *Int J Biotechnol Biochem*, 37 (2000) 201.
15 Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS & Tannenbaum SR, Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal Biochem*, 126 (1982) 131.
16 Karvonen MJ & Malm M, Colorimetric determination of fructose with indole. *Scand J Clin Lab*. 7 (1955) 305.
17 Polakoski KL & Zaneveld LJD, Biochemical examination of the human ejaculate. Techniques of human andrology, North Holland Pub1 Co Amsterdam, New York, Oxford, (1977) 265.
18 Amor H, Hammadeh ME, Mohd I & Jankowski PM, Impact of heavy alcohol consumption and cigarette smoking on sperm DNA integrity. *Andrologia*, (2022) e14434.
19 Schuppe HC, Pilatz A, Hossain H, Diemer T, Wagenlehner F & Weidner W, Urogenital infection as a risk factor for male infertility. *Dtsch Ärztebl Int*, 114 (2017) 339.
20 Jayasena CN, Sharma A, Abbara A, Luo R, White CJ, Hoskin SG, Khanjani S, Crawford MJ, Ramsay JW, Minhas S & Dhillo WS, Burdens and awareness of adverse self-reported lifestyle factors in men with sub-fertility: A cross-sectional study in 1149 men. *Clin Endocrinol*, 93 (2020) 312.
21 Vogt HJ, Heller WD & Borelli S, Sperm quality of healthy smokers, ex-smokers, and never-smokers. *Fertil Steril*, 45 (1986) 106.
22 Mak, V, Jarvi K, Buckspan M, Freeman M, Hechter S & Zini A, Smoking is associated with the retention of cytoplasm by human spermatozoa. *Urology*, 56 (2000) 463.
23 Boeri L, Capogrosso P, Ventimiglia E, Pederzoli F, Cazzaniga W, Chierigo F, Dehò F, Montanari E, Montorsi F & Salonia A, Heavy cigarette smoking and alcohol consumption are associated with impaired sperm parameters in primary infertile men. *Asian J Androl*, 21 (2019) 478.
24 Ramgir SS & Abilash VG, Impact of Smoking and Alcohol Consumption on Oxidative Status in Male Infertility and Sperm Quality. *Indian J Pharm Sci*, 81 (2019) 933.
25 Sermondade N, Elloumi H, Berthaut I, Mathieu E, Delarouzière V, Ravel C & Mandelbaum J, Progressive alcohol-induced sperm alterations leading to spermatogenic arrest, which was reversed after alcohol withdrawal. *Reprod Biomed Online*, 20 (2010) 324.
26 Maneesh M, Jayalekshmi H, Dutta S, Chakrabarti A & Vasudevan DM, Effect of chronic ethanol administration on testicular antioxidant system and steroidogenic enzyme activity in rats. *Indian J Clin Biochem*, 20 (2005) 62.
27 Calogero AE, Duca Y, Condorelli RA & La Vignera S, Male accessory gland inflammation, infertility, and sexual

dysfunctions: a practical approach to diagnosis and therapy. *Andrology*, 5 (2017) 1064.

28 Curtis KM, Savitz DA & Arbuckle TE, Effects of cigarette smoking, caffeine consumption, and alcohol intake on fecund ability. *Am J Epidemiol*, 146 (1997) 32.

29 Jensen TK, Gottschau M, Madsen JOB, Andersson AM, Lassen TH, Skakkebæk NE, Swan SH, Priskorn L, Juul A & Jorgensen N, Habitual alcohol consumption associated with reduced semen quality and changes in reproductive hormones; a cross-sectional study among 1221 young Danish men. *Br Med J Open*, 4 (2014) e005462.

30 Ricci E, Al Beitawi S, Cipriani S, Candiani M, Chiaffarino F, Viganò P, Noli S & Parazzini F, Semen quality and alcohol intake: a systematic review and meta-analysis. *Reprod Biomed online*, 34 (2017) 38.

31 Gaur DS, Talekar MS & Pathak VP, Alcohol intake and cigarette smoking: impact of two major lifestyle factors on male fertility. *Indian J Pathol Microbiol*, 53 (2010) 35.