Original Research Paper

Volume - 13 | Issue - 10 | October - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Anatomy

# EFFECT OF TOBACCO CHEWING, SMOKING AND ALCOHOL ON SEMINAL QUALITY AND SPERM DNA INTEGRITY IN SOUTH INDIAN POPULATION

**Dr. Raju Kumaran T** — M.D. (Anatomy), DNB. (Anatomy), Assistant Professor, Department of Anatomy, Velammal Medical College Hospital and Research Institute, Madurai, Tamilnadu, India

**Dr. Dharmaraj W Tamgire*** — M.S. (Anatomy), Additional Professor, Department of Anatomy, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, Puducherry. India *Corresponding Author

**Dr. Papa Dasari** — M.S. (Obstetrics and Gynaecology), Professor, Department of Obstetrics and Gynaecology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, Puducherry. India

**Dr. Prashant S Adole** — M.D. (Biochemistry), Additional Professor, Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, Puducherry. India

**Dr. Sree Rekha J** — M.D. (Pathology), Additional Professor, Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, Puducherry. India

**ABSTRACT** **Introduction:** Lifestyle factors such as tobacco chewing, smoking cigarettes, and alcohol intake are related to male infertility. Though most of the research has established adverse effects of tobacco chewing, smoking and alcohol on seminal quality and sperm DNA integrity, the quantum of studies in this part of the world is less, with variable outcomes. As environmental and geographical factors play a significant role in male fertility, we planned the study to ascertain the effect of tobacco chewing, smoking and alcohol on seminal quality and sperm DNA integrity in the South Indian Population. **Methods:** Male partners of infertile couples (n=106) attending the infertility clinic with abnormal semen analysis as per WHO criteria (2010) were recruited for the study from July 2018 to June 2020. Comet assay for sperm DNA fragmentation was done on the semen samples after semen analysis. **Results:** In the present study, we have observed a notable difference between sperm total motility, sperm morphology and sperm concentration among male infertile patients consuming alcohol, smoking and tobacco chewing with their counterparts, but no significance could be derived. The study also concludes that no significant difference exists between alcohol, smoking, tobacco chewing, and sperm DNA fragmentation as measured by comet assay among male infertile patients. **Conclusions:** Though most studies have reported adverse effects of tobacco chewing, smoking and alcohol on semen parameters and sperm DNA integrity, many studies have also reported no significant difference. Thus, this study serves as a pilot study for further studies on the quantity and time frame of exposure to tobacco chewing, smoking and alcohol among male infertile patients with a larger sample size.

**KEYWORDS :** Sperm DNA Fragmentation, Semen Analysis, Tobacco, Smoking, Alcohol

## INTRODUCTION

Infertility is defined as the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year.[1] It is a disorder that causes physiological and psychological trauma to an individual because of its social stigma. In recent years, there has been an alarming decrease in fertility in most parts of the world, of which the male factor is the sole cause in approximately 20% of cases.[2]

A recent study on the status of Infertility in India states that nearly 50% of infertility is related to reproductive anomalies in the male, and the rest is related to female factors. In 25%, no cause can be found, leaving it to be unexplained infertility.[3] This decrease in fertility has been attributed to several factors like environmental, genetic, socioeconomic, nutritional, lifestyle, and several unknown factors.[4]

Male factor infertility is ascertained when sperm parameters are below the average reference values specified by WHO.[5] Semen analysis with 89.6% sensitivity remains fundamental and is the most helpful investigation as it detects male infertility with a genuine problem in about 9 out of 10 males.[6] DNA integrity in the ejaculated mature spermatozoa as a cause for infertility due to male factors has been intensely studied in the past decade.[7]

Tobacco chewing and smoking remain the most abused substances worldwide. According to the WHO, one-third of men above 15 are smokers.[8] Tobacco chewing is predominant in developing nations and low socioeconomic groups, while smoking is equal in all socioeconomic strata.[9] Many substances like nicotine, carbon monoxide, mutagens such as benzopyrene, dimethyl nitrosamine and radioactive polonium are present in tobacco. These substances disrupt the microcirculation of the testis and cause DNA damage in germinal cells. Tobacco chewing is a systematic socioeconomic marker in India at individual and household levels. There is a difference of about 2.69 times between illiterate and graduates among those who smoke and chew tobacco.[10]

Tobacco smoking harms sperm, affecting semen parameters like sperm concentration, volume, motility, and morphology. It also damages the sperm's DNA, making it unfit for in vitro fertilisation. Smoking also affects sperm function and decreases its fertilising capacity. Oxidative sperm DNA damage is more profound in smokers than in non-smokers.[11]

Almost 60% of the global population above 15 in nearly all societies consume alcohol in one year.[12] Heavy intake of alcohol is associated with a decline in sperm quality and function, including a decrease in sperm count, immature testicular cells, sperm head breakage, distension of the middle piece and a lower percentage of normal morphological sperm. More significant sexual dysfunction is also caused by heavy alcohol intake in men.[13]

The decrease in semen quality and quantity, as assessed by the routine semen analysis, does not always give a definite diagnosis, as in 15% of male factor infertility cases, the results are expected.[14] In addition, with the advent of Intracytoplasmic Sperm Injection (ICSI), conventional sperm analysis has fallen out of favour, and more attention is being given to sperm molecular architecture, as the inherent integrity of sperm DNA plays a significant role in mammalian fertilisation and subsequent embryo development.[15]

Though most of the research has established adverse effects of tobacco chewing, smoking and alcohol on seminal quality and sperm DNA integrity, the quantum of studies in this part of the world is less, with variable outcomes. As environmental and geographical factors play a significant role in male fertility, we planned the study to ascertain the effect of tobacco chewing, smoking and alcohol on seminal quality and

sperm DNA integrity in the South Indian Population.

## METHODS

The present cross-sectional analytical study was undertaken in the Molecular Genetics and Cytogenetics Laboratory in a tertiary care institute in South India from July 2018 to June 2020. The Postgraduate Research Monitoring Committee (PGRMC) and the Institute Human Ethics Committee approved the study.

Among the male partners of the infertile couple who had attended the infertility clinic in the Obstetrics and Gynaecology Department, 106 were recruited for this study within the age group 21 to 50 years with abnormal semen parameters.

The study excluded men with reproductive organ disorders like testicular carcinoma, undescended testis, prostatitis, urinary tract infections, a history of chronic diseases, and diseases that lead to dyspermia.

The present study consisted of two procedures:
1. Semen analysis
2. Sperm DNA damage analysis

Semen samples were collected from the participants by masturbation after 2 to 3 days of sexual abstinence and received into the sterilised containers. Specimens were then kept at room temperature for 30 to 40 minutes, enabling the semen to liquefy. Conventional semen analysis was done in the Central Pathology Laboratory using Automated Semen Analyser SQA-V Gold Version 2.60. The analysis was done according to WHO (2010) guidelines.[16] Sperm parameters were considered abnormal if sperm concentration was <15 x$10^6$/ml, sperm total motility was <40% and sperm morphology was <4%.

Single-cell gel electrophoresis (Comet assay) is the easiest, most straightforward, sensitive, and rapid method for measuring DNA damage in replicating and non-replicating mammal cells. This study measured sperm DNA fragmentation in sperm cells as per the Singh et al. 2003 protocol.[17]

## RESULTS

The study recruited 106 males with abnormal semen parameters based on inclusion and exclusion criteria.

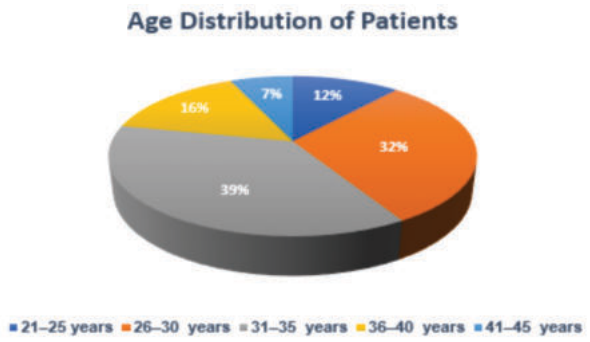


**Figure. 1** – Age distribution of patients

As per the pie chart diagram, the maximum number of patients was between 26 and 35 years old.

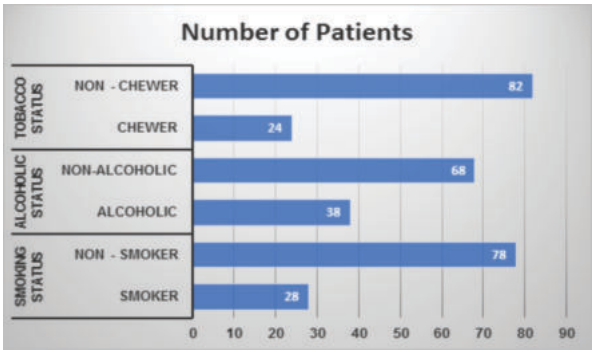


**Figure. 2** – Distribution of patients according to tobacco, alcohol, and smoking status

As per the bar diagram, the maximum number of patients among 106 were non-chewer, non-alcoholic and non-smoker.

**Table No.1:** Tobacco status-wise comparison on semen parameters

| Parameters | Tobacco Status (Mean ± SD) n=106 | | p-Value |
|---|---|---|---|
| | Non-Chewer n=82 | Chewer n=24 | |
| Sperm Total Motility | 22.01 ± 11.93 | 25.29 ± 14.90 | 0.266 |
| Sperm Morphology | 2.63 ± 2.21 | 2.42 ± 2.72 | 0.688 |
| Sperm Concentration | 56.60 ± 47.83 | 41.21 ± 37.85 | 0.151 |

Table No.1 compares sperm motility, sperm morphology, and sperm concentration among tobacco non-chewer and chewer groups. There was a decrease in sperm morphology and sperm concentration and an increase in sperm motility in tobacco chewers; we could note no significant difference between the two groups.

**Table No.2:** Tobacco status-wise comparison on comet parameters

| Parameters | Tobacco Status (Mean ± SD)n=106 | | p-Value |
|---|---|---|---|
| | Non-Chewer n=82 | Chewer n=24 | |
| Total Comet Length | 51.42 ± 12.40 | 49.56 ± 13.82 | 0.531 |
| Head Diameter | 26.12 ± 4.71 | 26.93 ± 7.21 | 0.515 |
| Percentage of DNA in Comet Head | 68.98 ± 13.05 | 68.78 ± 10.84 | 0.944 |
| Tail Length | 25.37 ± 11.33 | 22.76 ± 12.35 | 0.332 |
| Percentage of DNA in Comet Tail | 31.02 ± 13.05 | 31.22 ± 10.84 | 0.944 |

Table No.2 compares the comet length, head diameter of the comet, percentage of DNA in the comet head, tail length, and percentage of DNA in the comet tail among tobacco non-chewer and chewer groups. Apart from the DNA percentage in the comet head and tail, all other comet parameters show more sperm DNA damage in non-chewers. But the difference is not significant.

**Table No.3:** Smoking status-wise comparison on semen parameters

| Parameters | Smoking Status (Mean ± SD) n=106 | | p-Value |
|---|---|---|---|
| | Non-Smoker n=78 | Smoker n=28 | |
| Sperm Total Motility | 23.03 ± 13.06 | 22 ± 11.66 | 0.715 |
| Sperm Morphology | 2.50 ± 2.20 | 2.82 ± 2.65 | 0.532 |
| Sperm Concentration | 54.76 ± 46.30 | 48.54 ± 45.86 | 0.542 |

Table No.3 compares sperm motility, sperm morphology and sperm concentration among non-smoker and smoker groups. Though there was a decrease in sperm total motility and sperm concentration in smokers and a decrease in sperm morphology in non-smokers, we could not observe any significant difference between the groups.

**Table No.4:** Smoking status-wise comparison on Comet parameters

| Parameters | Smoking Status (Mean ± SD) n=106 | | p-Value |
|---|---|---|---|
| | Non-Smoker n=78 | Smoker n=28 | |
| Total Comet Length | 50.36 ± 12.42 | 52.76 ± 13.49 | 0.394 |
| Head Diameter | 25.95 ± 4.55 | 27.28 ± 7.13 | 0.263 |
| Percentage of DNA in Comet Head | 68.72 ± 12.57 | 69.55 ± 12.63 | 0.764 |
| Tail Length | 24.51 ± 11.38 | 25.54 ± 12.23 | 0.688 |
| Percentage of DNA in Comet Tail | 31.28 ± 12.57 | 30.45 ± 12.63 | 0.764 |

Table No.4 compares the comet length, head diameter of the comet, percentage of DNA in the comet head, tail length, and percentage of DNA in the comet tail among tobacco non-chewer and chewer groups. Comet parameters other than the total comet and tail sizes show more sperm DNA damage in non-smokers but did not show a significant difference between the two groups.

**Table No.5:** Alcoholic status-wise comparison on semen parameters

| Parameters | Alcoholic Status (Mean ± SD) n=106 | | P-Value |
|---|---|---|---|
| | Non-Alcoholic n=68 | Alcoholic n=38 | |
| Sperm Total Motility | 21.71 ± 13.15 | 24.63 ± 11.68 | 0.256 |
| Sperm Morphology | 2.37 ± 2.07 | 2.97 ± 2.70 | 0.199 |

| | | | |
|---|---|---|---|
| Sperm Concentration | 57.76 ± 47.59 | 44.79 ± 42.48 | 0.165 |

Table No.5 compares sperm motility, sperm morphology and sperm concentration among non-alcoholic and alcoholic groups. A decrease in sperm concentration among alcoholics and a reduction in sperm total motility and concentration among non-alcoholics showed no significant difference.

**Table No.6:** Alcoholic status-wise comparison on comet parameters

| Parameters | Alcoholic Status (Mean ± SD) n=106 | | P |
|---|---|---|---|
| | Non-Alcoholic n=68 | Alcoholic n=38 | -Value |
| Total Comet Length | 52.05 ± 12.47 | 49.12 ± 13.03 | 0.256 |
| Head Diameter | 26.84 ± 5.51 | 25.35 ± 4.97 | 0.172 |
| Percentage of DNA in Comet Head | 67.90 ± 12.41 | 70.79 ± 12.70 | 0.258 |
| Tail Length | 25.28 ± 11.46 | 23.88 ± 11.85 | 0.552 |
| Percentage of DNA in Comet Tail | 32.10 ± 12.41 | 29.22 ± 12.70 | 0.258 |

Table No.6 compares the comet length, head diameter of the comet, percentage of DNA in the comet head, tail length, and percentage of DNA in the comet tail among non-alcoholic and alcoholic groups. Though all comet parameters except the head diameter of the comet show increased sperm DNA damage in non-alcoholics, no significant difference could be observed between the two groups.

## DISCUSSION

We conducted this study to determine any relationship between tobacco, alcohol and smoking with seminal parameters and sperm DNA fragmentation among male infertile patients in the South Indian Population.

Various studies have demonstrated the association of tobacco chewing with abnormal semen parameters. Studies contradicting the above have also been reported. Sperm quality deteriorated with increased levels of addiction towards tobacco chewing.[18] Inverse relationships have been reported between semen parameters and tobacco chewing depending on the severity and increased duration.[11] Abnormal semen parameters, including volume, count, motility, and deranged sperm DNA integrity, have been observed with tobacco chewing.[19] Dikshit RK et al. study on 119 tobacco chewers showed no statistical significance in ejaculate volume, total count, sperm density, morphology, and motility among tobacco chewers.[20] A previous study also supports that not all the semen parameters are affected by tobacco chewing in male infertile patients.[21] Our results count on the above research regarding sperm DNA integrity and semen parameters, as we noted no significant difference among tobacco-chewing and non-chewing patients.

No significant effect was noted on sperm morphology, sperm motility, and sperm density in smoking individuals.[22] A study with 219 smokers observed no significant difference in semen quality among smokers and non-smokers.[20] Jenkins TG et al. noticed a modest decrease in semen parameters and marginal significant sperm DNA damage among smokers and never-smokers. Smoking alters sperm DNA methylation, significantly affecting its integrity. A substantial reduction in progressive motility of sperm was observed in moderate and heavy smokers, with no significant changes in mild smokers. Semen parameters and sperm DNA damage were not affected in short-term smokers. Studies also report that smoking harms sperm DNA integrity.[23] An inverse association was noted between progressive motility and concentration of sperm and DNA fragmentation.[24]Recent meta-analyses have shown that smoking adversely affects the semen quality.[25,26] Studies also report no statistical difference between smoking and semen parameters and sperm quality and no significance between smoking and sperm DNA fragmentation.[27–29] We also noticed no significant difference between semen parameters and sperm DNA damage among smokers and non-smokers in male infertile patients.

There are sparse studies to determine the relationship between sperm DNA fragmentation and consumption of alcohol.[30] Though studies have confirmed the negative impact of alcohol on both semen parameters and sperm DNA integrity, most studies have reported that a decrease in semen quality and sperm DNA damage occurs only in heavy alcohol drinking and not in all alcohol consumption subjects.[13,31,32] A recent meta-analysis by Tung et al. showed no statistical significance between sperm density, count, motility, and alcohol intake. Further, the same study also concludes that drinking alcohol did not cause considerable damage to fragment sperm DNA.[33] Our analysis also supports that alcoholism among male infertile patients has no significant difference in semen parameters and sperm DNA fragmentation.

### Limitations:

Being a single-centre, time-bound study, there are a few limitations. The major limitation is we need to define the quantum of tobacco chewing, smoking and alcohol intake and match the confounding factors. Non-uniformity in the sample size of groups is another major limitation of our study. The sample size was disproportionate as we adopted the simple random sampling technique in our cross-sectional analytical study. BMI, occupation, exposure to pesticides, etc., were not considered, which can be a potential confounder for the study results. We can avoid these limitations by conducting the study in a larger sample with an increased study duration.

## CONCLUSION

The present study's findings have highlighted no significant difference between sperm total motility, sperm morphology and sperm concentration among male infertile patients consuming alcohol, smoking and tobacco chewing with their counterparts. The study also concludes that there is no statistical significance between alcohol, smoking, tobacco chewing, and sperm DNA fragmentation as measured by comet assay. Though studies contradict the present study, it is also supported by a few studies accepting the same. Thus, this study serves as a pilot study for further studies on the quantity and time frame of exposure to tobacco chewing, smoking and alcohol among male infertile patients with a larger sample size.

**Funding:** Intramural Grant

**Conflict Of Interest:** None

## REFERENCES

1. Zegers-Hochschild, F., Adamson, G. D., De Mouzon, J., Ishihara, O., Mansour, R., Nygren, K., Sullivan, E., & Vanderpoel, S. (2009). International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009□. Fertility and Sterility, 92(5), 1520–1524. https://doi.org/10.1016/j.fertnstert.2009.09.009
2. Kumar, N., & Singh, A. (2015). Trends of male factor infertility, an important cause of infertility: A review of literature. Journal of Human Reproductive Sciences, 8(4), 191. https://doi.org/10.4103/0974-1208.170370
3. Kumar TCA (2014). Fertility and In-vitro fertilisation in India. Current Science. 86, 2546.
4. Fisch, H., & Goluboff, E. T. (1996). Geographic variations in sperm counts: A potential cause of bias in studies of semen quality. Fertility and Sterility, 65(5), 1044–1046. https://doi.org/10.1016/S0015-0282(16)58284-3
5. Esteves, S. C. (2014). Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. International Braz j Urol, 40(4), 433–453. https://doi.org/10.1590/S1677-5538.IBJU.2014.04.02
6. Butt, F., & Akram, N. (2013). Semen analysis parameters: Experiences and insight into male infertility at a tertiary care hospital in Punjab. J Pak Med Assoc, 63.
7. Sakkas, D., Mariethoz, E., Manicardi, G., Bizzaro, D., Bianchi, P. G., & Bianchi, U. (n.d.). Origin of DNA damage in ejaculated human spermatozoa.
8. Saleh, R. A., Agarwal, A., Sharma, R. K., Nelson, D. R., & Thomas, A. J. (2002). Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: A prospective study. Fertility and Sterility, 78(3), 491–499. https://doi.org/10.1016/S0015-0282(02)03294-6
9. Sinha, D., Gupta, P., Ray, C., & Singh, P. (2012). Prevalence of smokeless tobacco use among adults in WHO South-East Asia. Indian Journal of Cancer, 49(4), 342. https://doi.org/10.4103/0019-509X.107726
10. Patel, K.K., Mehta, N., & Mehta, A. (2015). A study on effects of tobacco chewing on various semen parameters. *Indian Journal of Applied-Basic Medical Sciences*, 67-74.
11. Patel, D. K. (2012). A Study on Effects of Cigarette Smoking on Various Semen Parameters. *International Journal of Scientific Research*, *2*(11), 423–425. https://doi.org/10.15373/22778179/NOV2013/139
12. World Health Organization. (2018). Global status report on alcohol and health 2018. World Health Organization. https://apps.who.int/iris/handle/10665/274603
13. Trautman, A., Gurumoorthy, A., & Hansen, K. A. (2023). Effects of alcohol use on sperm chromatin structure, a retrospective analysis. Basic and Clinical Andrology, 33(1), 14. https://doi.org/10.1186/s12610-023-00189-9
14. Aydos, O. S., Yukselten, Y., Kaplan, F., Sunguroglu, A., & Aydos, K. (2015). Analysis of the correlation between sperm DNA integrity and conventional semen parameters in infertile men. Türk Üroloji Dergisi/Turkish Journal of Urology, 41(4), 191–197. https://doi.org/10.5152/tud.2015.98475
15. Agarwal, A. (2003). Role of sperm chromatin abnormalities and DNA damage in male infertility. Human Reproduction Update, 9(4), 331–345. https://doi.org/10.1093/humupd/dmg027
16. Lu, J.-C., Jing, J., Dai, J.-Y., Zhao, A. Z., Yao, Q., Fan, K., Wang, G.-H., Liang, Y.-J., Chen, L., Ge, Y.-F., & Yao, B. (2015). Body mass index, waist-to-hip ratio, waist circumference and waist-to-height ratio cannot predict male semen quality: A report of 1231 subfertile Chinese men. Andrologia, 47(9), 1047–1054. https://doi.org/10.1111/and.12376
17. Lu, J.-C., Jing, J., Chen, L., Ge, Y.-F., Feng, R.-X., Liang, Y.-J., & Yao, B. (2018). Analysis of human sperm DNA fragmentation index (DFI) related factors: A report of 1010 subfertile men in China. Reproductive Biology and Endocrinology, 16(1), 23. https://doi.org/10.1186/s12958-018-0345-y
18. Said, T. M., Ranga, G., & Agarwal, A. (2005). Relationship between semen quality and tobacco chewing in men undergoing infertility evaluation. Fertility and Sterility, 84(3), 649–653. https://doi.org/10.1016/j.fertnstert.2005.03.052

19. Sunanda, P., Panda, B., Dash, C., Ray, P., Padhy, R., & Routray, P. (2014). Prevalence of abnormal spermatozoa in tobacco chewing sub-fertile males. Journal of Human Reproductive Sciences, 7(2), 136. https://doi.org/10.4103/0974-1208.138873
20. Dikshit, R. K., Buch, J. G., & Mansuri, S. M. (1987). Effect of tobacco consumption on semen quality of a population of hypofertile males. Fertility and Sterility, 48(2), 334–336. https://doi.org/10.1016/S0015-0282(16)59369-8
21. Aggarwal, N., Vohra, R. R., Priyadarshi, S., Vyas, N., Yadav, S. S., & Tomar, V. (2017). Study to evaluate Association of Tobacco Chewing and Smoking with Semen Parameters. International Journal of Infertility & Fetal Medicine, 8(3), 97–100. https://doi.org/10.5005/jp-journals-10016-1156
22. Vogt, H.-J., Heller, W.-D., & Borelli, S. (1986). Sperm quality of healthy smokers, ex-smokers, and never-smokers. Fertility and Sterility, 45(1), 106–110. https://doi.org/10.1016/S0015-0282(16)49106-5
23. Jenkins, T. G., James, E. R., Alonso, D. F., Hoidal, J. R., Murphy, P. J., Hotaling, J. M., Cairns, B. R., Carrell, D. T., & Aston, K. I. (2017). Cigarette smoking significantly alters sperm DNA methylation patterns. Andrology, 5(6), 1089–1099. https://doi.org/10.1111/andr.12416
24. Cui, X., Jing, X., Wu, X., Wang, Z., & Li, Q. (2016). Potential effect of smoking on semen quality through DNA damage and the downregulation of Chk1 in sperm. Molecular Medicine Reports, 14(1), 753–761. https://doi.org/10.3892/mmr.2016.5318
25. Sharma, R., Harlev, A., Agarwal, A., & Esteves, S. C. (2016). Cigarette Smoking and Semen Quality: A New Meta-analysis Examining the Effect of the 2010 World Health Organization Laboratory Methods for the Examination of Human Semen. European Urology, 70(4), 635–645. https://doi.org/10.1016/j.eururo.2016.04.010
26. Bundhun, P. K., Janoo, G., Bhurtu, A., Teeluck, A. R., Soogund, M. Z. S., Pursun, M., & Huang, F. (2019). Tobacco smoking and semen quality in infertile males: A systematic review and meta-analysis. BMC Public Health, 19(1), 36. https://doi.org/10. 1186/s12889-018-6319-3
27. Lewin, A., Gonen, O., Orvieto, R., & Schenker, J. G. (1991). Effect of Smoking on Concentration, Motility and Zona-Free Hamster Test on Human Sperm. Archives of Andrology, 27(1), 51–54. https://doi.org/10.3109/01485019108987651
28. Osser, S., Beckman-Ramirez, A., & Liedholm, P. (1992). Semen quality of smoking and non-smoking men in infertile couples in a Swedish population. Acta Obstetricia et Gynecologica Scandinavica, 71(3), 215–218. https://doi.org/10. 3109/00016349209009921
29. Sergerie, M., Ouhilal, S., Bissonnette, F., Brodeur, J., & Bleau, G. (2000). Lack of association between smoking and DNA fragmentation in the spermatozoa of normal men*. Human Reproduction, 15(6), 1314–1321. https://doi.org/10.1093/humrep/15.6.1314
30. Jaafar, F. H. F., Osman, K., Kumar, J., & Ibrahim, S. F. (2017). Effect of Alcohol Consumption on the Sperm DNA Integrity: A Systematic Review. Journal of Agricultural Science, 9(13), 136. https://doi.org/10.5539/jas.v9n13p136
31. Boeri, L., Capogrosso, P., Ventimiglia, E., Pederzoli, F., Cazzaniga, W., Chierigo, F., Dehò, F., Montanari, E., Montorsi, F., & Salonia, A. (2019). Heavy cigarette smoking and alcohol consumption are associated with impaired sperm parameters in primary infertile men. Asian Journal of Andrology, 21(5), 478. https://doi.org/10.4103/aja.aja_110_18
32. Amor, H., Hammadeh, M. E., Mohd, I., & Jankowski, P. M. (2022). Impact of heavy alcohol consumption and cigarette smoking on sperm DNA integrity. Andrologia, 54(7). https://doi.org/10.1111/and.14434
33. Nguyen-Thanh, T., Hoang-Thi, A.-P., & Anh Thu, D. T. (2023). Investigating the association between alcohol intake and male reproductive function: A current meta-analysis. Heliyon, 9(5), e15723. https://doi.org/10.1016/j.heliyon.2023.e15723