

EFFECT OF ALCOHOL INTAKE AND CIGARETTE SMOKING ON SPERM PARAMETERS AND MALE FERTILITY: A PROSPECTIVE OBSERVATIONAL STUDY

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Abstract

Introduction: On average, around 15% of all couples face difficulty in starting a family. Tobacco smoking and alcohol has many harmful effects on semen parameters (i.e. lower semen concentration, impaired motility of spermatozoa and increased sperm morphology defects in head, neck and tail) resulting in male infertility. **Materials & Methods:** This study was conducted at the central laboratory, department of Pathology, Jaipur National University Institute for Medical Sciences and Research Center (JNUIMSRC), Jaipur (Rajasthan) in which total 150 cases of male infertility was enrolled from January 2021 to September 2022 presented to OBGY Department (OPD) of our institute. The study subjects were divided into 3 groups: Alcoholic, smokers and controls. The semen samples were collected and microscopic examination for sperm count, sperm motility, morphology and vitality were done under 40X and 100X. Effect of smoking and alcohol were examined on various semen parameters. The data set has been analyzed by the Fischer exact test and chi square test using R 3.4.3 version statistical software and MS Excel. P-value <0.05 was considered statistically significant. **Results:** this study was conducted on 150 subjects, out of which 50 subjects were alcoholics, 50 smokers and 50 subjects were categorized as controls. Out of 50 alcoholics, 19 cases were come in asthenozoospermia and 9 were in oligozoospermia in comparison to controls. Among 50 smokers, 20 cases had asthenozoospermia and 7 were positive for oligozoospermia. P value (0.9223) was not significant. In 50 alcohol consumers, 7(7%), 8(8%) and 2(2%) patients had 10-19, 50-59 and 90-99 million/ml sperm count. Out of 50 controls, 8(8%), and 7(7%) had 60-69, and more than 100 million/ml sperm count. P value (0.328) was not significant. In 50 smokers, 10(10%), 8(8%) and 4(4%) patients had 30-39, 60-69, and more than 100 million/ml sperm count. **Conclusion:** Asthenozoospermia was the most common anomaly of semen analysis in alcoholic and smokers' infertile patients followed by oligozoospermia. Deterioration in semen quality appeared in direct proportion to the quantity of alcohol intake and cigarettes smoked and age.

INTRODUCTION

Nowadays Infertility has become ominous health issue. On an average, around 15% of all couples' face difficulty in starting a family and this makes a feeling of great personal failure. High significant relationship had been observed between variability in semen quality including sperm motility count and morphology.^[1] When any couple has the inability to conceive after 12 months of unprotected intercourse defined as Infertility.^[2] Infertility problems originates from both men and women 30-40% and around half

(20%) of infertility problems are contributed by male factors.^[3] It has drawn the attention to the impact of lifestyle status and various environmental factors such as alcohol intake, smoking, diet, obesity, recreational drug use and exposure to environmental toxins on reproductive system of infertile men.^[4,5] It was noted that if evaluation of normal spermatozoa morphology is done under strict criteria, these parameters has an excellent predictive value in fertilization.^[6] Patients with <4% normal forms had a fertilization rate of 7.6%. Patients with 4 to 14% normal forms had a significantly better fertilization

rate than above and cases with >14% normal forms shows high fertilization rate than above both conditions.^[7]

Tobacco smoking has many harmful effects on semen parameters (i.e. lower semen concentration, impaired motility of spermatozoa and increased sperm morphology defects in head, neck and tail) resulting in male infertility.^[8,9] Various other factors like environmental and occupational agents affect male infertility which may result in genetic disorders in germ cells.^[10,11]

Sedentary work profile are frequently related with testicular over heat and possibly increase risk to harm sperm DNA.^[12-14] Alcohol toxins affect the leydig cells and decrease blood levels of testosterone hormone, by lowering its production and enhancing its metabolic clearance.^[15] The sertoli cells functions are also influenced by alcohol, probably by increasing damage to some of the proteins needed for spermatozoa cells formation that sertoli cells provide.^[16]

So, alcohol enhances the reduction of level of follicle stimulating hormone (FSH), testosterone and LH. It is not only hampering the sperm morphological development and maturation by producing significant terato-zoospermia, also slows down the spermatozoa production by testicular germ cells, particularly in heavy alcohol users.^[17]

Toxin in cigarettes smoke directly affects to spermatozoa, probably tilts the delicate balance of reactive oxygen species (ROS) that are created by sperm for their specified functions like decapitation. Increased quantities of ROS have been shown to be detrimental to the DNA of spermatozoa, thus producing negative effect on the viability and morphology of spermatozoa.^[18]

MATERIALS AND METHODS

It was prospective observational study in which total 150 cases of male infertility was enrolled from January 2021 to September 2022. This study was conducted at the central laboratory, department of Pathology, Jaipur National University Institute for Medical Sciences and Research Center (JNUIMSRC), Jaipur (Rajasthan).

The infertile male partners of couples were included in the study who was taking treatment for primary infertility at the Obstetrics and Gynecology patient Department (OPD) of our institute. The institutional ethics committee cleared the protocol and the information pertaining to the patients and controls. Informed consent was obtained from all patients and controls in accordance with institutional ethical committee guidelines.

The detail history of subjects including gender, age, height, weight, education, occupation, cigarette smoking, alcohol consumption, duration of active married life and family history were recorded.

The study subjects were divided into 3 groups:

Group A Strict non-alcohol consumer and non-smokers who considered as controls,

Group B Alcohol users but strict non-smokers,

Group C Smokers (these were strict non-alcohol consumers).

Following strict criteria of 2 to 7 days of abstinence: The samples were collected by masturbation. Samples were obtained in universal sterile containers labelled with name of patient, registration number, time and date of collection. Samples with partial spillage were rejected. All samples were kept at $37^{\circ} \pm 2^{\circ}\text{C}$ temperatures and processing was done as early as possible after complete liquefaction. Following tests were done on seminal fluid:

Physical examination Viscosity, volume, liquefaction time, viscosity, pH and color.

Biochemical Analysis Fructose test.

Microscopic evaluation: Sperm vitality, count, morphology, motility and headless spermatozoa, proportion of white cells. Microscopic evaluation is the most essential test in semen analysis for infertility.

Sperm Motility

Motile & non motile sperms were counted under high power magnification (40x) of microscope in a wet preparation. Motile spermatozoa were observed and calculated in percentage. Normal motility criteria: $\geq 30\%$ of sperms should have progressive motility. If the motility of spermatozoa is $<40\%$, then viable sperms were determined by examining it in eosin-nigrosin preparation.

Sperm Viability or Vitality

One drop of semen was mixed with 1 drop of eosin-nigrosin solution and incubation was done for 30 seconds. The air - dried smear were examined under $\times 100$ oil immersion. Unstained sperms were classified as viable and red sperms were classified as dead or non-viable. Minimum 200 spermatozoa were examined. The result was represented as a proportion of viable sperms against non-viable.

Sperm Count

Principle

The sperm count was done after liquefaction in a counting chamber following 1:20 dilution with sodium bicarbonate formalin diluting fluid and the total number of spermatozoon were reported in million/ml ($10^6/\text{ml}$). The chamber was examined Using the $40\times$ magnification, number of spermatozoon were counted in large corner squares. Normal sperm count is ≥ 16 million/ml. Sperm count <16 million/ml might be associated with infertility in males.

Statistical Analysis

The data set were analyzed by the Fischer exact test and chi square test using R 3.4.3 version statistical software and MS Excel. P-value <0.05 was considered as statistically significant, values lesser than 0.0001 will be considered as highly statistically significant)

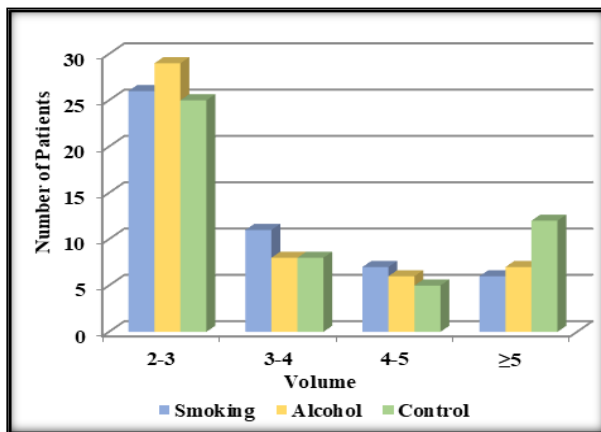


Figure 1: Number of patients according to semen volume and lifestyle status.

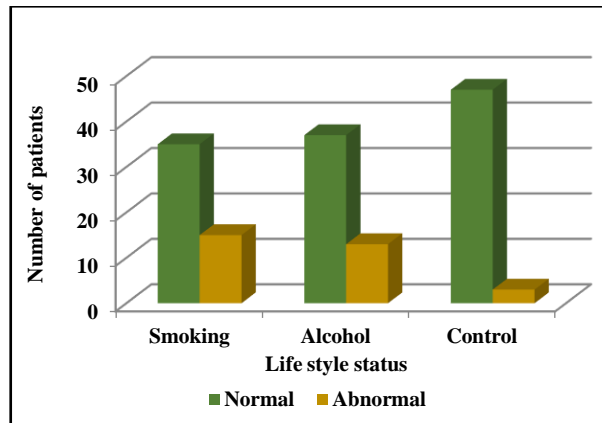


Figure 3: Distribution of Number of patients with normal and abnormal Viability and lifestyle status.

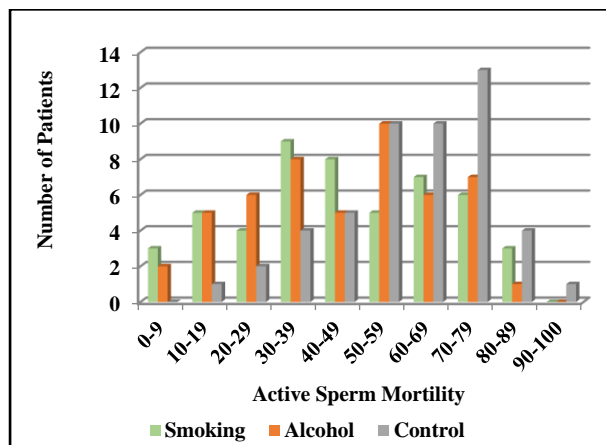


Figure 2: Relationship between lifestyle status and active sperm motility.

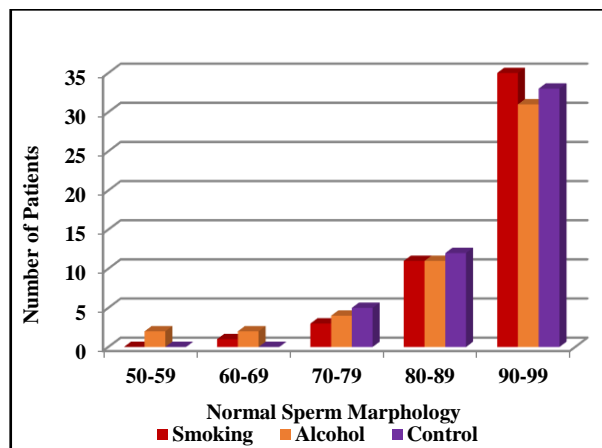
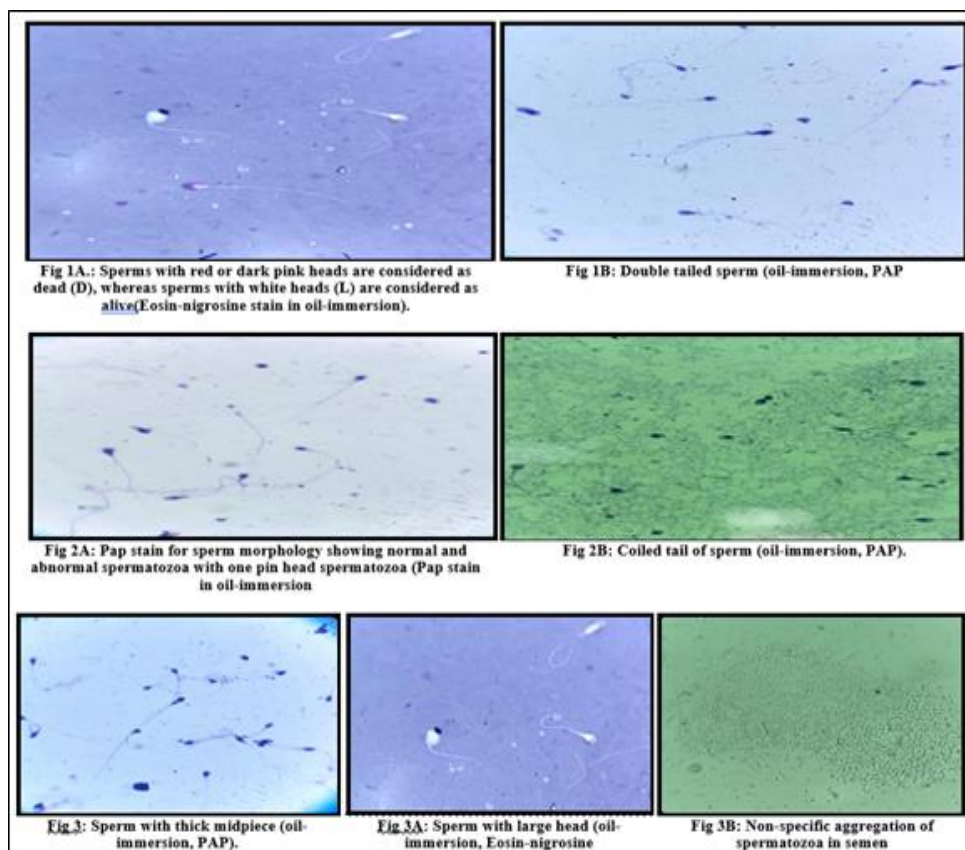


Figure 4: Lifestyle status wise number of patients according to their normal sperm morphology.



RESULTS

Table 1 showed that this study was conducted on 150 subjects, out of which 50 subjects were alcohol consumers and 50 smokers and 50 subjects were apparently healthy, categorized as controls. Out of 50 alcohol consumers, 25(16.67%) were mild and 6(4%) were heavy consumers. Out of 50 smokers, 26(17.33%) were light and 14(9.33%) were moderate smokers.

Table 2 depicted that out of 50 alcoholic, 19 cases were come in asthenozoospermia and 9 were in oligozoospermia in comparison to controls. Among 50 smokers, 20 cases had asthenozoospermia and 7

were positive for oligozoospermia. P value (0.9223) was not significant. Present study showed that in 50 alcohol consumers, 7(7%), 8(8%), 8(8%) and 2(2%) patients had 10-19, 20-29, 50-59 and 90-99 million/ml sperm count respectively. Out of 50 controls, 8(8%), 6(6%) and 7(7%) had 60-69, 80-89 and more than 100 million/ml sperm count respectively. P value (0.328) was not significant.

Present study showed that in 50 smokers, 10(10%), 3(3%), 8(8%) and 4(4%) patients had 30-39, 60-69, 50-59 and more than 100 million/ml sperm count respectively. Out of 50 controls, 8(8%), 6(6%) and 7(7%) had 60-69, 80-89 and more than 100 million/ml sperm count respectively. P value (0.653) was not significant.

Table 1: Lifestyle status of all infertile patients

Lifestyle status		Number of Cases
Controls (Non-Alcoholic and Non-Smokers)		50 (33.33%)
Alcohol Consumers	Mild	25 (16.67%)
	Moderate	19 (12.67%)
	Heavy	6 (4.00%)
Smokers	Light	26 (17.33%)
	Moderate	14 (9.33%)
	Heavy	10 (6.67%)

Table 2: Sperm variables for all patients with lifestyle status.

Sperm variables	Number of patients				P-value
	Alcoholic	Smoker	Control	Total	
A	19 (27.94%)	20 (29.41%)	5 (7.35%)	44 (64.71%)	0.9223
O	9 (13.24%)	7 (10.29%)	1 (1.47%)	17 (25%)	
A+O	3 (4.41%)	4 (5.88%)	0 (0%)	7 (10.29%)	
T	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
A+T	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
O+T	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
A+O+T	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Total	31 (45.59%)	31 (45.59%)	6 (8.82%)	68(100%)	

A= Asthenozoospermia O= Oligozoospermia T= Teratozoospermia

*Fischer Exact Test * P value non-Significant

DISCUSSION

Present study was conducted on 150 subjects, out of which 50 subjects were alcohol consumers and 50 smokers and 50 subjects were controls. Out of 50 alcohol consumers, 25(16.67%) were mild and 6(4%) were heavy consumers. Out of 50 smokers, 26(17.33%) were light and 14(9.33%) were moderate smokers [Table 1]. Dushyant S G²⁰ et al performed this study on 100 alcoholic and 100 cigarette smoker men were following WHO guidelines and compared them with 100 strict non-alcoholic and non-smoker males in Department of Pathology. Out of them, 40 were mild, 35 were moderate and 25 were heavy alcohol consumers. In 100 smokers, 49 were light, 26 were moderate and 25 were heavy smokers.

Present study showed that out of 50 controls, 24(16%) cases were in 25-29 years group and 12(8%) were in 30-34 years age groups respectively. In 50 alcohol consumers, 19(12.67%) cases were in 25-29 years group and 17(11.33%) were in 30-34 years and out of 50 smokers, 17(11.33%) cases were in 25-29

years and 20(13.33%) were in 30-34 years respectively. In the study, most of patients were in 25-29 years age group. Association between alcoholic, smoker and controls was significant(p=0.03789) [Table 2]. Tandel H K et al²² conducted their study on 200 cases of infertility. Out of which 112(56%) patients was in 26–30 years age group. The age group of presentation was 21 to 40 years with commonest age group was 25–30 years (mean age 28.8 years) in the study.

Semen Variables: (Sperm motility, sperm count and sperm morphology)

Present study depicted that out of 50 alcoholic, 19 cases were come in asthenozoospermia and 9 were in oligozoospermia in comparison to controls. Among 50 smokers, 20 cases had asthenozoospermia and 7 were positive for oligozoospermia. P value (0.9223) was insignificant. In the present study out of 50 controls, normozoospermia was observed in 44 subjects. Out of the remaining controls, all the 3 semen parameters were observed; asthenozoospermia (A) was seen in the most of cases in comparison to oligo (O) and teratozoospermia (T) that was similar to the study of Dushyant S G et al.^[20]

Gaur D S et al^[19] studied on 100 cigarette smokers and 100 strictly non-smokers primary infertility patients. Fisher's exact test showed that the incidences of both isolated asthenozoospermia (p=0.0015) and astheno-zoospermia with teratozoospermia (p=0.0106) among smokers was significant.

Liquefaction time

Present study showed that 18 (12%) and 12 (8%) alcohol consumers had 25-29 and 30-34 min. liquefaction time. Out of 50 smokers, 13 (8.67%) and 10 (6.67%) smokers had 20-24 and 30-34 min. liquefaction time. Among 50 controls, 17(11.33%) had 25-29 min. liquefaction time. P value (0.1229) was non-significant.

Semen Volume:

Present study showed that in 50 alcohol consumers, 29(29%) observed 2-3 ml volume and 7(7%) had more than 5 ml volume of semen. In 50 smokers, 26(26%) had 2-3 ml volume and 6(6%) had more than 5 ml volume of semen. Out of 50 controls, 25(25%) had 2-3 ml and 12 had ≥ 5 ml semen volume analysis respectively. Association between alcoholic and control patients according to their volume parameter of semen analysis, p value was not significant (0.997). Association between smoker and control patients according to their volume parameter of semen analysis, p value was insignificant (0.5402). KJ Joo et al^[21] conducted their study on 62 Semen samples were collected from healthy men. They were participating Chung Ang University College of Medicine, Seoul, republic of Korea for routine medical screening in Jan. 2005. Semen volume was reduced in smokers than in non-smokers, but these association was not statistically significant similar to the present study.

Agglutination

Present study showed that in 50 alcohol consumers, 11(11%) patients had agglutination. In 50 smokers, 17(17%) patients had agglutination. Statistical analysis showed that p value was observed to be significant (<0.05) for association between alcoholic, smoker and control infertile patients for agglutination.

Sperm count

Present study showed that in 50 alcohol consumers, 6(6%), 9(9%), 8(8%) and 2(2%) patients had 10-19, 20-29, 50-59 and 90-99 million/ml sperm count respectively. Out of 50 controls, 8(8%), 6(6%) and 7(7%) had 60-69, 80-89 and more than 100 million/ml sperm count respectively. Among 50 smokers, 10(10%), 3(3%), 8(8%) and 4(4%) patients observed 30-39, 60-69, 50-59 and more than 100 million/ml sperm count respectively. Association between count in million and alcoholic and control infertile patients was insignificant. P value (0.6538) was statistically non-significant for relationship between smoker and control with sperm count. Pajarinen J et al^[23] analyzed the testicular tissue morphology and spermatogenesis in 195 men, with (CASA) computer-assisted microscopy in the autopsy study. They observed that long average daily

alcohol consumption of <40 gm of alcohol did not to be associated with anomalies of spermatogenesis in favor of the present study.

Sperm motility

Present study showed that in 50 alcohol consumers, 6(6%), 8(8%) and 10(10%) patients had 20-29, 30-39 and 50-59 percent motility respectively. 10(10%), 5(5%) and 13(13%) observed 50-59, 40-49 and 70-79 percent sperm motility, among of 50 controls, respectively. In 50 smokers, 6(6%), 8(8%) and 9(9%) patients had 70-79, 40-49 and 30-39 percent motility respectively. Statistical analysis showed that p-value was found significant (less than 0.05) for association between alcoholic, smoker and control infertile patients according to their active sperm motility. Ramirez N et al^[25] evaluated the synergistic effects of the aging, use of alcohol and body mass index (BMI) on the seminal quality. They concluded that age was the most impactful factor to effect on sperm viability, motility and morphology without any effects on spermatozoa count.

Sperm viability

In Present study showed that in 50 alcohol consumers, 37(37%) were normal and 13(13%) patients had abnormal viability respectively. Out of 50 controls, 47(47%) were normal and 3(3%) were abnormal viability respectively. In 50 smokers, 35(35%) were normal and 15(15%) patients were abnormal viability respectively. Association between normal and abnormal viability wise alcoholic, smoker and control infertile patients were statistically insignificant (p values were 0.2579 & 1.00).

Sperm morphology

Present study showed that in 50 alcohol consumers, 31(31%) and 11(11%) patients had 90-99 and 80-89 normal sperm morphology respectively. 12(12%) and 33(33%) found 80-89 and 90-99 normal sperm morphology, among of 50 controls respectively. In 50 smokers, 35(35%) and 11(11%) patients had 90-99 and 80-89 normal sperm morphology respectively. Association between alcoholic, smokers and control infertile patients for sperm morphology were not significant. KJ Joo et al^[21], Guo H et al^[24] found that heavy smoking cases was related with decreased number of sperm counts and consumption of alcohol was associated with more numbers of morphological abnormal spermatozoa.

CONCLUSION

Alarming numbers of the population from North side of India includes the state of Rajasthan and adjoining regions presently suffering from male infertility. For early identification and proper, rapid treatment of patients suffering from male infertility, it is utmost to understand the epidemiology as well as risk factors of infertility. Early diagnosis and proper management are important for Infertility in the present scenario. The aim of present study was to detect effects of smoking and alcohol on infertility. The study was conducted on 50 alcohol consumers, 50 smokers and 50 controls.

In our study, Asthenozoospermia was the most common anomaly of semen analysis in alcoholic and smokers infertile patients. Its presence denoted as subtle, 'early indicator' of reduction in the semen quality, which has to be gets overlooked, if the semen sample shows adequate sperm count and normal morphology. The second most common variable was observed oligozoospermia in alcoholic and smoker, whether present individually or in combination with asthenozoospermia.

Alcohol consumption produces a progressive decrease in sperm motility in moderate users with sperm count. Deterioration in semen quality appeared in direct proportion to the quantity of alcohol intake and cigarettes smoked. The results of various anomalies of semen were insignificant with lifestyle factors such as smoking and alcohol consumption. So, alcohol and smoking do not seem to play a pivotal role in the etiology of poor semen quality in our study, but a pattern of excessive alcohol and smoking consumption may decrease motility and count of sperm with normal morphology.

Age factor was considered in this study showed a synergistic negative impact on semen quality. all persons above 21 and above years undergo examination by healthcare practitioners which assist in early diagnosis of infertility. Increase literacy rate and make people aware about the harmful effects of tobacco chewing, smoking and alcohol use. Our study has implications for practice also. Tobacco smoking and alcohol consumption are modifiable lifestyle habits. Public health programs directed towards these habits are expected to have an important impact on male fertility.

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