

Study on Effect of Cigarette Smoking on Semen Quality of Infertile Men

Gaisamudre Karuna B.¹, Waghmare Anil R.²

¹Assistant Professor, Dept. of Physiology, MIMSR Medical College, Latur

²Assistant Professor, Dept. of Physiology, B.J. Govt. Medical College, Pune

ABSTRACT

Background : The highest prevalence of smoking is observed in young adult males during their reproductive period. Smoking has been suggested to contribute to a number of diseases including male infertility.

Aim & Objectives : The present study was aimed at studying effect of cigarette smoking on semen quality (Sperm motility and vitality).

Materials & Methods : A total of one hundred Infertile men (50 Nonsmokers and 50 Smokers) between the age group 20-45 years were taken into study. The Sperm motility and vitality in the Infertile Nonsmokers and Infertile Smokers group were compared using Z Test. Infertile Smokers which were divided into Group A (≥ 1 and ≤ 10 cigarettes/ day), Group B (>10 and <20 cigarettes/ day) and Group C (≥ 20 cigarettes/ day) and were analyzed for Sperm motility and vitality by ANOVA Test.

Results : We observed that Sperm motility ($p < 0.01$) and Sperm vitality ($p < 0.01$) was significantly lower in Infertile Smokers group than Infertile Nonsmokers group. We also observed that Sperm motility ($p < 0.05$) and Sperm vitality ($p < 0.01$) was significantly decreased in accordance with the severity of smoking.

Conclusion : Cigarette smoking adversely affects Sperm motility and vitality and in turn semen quality.

Key Words : Smokers, Nonsmokers, Sperm motility, Sperm vitality.

INTRODUCTION:

Infertility is one of the most tragic of all marital problems. Facing infertility can be very difficult for both men and women. It is emotionally stressful and physically taxing in most couples. Infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse.¹ Male infertility plays a key role in conception difficulties of up to 40% infertile couples.² The term 'male infertility' does not

constitute a defined clinical syndrome but rather a collection of different conditions exhibiting a variety of etiologies and varying prognosis.³ Although in some men a specific disorder may be present, in majority no apparent reason for infertility could be found. This has drawn attention to the impact of lifestyle and environmental factors, especially diet, obesity, smoking, alcohol intake, recreational drug use, and exposure to environmental toxins, on the reproductive health of such men.²

Address for correspondence:

Waghmare Anil R.D-8, Vishnu Sadashiv Sassoon Doctors Quarters, MangalwarPeth, Pune 411001.

Email id: dranilwaghmare79@gmail.com

It has been reported that tobacco smoke contains some of the most deadly toxic chemicals. Smokers inhale directly and absorb the following substances: Nicotine, Carbon monoxide, Nitrogen Oxide, mutagenic pyrolysis-derived compounds and Cadmium. Most of the are known to be mutagens and carcinogens, directly affecting male and female gametes and embryos.⁴ Although much is known about the carcinogens in tobacco cigarette smoke and their resultant effects on organs like lungs and urinary bladder, their effects on fertility status have been less documented.²

Cigarette smoking is an avoidable lifestyle factor observed to have a negative impact on male fertility.⁵ The aim of our study was to compare Sperm motility and vitality of Infertile men who were cigarette smokers with Infertile Non-smoking men, in order to ascertain the effect of cigarette smoking on the quality of seminal fluid (Sperm motility and vitality).

AIM AND OBJECTIVES:

AIM:

To study the effect of cigarette smoking on semen quality (Sperm motility and vitality) of infertile men

OBJECTIVES:

Primary: To study semen quality (Sperm motility and vitality) in infertile nonsmoker and infertile smoker men.

Secondary: To compare semen quality (Sperm motility and vitality) in infertile nonsmoker and infertile smoker men.

MATERIAL AND METHODS:

The study protocol was approved by the Institutional Ethical Committee. Before enrollment in the study, informed written consent was obtained from each subject.

A total of one hundred men (fifty infertile nonsmokers and fifty infertile smokers) between the age group 20-45 years were taken into study. The study was undertaken for a duration of 12 months.

Infertile nonsmokers were the men who had never smoked.

Infertile smokers were the men who smoke cigarettes since 5 years or more and smoking till date.

The infertile smokers were in turn divided into following groups:

Group A (n = 28) - (≥ 1 and ≤ 10 cigarettes/day)

Group B (n = 17) - (> 10 and < 20 cigarettes/day)

Group C (n = 5) - (≥ 20 cigarettes/day)

n = Number of subjects

Exclusion Criteria:

1. History of tobacco chewing and alcohol intake.
2. History of injury to testes, varicocele, hydrocele or undescended testes.
3. History of any chronic illness like Tuberculosis, diabetes, hypertension and thyroid disease.
4. History of UTI, occupational exposure to chemicals or excess heat.
5. Azoospermic Subjects.
6. History of taking drugs like Vitamin E, Vitamin C or glutathione supplementation.

Sample collection and semen analysis :

Semen samples were collected by masturbation into a sterile, wide mouthed container, after at least

72 hours (3 days) of sexual abstinence. Samples were allowed to liquefy at room temperature (25°C) for at least 45 minutes. After liquefaction, samples were analyzed for Sperm motility and vitality according to World Health Organization (WHO) guidelines.⁶

Sperm Motility Procedure:

- ❖ The liquefied semen sample was mixed well.
- ❖ The analysis was carried out on 10 μ depth chamber which allows the spermatozoa to swim freely.
- ❖ A standard volume of semen, i. e. 10 μl, was placed onto a clean glass slide.
- ❖ It was covered with a cover slip which helps to spread the sample.
- ❖ The slide was examined with optics at ×200 or ×400 magnifications.
- ❖ At least 200 spermatozoa for the percentage of different motile categories were evaluated.

Categories of sperm movement:

- a) Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed.
- b) Non-progressive motility (NP): all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed.
- c) Immotility (IM): no movement.
 - ✘ Progressive and Non progressive motility gives the total motility of sperms.
 - ✘ The lower reference limit for total motility (PR + NP) is 40%

Sperm Vitality Procedure:

Vitality test using Eosin–Nigrosin:

Requirements: Liquefied semen, Reagents (Eosin reagent and Nigrosin Reagent)

Procedure:

1. The liquefied semen sample was mixed well.
2. One drop of liquefied semen was taken in a porcelain spot plate well.
3. Two drops of the eosin reagent was added to it and mixed well.
4. Three drops of nigrosin reagent was added to it and mixed well.
5. From the above suspension, a smear was made on a glass slide and allowed to dry in air.
6. It was examined immediately after drying.
7. The slide was examined under oil immersion.
8. 200 spermatozoa were evaluated.

Spermatozoa with red or dark pink heads were considered dead (membrane-damaged), whereas spermatozoa with white heads or light pink heads were considered alive (membrane intact).

The sperm vitality (membrane-intact spermatozoa) was expressed in percentage.

The lower reference limit for vitality (membrane-intact spermatozoa) is 58%.

RESULTS:

In the present study, all the calculations and statistics were done using Microsoft Excel 2007 and “graph pad prism 5 software” version 5.01 was used. A 'p' value of less than 0.05 ($p < 0.05$) was considered to be statistically significant. A 'p' value of less than 0.01 ($p < 0.01$) was considered to be statistically highly significant.

For each parameter, the mean value and standard deviation were calculated. Z test was applied to study the difference between Infertile Nonsmokers group and Infertile Smokers group. Sperm motility and vitality in all three groups of Infertile Smokers were compared using one way ANOVA (analysis of variance) test. The observations and results of the present study were tabulated as below:

Table 1. Comparison of sperm motility and sperm vitality in infertile nonsmokers and infertile smokers group :

Parameter	Nonsmokers N=50	Smokers N=50	P value
Sperm motility(%)	45.7 ± 18.84	35.6 ± 19.07	<0.01**
Sperm vitality(%)	51.14 ± 18.31	39.92± 19.34	<0.01**

* Data presented as Mean ± SD, ** Significant N = sample size

Table 2. Comparison of semen quality (Sperm motility and vitality) in three groups of infertile Smokers:

Group	N	Sperm motility(%)	P Value	Sperm vitality(%)	P Value
A	28	39.28±19.98	<0.05**	46.85±18.15	<0.01**
B	17	35.88±16.22		36.23±16.34	
C	5	14±5.47		13.6±5.41	

* Data presented as Mean ± SD, ** Significant. N = sample size

DISCUSSION:

Cigarette smoking is a serious health problem of most societies. Consumption of tobacco exerts widely adverse effects on different aspects of health.

In the present study, the mean values of sperm motility in infertile smokers group was 35.6 ± 19.07 and in nonsmokers group the value was 45.7 ± 18.84. The intergroup comparison of the sperm motility has shown that the sperm motility was decreased in infertile smokers group. The

difference of the sperm motility in both the groups was statistically significant (p<0.01) (Table 1).

The mean values of sperm motility in different groups of Infertile Smokers were:

- i) Group A (Mild smokers) – 39.28 ± 19.98
- ii) Group B (Moderate smokers) - 35.88 ± 16.22
- iii) Group C (Heavy smokers) - 14 ± 5.47

The Sperm motility decreased with the severity of smoking and these values were statistically significant (p<0.05) (Table 2).

The following studies support the same findings:

Kulikauskas et.al.⁷ showed that Cigarette smoking has been associated with alterations in motility.

Saaranen et al⁸ studied the motility decreased more rapidly in heavy Smokers (>16 cigarettes/day) than Non smokers.

Zhang et al⁹ showed that the forward progression of sperm was negatively correlated with the amount and duration of cigarette smoking.

Mehranian⁵ have also found similar results.

Cotinine concentration in seminal plasma are considered as a biomarker for smoking.¹⁰ The concentration of cotinine and hydroxy cotinine in the seminal plasma is significantly correlated with total motility of spermatozoa.¹¹

There is clear correlation between seminal plasma zinc levels and the extent of smoking.¹²

Decrease in Zn and Cu level leads to lowering of SOD activity which is closely related to Sperm motility. In addition, smoking increases the production of free radicals (ROS) that augment the consumption of superoxide dismutase SOD.

The sperm mitochondrial membrane is a major target of ROS.¹⁴

Disruption of sperm mitochondrial function directly affects sperm motility by decreasing the intra-cellular ATP level and also subsequently affects sperm motility.¹⁵

ROS induced peroxidation of sperm membrane decreases its flexibility and therefore tail motion.¹⁶

Also it has been found that smoking reduces sperm motility through increased seminal oxidative stress and DNA damage.¹⁷

The results obtained in the present study showed that the mean \pm SD of Sperm vitality in Nonsmoker men was 51.14 ± 18.31 and in Smoker men the value was 39.92 ± 19.34 . The intergroup comparison of the Sperm vitality has shown that the Sperm vitality was decreased in Infertile Smokers group. The difference of the mean Sperm vitality in both the groups was statistically significant ($p < 0.01$) (Table 1). The individuals with cigarette smoking thus are related with the reduced Sperm vitality.

The mean values of Sperm vitality in different groups of Infertile Smokers were:

- I) Group A (Mild smoker) - 46.85 ± 18.15
- ii) Group B (Moderate smokers) - 36.23 ± 16.34
- iii) Group C (Heavy smokers) - 13.6 ± 5.41

The Sperm vitality decreased in accordance with severity of smoking and these values were statistically significant ($p < 0.01$) (Table 2).

The above results of the present study are in accordance with the following studies:

Zavos et al¹⁸ found detrimental effects of smoker's seminal plasma on nonsmoker's sperm viability.

Zhang et al¹³ showed that the sperm viability was negatively correlated with the amount and duration of cigarette smoking.

Mehranian⁵ in his study showed that viability was much lower in the smokers than in the nonsmokers ($p < 0.01$).

In the category of environmental factors, nicotine was shown to be a potential oxidant agent, which affects plasma membrane and DNA integrity of sperms. In addition there was a strong negative correlation between lipid peroxidation (LPO) and percentage of viable sperm cells. Many studies have suggested a detrimental direct effect of Cotinine on sperm membrane permeability and sperm membrane function. Thus, the spermatozoa may not have optimal ability to undergo capacitation and hyperactivation within the female reproductive tract.¹⁹ Smoking causes impairment in membrane integrity by elevation in MDA (Malondialdehyde) levels which declines Sperm viability.²⁰

The presence of tobacco smoke constituents in seminal plasma could provide a warning of the adverse effects of cigarette smoke on the physiology of reproduction. Interventional steps can be taken to correct the effects and to quit smoking.

The clinical significance of the present finding is to develop effective interventions aimed at helping patients to stop smoking for the benefits of their general health and fertility. Hence we suggest that every smoker should be encouraged to stop smoking especially if pregnancy is planned.

CONCLUSION:

It is concluded that cigarette smoking adversely affects Sperm motility and vitality and in turn semen quality. Deterioration in semen quality appears in direct proportion to the number of cigarettes smoked.

REFERENCES:

1. Olayemi FO. A Review on some causes of male infertility. African Journal of Biotechnology. 2010 May 17;9(20): 2834-2842.
2. Gaur DS, Talekar MS, Pathak VP. Alcohol intake and cigarette smoking: Impact of two major lifestyle factors on male fertility. Indian journal of Pathology & Microbiology. 2010; 53(1): 35-40.
3. Brugo-Olmedo S, Chillik C, Kopelman S. Definition and causes of infertility. Reprod Biomed Online. 2001; 2(1):173-185
4. Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. Frontiers in Bioscience. 1996 Aug; 1:e78-86.
5. Mehrannia T. The effect of cigarette smoking on semen quality of infertile men. Pakistan journal of medical sciences quarterly. 2007 Oct-Dec;23(1)5:717-719.
6. WHO laboratory manual for the Examination and processing of human semen. Fifth edition: WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int), 2010: pp.10-32.
7. Kulikauskas V, Blaustein D, Ablin RJ. Cigarette smoking and its possible effects on sperm. Fertil Steril. 1985;44(4):526-528.
8. Saaranen.M, Suonio S, Kauhanen O, Saarikoski S. Cigarette smoking and semen quality in men of reproductive age. Andrologia. 1987 Nov-Dec;19(6):670-6.
9. Zhang JP, Meng QY, Wang Q, Zhang LJ, Mao YL, Sun ZX. Effect of smoking on semen quality of infertile men in Shandong, China. Asian j androl. 2000 Jun;2(2):143-6.
10. Hammadeh ME, Hamad MF, Montenarh M, Fischer-Hammadeh C. Protamine contents and P1/P2 ratio in human spermatozoa from smokers and non-smokers. Human Reproduction. 2010Nov; 25(11):2708–2720.
11. Pacifici R, Altieri I, Gandini L, Lenzi A, Pichini S, Rosa M et al. Nicotine, cotinine, and tans-3-hydroxycotinine levels in seminal plasma of smokers: Effects on sperm parameters. Ther Drug Monit. 1993 Oct;15(5):358-63.
12. Liu RZ, Gao JC, Zhang HG, Wang RX, Zhang ZH, Liu XY. Seminal plasma zinc level may be associated with the effect of cigarette smoking on sperm parameter. J int med res. 2010 May-Jun;38(3):923-8.
13. Zhang JP, Meng QY, Wang Q, Zhang LJ, Mao YL, Sun ZX. Effect of smoking on semen quality of infertile men in Shandong, China. Asian j androl. 2000 Jun;2(2):143-6.
14. Sikka SC. Relative impact of oxidative stress on male reproductive function. Curr Med Chem. 2001Jun;8(7): 851-62.

15. De Lamirande E, Jiang H, Zini A, Kodama H, Gagnon C. Reactive oxygen species and sperm physiology. *Rev Reprod.*1997 Jan;2(1): 48-54.
16. Jones R, Mann T, Sherins R. Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. *Fertil Steril.*1979 May;31(5): 531-537.
17. Mostafa T. Cigarette smoking & male infertility. *Journal Of Advance research.* 2010 July;1(3):179-186.
18. Zavos PM, Correa JR, Antypas S, Zarmakoupis-Zavos PN, Zarmakoupis CN. Effects of seminal plasma from cigarette smokers on sperm viability and longevity. *Fertil Steril.*1998 Mar;69(3):425-9.
19. Sofikitis N, Takenaka M, Kanakas N, Papadopoulos H, Yamamoto Y, Drakakis P et al. Effects of cotinine on sperm motility, membrane function, and fertilizing capacity in vitro. *Urol Res.* 2000 Dec;28(6):370–5.
20. Mehran A, The toxic effect of seminal plasma from smokers on sperm function in non-smokers. *Zhonghua Nan KeXue.* 2005 Sep;11(9):647-51.

Received on 12/07/2017 Revised on 28/08/2017 Accepted on 31/08/2017