

# A Comparative Research on Effects of Cigarette Smoking on Male Fertility Followed by the Fragmentation of DNA

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**Abstract:** *Cigarette smoking plays a major role in infertility of men due to presence of >4000 of chemical compounds which affects the DNA of male. Damaged DNA affects the concentration of sperm and its motility. Some time smoking can affect the morphological characteristics of sperms. Many studies shows that the chemical compounds present in cigarette such as nicotine, nitrosamines, polycyclic aromatic hydrocarbons attach with the membrane and starts doing abnormal activities in the body, specially related to the human reproductive system. It is better to stop the intake on cigarette for the betterment of reproductive health. Objectives: To find out the known effects of smoking on male infertility by comparing them with non smoking males.*

**Keywords:** Male infertility, DNA fragmentation, Reactive Oxygen Species (ROS)

## 1. Introduction

Smoking is the responsible for increasing lungs disease rates in developing countries and has a negative impact on reproduction and fertility in males. The semen of smokers creates poisonous compounds surroundings for sperm. Cigarette smoke consists of quite 4 thousand completely different chemical compounds for example; nicotine, nitrosamines, polycyclic aromatic hydrocarbons, cadmium and carbon monoxide. These all are responsible for sperm DNA damaging and harms the fertility of smokers. The sperm DNA integrity play key role in transmission of correct hereditary data, and the standard chromatin structure for the fertility of sperm. A study conducted by Evenson and Wixon in 2006, in which they explained that normal morphology of sperm is important in assisted reproductive technology (1)

Smoking can be one of the main reasons that lead to male infertility. Especially when it involves sperm DNA fragmentation. DNA damage can be evaluated by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay method, comet assay, and sperm chromatin structure assay (SCSA). When free radicals destroy Reactive Oxygen Species (ROS), DNA fragmentation occurs. When cells are damaged ROS stimulate antioxidant capacity to prevent oxidation, oxidative stress develop, and its ultimately caused programmed cell death. Morphological Nuclear integrity and motility of sperm cells are affected by smoking, and are associated with high levels of reactive oxygen species (ROS) and oxidative stress in sperm, so smoking can cause DNA fragmentation (2, 3).

Minor sperm DNA disruption can be able to renew by post-replication DNA repair mechanisms, but major DNA disruption not even possible to repair. Infertile men can have normal sperm with DNA damage which may result into miscarriage or child birth has severe or mild congenital malformations which may increased susceptibility to certain cancers (retinoblastoma) (4, 5).

Chromosomal mutations in men is one of the main reason of male infertility, DNA fragmentation is that the breaking of DNA strands into little fragments. Breaks can be occurs within the single or double stranded of the DNA and it's termed as "nicks", insertion or deletions. DNA fragmentation is associated with nicking endonuclease - mediated strand which may result into apoptosis (6).

## 2. Review of Literature

In year 1985 Kulikauskas et al. evaluated the sperm quality of 103 smokers and 135 non - smokers. It was found that the sperm density and motility of smokers were lower than those of non - smokers. There is no significant difference seen in Morphology of smokers and non - smokers semen sample. Smoke precipitates may be the cause of the decrease in sperm motility and density (7).

In 2017 Osama A studied on 37 infertile smokers and 45 non - smokers men. This study evaluated that Smoking has no effect on DNA fragmentation. 37.8% sperm DNA fragmentation was found in smokers and almost same 37.9% of sperm DNA fragmentation was found in non - smokers. The rate of DNA fragmentation was 45.7% in patients with non - motile form, and the DNA fragmentation rate was 32.7% in patients with the motile form. This suggests that if the DNA fragmentation is high, then the motility decreases which shows that DNA fragmentation has an impact on sperm count (8).

A study conducted in 2007 showed that smoking cigarettes affects the parameters of sperm such as smokers had a lower sperm count, less semen volume and motility of sperm decreases as compared to non - smokers (9).

In a 2015, a study by Boushaba and Belaaloui, revealed the link between DNA fragmentation and sperm parameters. 26 men, who were about 37 year old, underwent semen analysis for various parameters (motility, count, morphology) including DNA fragmentation analysis. According to this

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study, 19 males had 73% DNA fragmentation, and 7 males had 10.14% DNA fragmentation (10).

The study was conducted on 97 men from the age of 19 to 39. Of those, 69 were non - smokers, 17 were light smokers, and 11 were heavy smokers. As a result of the TUNEL assay method, there was no significant difference among the participants in terms of age or sperm parameters in the study. The analysis of the 97 males and their data showed that there were no significant differences in DNA fragmentation between the three groups. The percentage of fragmentation in non - smokers was 20.41 percent, light smokers had 11.66 percent, and smokers had 12.11 percent (11).

### 3. Material and Methods

The TUNEL test is processed according to the literature (procedure is not mentioned in this article). This study included 50 infertile men (25 smoker and 25 non - smoker). Samples were collected in the sterile container after 3 days abstinence. All samples DNA fragmentation evaluated by TUNEL assay method.

#### TUNEL Assay

The TUNEL test is a Nick End - labeled dUTP terminal deoxynucleotidyl transferase (TdT), which is used to detect apoptotic cells that undergo a large amount of DNA fragmentation in the last stage of apoptosis. The TUNEL test procedure is based on TdT and its ability to inactivate the 3' - hydroxyl end of double - stranded DNA (12).

DNA degrades with the help of biochemical marker of apoptosis enzyme endonuclease and then formed double stranded oligonucleotides fragments. DNA fragments are 180 to 200 bp in size. The TUNEL assay uses terminal deoxynucleotidyl transferase (TdT) to identify double - stranded blunt ends. A reaction is catalyzed by enzyme catalyzing addition of fluoresce in - labeled deoxynucleotides (dUTPs) to the DNA end, which can then be visualized using a fluorescent microscope and immunohistochemistry (13.)

#### Requirements

Semen sample, TUNEL Assay kit, 4% Paraformaldehyde.0.25 % Triton X - 100. Bovine serum albumin (BSA), Agar media, Makler counting chamber, tissue paper, Distilled water, Incubator (37°C), Hot plate, Eppendorfs, Centrifuge machine, heating blocks, Centrifuge tubes, Light microscope, Fluorescence microscope, Microscopic slides, Micropipettes and tips. Aluminum foil, marker, refrigerator, Computer, Computer analyze software.

### 4. Results and Discussion

The current research study included the 50 infertile men.50 Samples were examined for the DNA fragmentation in which 25 were collected from smokers and 25 were from non smokers. All the semen samples were processed

according to the literature of TUNEL Assay kit. Semen samples were categorized according to the age (22 - 30years, 30 - 38 years and 38 - 46 years), smokers and non smokers, smokers and non smoker with age groups. Smokers having the avg. DNA fragmentation rate of 36.71% and non smokers having the avg. DNA fragmentation rate of 39.60% (Chart 1.0).

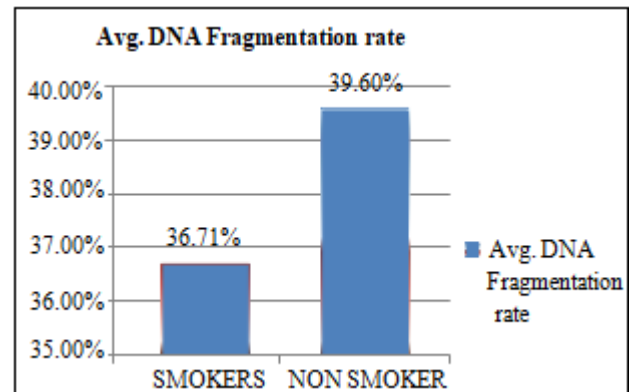


Chart 1: Avg. DNA fragmentation rate in between Smokers and Non - smokers

The significant difference in between the DNA fragmentation rate of smoker and non smokers is 2.89%. The DNA fragmentation rate also categorized according to the different age groups. Age group of 22 - 30 years (smokers), 30 - 38 years (smokers), 38 - 46 years (smokers) observed as an avg. DNA fragmentation rate of 34.73%, 36.71%, 39.79% respectively (Chart 2.0).

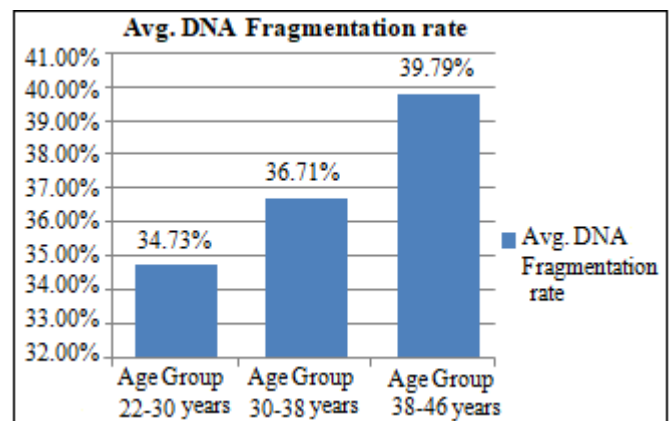
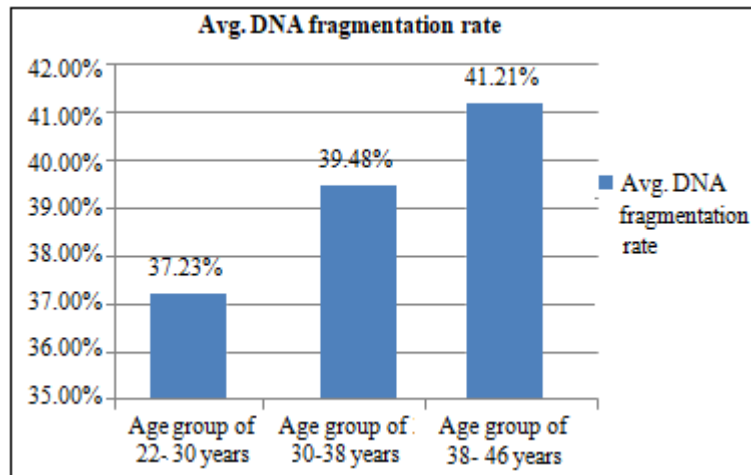


Chart 2: Avg. DNA fragmentation rate in between different age groups (Smokers)

Age group of 22 - 30 years (Non - smokers), 30 - 38 years (Non - smokers), 38 - 46 years (Non - smokers) observed as an avg. DNA fragmentation rate of 37.23%, 39.48%, 41.21% respectively (Chart 3.0). The lowest DNA fragmentation rate was found in Smoker males with age group of 22 - 30 years and the highest DNA fragmentation rate was observed in non smoker males with the age group of 38 - 46 years.



**Chart 3:** Avg. DNA fragmentation rate in between different age groups (Non - Smokers)

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