

ORIGINAL RESEARCH

The comparison of cigarette consumption towards semen analysis in Andrology Polyclinic of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia in 2017

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Article Info

Article history:

Received Jan 10, 2021

Revised Mar 24, 2021

Accepted Jun 9, 2021

Published Jul 1, 2021

Keywords:

Healthy lifestyle

Tobacco use

Tobacco addiction

Tobacco smoking

Sperm quality

Sperm quantity

Male infertility

Semen analysis

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ABSTRACT

Background: The habit of smoking tobacco is a lifestyle that has become a necessity for some people. Sometimes, social needs is the main reason for someone to have a smoking habit. Based on this problem, the level of cigarette consumption in the community has increased. Nowadays, the total number of couples visiting an infertility clinic is on the rise. This have led to an assumption among the community that smoking tobacco is one of the main factors causing male infertility. **Objective:** to compare light smokers and heavy smokers towards the results of semen analysis in infertile male patients. **Materials and Methods:** This study was an observational analytics, with a retrospective-cross sectional. The instrument used in this study was taken from medical records of the patients who visited the Andrology Clinic of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, for the period from January to December 2017. The observed semen analysis parameters were concentration, motility, and morphology for microscopic examination and liquefaction, pH, and volume for macroscopic examination, by following the inclusion and exclusion criteria. **Results:** Ninety-two samples of infertile male participants (50 light smokers and 42 heavy smokers) were included in this study. Semen analysis on its concentration, motility, spermatozoa morphology, as well as liquefaction, pH, and volume) towards the number of cigarette consumption showed no significant difference ($p > 0.05$) between light smokers and moderate smokers. **Conclusion:** There was no significant difference between the light smoker and heavy smoker groups on the results of the semen analysis.

How to cite:

Saputra, Y.W.E, Wigati, K.W., Yudiwati, R., Tirthaningsih, N.W. 2021. The comparison of cigarette consumption towards semen analysis in Andrology Polyclinic of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia in 2017. *Majalah Biomorfologi*, 31(2): 31-38.

Majalah Biomorfologi (Biomorphology Journal) p.ISSN:0215-8833, e.ISSN: 2716-0920

doi: [10.20473/mbiom.v31i2.2021.31-38](https://doi.org/10.20473/mbiom.v31i2.2021.31-38)



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BACKGROUND

Every married couple in the world would like to have children, but this does not correspond to world conditions, since infertility in the world itself is still a problem for married couples. It can be seen that one in seven couples worldwide suffer from fertility disorders (Jo, et al, 2015). The World Health Organization (WHO) has defined infertility as the inability of a couple to become pregnant after 12 months or more of regular unprotected sex (Kumar, et al., 2015). According to studies, about 48.5 million (or 15%) couples in the world suffer from infertility, and the proportion of men is 20-30% of cases of infertility and is about 40-50%. 50% of all possible cases may occur (Agarwal, et al., 2015), up to 2% have spermatozoa with suboptimal parameters (Kumar, et al., 2015). Although semen analysis is considered the gold standard test used to diagnose male fertility, it cannot determine if there are molecular abnormalities that could be causing unexplained infertility (Pizzol, et al., 2014).

Based on World Health Organization data, the total number of smokers in Indonesia ranks third after China and India, with 34.7% of the total population or around 82 million people (Apriora, et al., 2015). Tobacco smoking is considered to be one of the main factors that often causes male infertility (Daumler, et al., 2016), and about 120,000 young men (aged 30 to 50 years) in the UK are impotent because of the impact of this smoking habit. Infertility in men (about 50% of infertility cases between partners (Kovac, et al., 2013)) gradually leads to psychological effects such as depression or others, and this can be a potential sign of serious future consequences.

The effects of tobacco smoking on semen quality and semen quantity are now rarely studied in a coherent manner. Therefore, by carrying out a comparison of semen parameters between light smokers and heavy smokers, we aimed to systematically investigate the impact of tobacco cigarette consumption towards semen quality in infertile male participants.

OBJECTIVE

The purpose of this study was to compare light smokers and heavy smokers towards the results of semen analysis in infertile male patients who underwent semen analysis at the Andrology Polyclinic of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

MATERIALS AND METHODS

This study is an observational analytic study with clinical observational study in infertility patients who have a smoking habit. The sample used in this study was 92 infertile men, consisting of 50 light smokers (total cigarette consumption of 1-10 cigarettes a day) and 42 heavy smokers (total cigarette consumption of 11-20 cigarettes a day).

The subjects taken from medical record data were selected using inclusion criteria (patients who underwent semen analysis) and exclusion criteria (patients with no history of varicocele, patients with infection, patients with immunological disorders, endocrine disorders, iatrogenic disorders, systemic disorders, congenital abnormalities, and erectile dysfunction). The data that had been collected were examined by applying the Kolmogorov-Smirnov normality test. If the data distribution was not normal, the Mann-Whitney test would be carried out, but if the data distribution was normal, the hypothesis testing would be carried out using the unpaired t test. All test results were conducted by employing the Statistical Package for the Social Sciences (SPSS) application.

RESULTS

Frequency distribution of semen analysis results

The results of the study on 92 male patients with a diagnosis of infertility are displayed on the following frequency distribution data:

Table 1. Frequency distribution of semen analysis results

Category	Total (n)	Normal (n)	Abnormal (n)
Concentration	92	58	34
Motility	92	68	24
Morphology	92	23	69
Liquefaction	92	91	1
pH	92	83	9
Volume	92	86	6

The distribution data above shows that morphology has high abnormal values, while other categories have values within normal limits.

Comparative test results of cigarette consumption effect towards the results of semen analysis

Based on the results of the study on 92 male patients with a diagnosis of infertility, the comparative data between the number of cigarettes consumed and the results of semen analysis are obtained and displayed below.

Table 2. Comparative test results of cigarette consumption effect towards the results of semen analysis

Categories	1-10 cigarettes/day		11-20 cigarettes/day		p value
	N	Mean Rank	N	Mean Rank	
Concentration	50	46.61	42	46.37	0.996
Motility	50	48.09	42	44.61	0.530
Morphology	50	47.58	42	45.21	0.664
Liquefaction	50	46.30	42	46.74	0.919
Ph	50	45.92	42	47.19	0.811
Volume	50	2.948	42	2.866	0.170

The comparative test data above show that the effect of the number of cigarette consumption towards the results of the semen analysis which includes, concentration, motility, morphology, liquefaction, pH and volume have the overall value of $p > 0.005$. Therefore, it can be concluded that there is no significant difference between smokers who consume 1-10 cigarettes/day and those who consume 11-20 cigarettes/day.

DISCUSSION

Infertility cases alone do not close the possibility of occurring in men, because from research that has been done there are about 40-50% of cases found to be identified and obtained results that this male factor has contributed to the occurrence of infertility (Agarwal, et al., 2015), other studies show about 15% of people at the world level obtained results that infertility caused by smoking habits, where men contribute by 50-60%, most of the other reasons are due to the high risk of damage to male germ cells that accumulate in mutations in each division of spermatogenesis processes (Harlev, et al., 2015). Other research conducted has shown that there is evidence of a decline in the quality of human sperm that has increased at the world level over the last few times (Sengupta, et al., 2017). Some reports from Levine's group conducted systematic reviews and meta-regression analyses of the current state of sperm count in men. This comprehensive study involved a total of 42,935 men with samples taken over 40 years. In this report, they reported that there was a significant decrease between 50-60% in sperm count among men from North America, Europe, Australia, and New Zealand (Levine, et al., 2017). In addition, fertility disorders can also be caused by several factors such as exposure to occupational and industrial toxins, drugs consumed, smoking habits, stress levels, and radiation exposure that can cause hormonal changes that can lead to obesity, changes in spermatogenesis can also be one of the factors that can cause infertility (Szkodziak, et al., 2016; Kovac, et al., 2015). One has reported that this modern lifestyle factor, can be one of the factors that have a greater contribution to the occurrence of fertility disorders when compared to cases with the presence of genetic factors, and therefore, it requires special attention to be able to maintain better lifestyle habits (Dai, et al., 2015)

When a person is in the habit of smoking, cigarette smoke generated during smoking is a carcinogen and at the same time a somatic cell mutagen that can cause active and passive health problems in a person. This is evidenced by strong evidence presented in recent years, which has confirmed that the resulting cigarette smoke causes direct active problems, which can include various types of cancer and cardiovascular disease, but if passive effects arise from the smoking habit. This is similar to the existence of Reproductive health problems that may arise from this smoking habit, which has not been specifically reported (Sharma, et al., 2016). Recent research has shown that smoking can affect sperm count, sperm motility, and sperm morphology. In addition, smoking can also increase the oxidative state of sperm, which can cause fragmentation of sperm DNA and damage to sperm DNA, and this may be one of the reasons why a person experiences infertility problems (Dai, et al., 2015; Sharma, et al., 2016).

Assessment of sperm parameters includes sperm count, sperm morphology, and different types of motility (Agarwal, et al., 2015). One study found that the percentage of rapid motility and sperm concentration in the infertile group that included smokers was lower than in the infertile group that did not smoke. Based on strict criteria, Kruger confirmed that the percentage of normal sperm morphology in infertile smokers is relatively lower than in other groups. In addition to infertile smokers, infertile non-smokers obtained results that showed that sperm morphology was lower compared to fertile smokers and control subjects. In addition to morphology, the semen volume score was also low in infertile smokers with an average ejaculation volume of 1.1 ml and a minimum of 0.4 ml. But this is different in fertile smokers and infertile nonsmokers, with sperm volume being close to the results of control subjects (non-fertile smokers), which were considered normal according to the WHO standard sperm parameters (Ranganathan, et al., 2018).

A meta-analytical study that has ever been conducted with men from 26 countries/regions has shown that a person who has a smoking habit causes a decrease in sperm quality in fertile men and infertile men (Li, et al., 2011). Results. The sperm concentration in male smokers is usually 13-17% lower than in non-smokers. In addition, smoking adversely affects sperm count, motility, morphology. Decreased sperm quality was also found more often in men with heavy smokers (> 20 cigarettes per day) and moderate smokers (10-20 cigarettes per day) compared with men who smoked lightly (1-10 cigarettes per day). This higher effect size in infertile men compared to the general population (Sharma, et al., 2016).

Studies in animal models of mice have shown that Cd²⁺ can influence and modulate calcium (Ca²⁺), potassium (K⁺), and zinc (Zn²⁺) channels present in sperm semen (Zakaria, et al., 2015). affect sperm capacity and fertilization activity, and in the case of many primary acrosomes, this reaction causes damage to mature sperm (Wang, et al., 2017). In particular, an increase in Cd levels can impair sperm motility and can also affect sperm concentration in some men who are associated with oligoasthenospermia (Basse, et al., 2013). Studies report that the released Cd²⁺ can deplete the concentration of Zn²⁺ in the seed, altering Ca²⁺ in the ATPase system and affecting sperm motility. In addition, if there is a decrease in the quality of the cement, it will be due to the suppression of the Cd antioxidants. However, the effect of cadmium on cement quality is poorly understood (Gilany, et al., 2015).

In addition to being found in cigarettes, this zinc content is an important trace mineral that usually accumulates in the testes during the primary spermatogenesis process and is a key factor in regulating the proliferation of spermatogonial cells and is usually able to maintain germ cells in the testes. meiotic stage (Sridharan, et al., 2018). The analysis of markers, which was carried out on seeds, shows that at lower concentrations of zinc in the seed, a negative correlation with the status of ROS levels (high) in infertile smokers will be negative. This zinc ion is usually required to maintain the stability of the sperm membrane across the sulfhydryl bridge located in the middle of the sperm (Miyamoto, et al., 2017). In addition, the negative correlation between zinc and ROS observed among infertile smokers may play an important role in impaired fertility. In addition, this zinc concentration may also play an important role in spermatogenesis, prostate secretion (Jahan, et al., 2014), and sperm chromatin physiology (Zhu, et al., 2013), as well as testicular function (Zhao, et al., 2016). Consequently, a decrease in the level of zinc in semen can impair all sperm functions. In healthy people, this seminal zinc usually promotes the development of seminal proteins such as seminogelin (I and II) and other enzymatic proteins capable of supporting sperm.

In this case, according to the studies carried out, it is argued that the enzymatic function of the seed protein should be reversed due to the absence of zinc ion in the seminal plasma, therefore, in this case,

this will lead to a violation of the morphology. to maintain normal sperm in infertile smokers (Ranganathan, et al., 2018). This total seminal protein can also help maintain sperm fluidity and may also aid in the movement of sperm to the egg, passing through the process of overactive capacity with the help of seminal zinc (the "coagulation" process). But in infertile smokers, both due to semen content, zinc content, and an increase in total protein, it is expected that a decrease in prostate secretion will lead to changes in testicular function (Jahan, et al., 2014). Another study also showed that smoking lowers the level of DNA repair proteins that are found in seminal plasma, and these damaged proteins (such as the Chk1 protein) cannot return to their original form and begin to induce apoptosis in sperm. Semen protein levels are dependent on seminal plasma zinc (Cui, et al., 2016). Studies have also shown that infertile smokers will have very low total seminal protein, and if the zinc concentration is high, this will usually maintain the fluidity of the protein in the seminal plasma, but when the Zn concentration decreases, the protein content in the semen decreases and eventually ceases to support sperm (Ranganathan, et al., 2018). In addition to zinc content, free radicals (ROS) and antioxidants also affect the total amount of protein in sperm (Chen, et al., 2017).

Clinical studies have shown that in infertile smokers with changes in sperm morphology, namely, with damaged heads, midsections, tails of spermatozoa with high slow sperm motility, this leads to the impossibility of ovulation or natural pregnancy. In this case, the only way to rely on artificial methods of conception is IVF and GIFT. However, in some cases, this procedure can potentially lead to an abnormal shape of the fetus (Davar, et al., 2012). Also, studies conducted in animal models of mice revealed an abnormal sperm head shape and sperm count obtained after daily consumption of tobacco-rich cigarettes (Wu, et al., 2017). Studies report that smoking has a negative effect on sperm morphology in immobility or impotence in some individuals. In our study, sperm morphology showed that between the three groups of subjects, the results were not significant between the groups of subjects. The significant effect of this smoking habit has strong evidence that the group of infertile smokers has comparatively higher scores for abnormal sperm morphology, such as tubeloid shape, altered shape, striped tail with protruding head and cross-section. Damaged middle mitochondria, which will eventually be able to produce slow motility or in immotile sperm (Kumar, et al., 2015). Our analysis of these semen parameters, as well as the effects of smoking, is consistent with previous studies (Harlev, et al., 2015; Adewoyin, et al., 2017; Bisht, et al., 2017), which states that (specify what they report). Although several reports also report on the negative impact of smoking on sperm quality (Lotti, et al., 2015; Povey, et al., 2012). To further validate our claim and reinforce our findings, we conducted an original study of markers from all three groups of active smokers.

Previous research has shown that this antioxidant is useful in balancing homeostatic mechanisms and has the function of protecting the functional integrity of the sperm from damage to the sperm. This will have adverse consequences if someone has a smoking habit because this smoking habit creates physiological oxidants that can cause an antioxidant imbalance in the sperm nucleus, which can cause damage to sperm DNA (Parameswari, et al., 2016). However, various enzymatic (SOD, CAT) and non-enzymatic (Vit-C and E) antioxidants, in which seminal plasma usually protects sperm from O₂ toxicity, and are also capable of inactivating ROS, which can be damaged by lipid peroxidation processes. Analysis of enzymatic and non-enzymatic antioxidants usually shows a greater decrease in vitamin E in the infertile smoker group compared to other groups, followed by a decrease in catalase levels. Another study found that the net effect of smoking would lead to a decrease in SOD catalase, while at the same time Vit-E was reduced compared to Vit-C and could be detected within 12 hours of poisoning. Likewise, an antioxidant assessment in the test groups showed lower levels of antioxidants, Vit-C, and SOD with increased ROS levels in infertile smokers, which should be the reason for the decline in sperm morphology and sperm count by the results. In addition, in the setting of elevated ROS levels observed in the group of infertile smokers, this should affect polyunsaturated fatty acids and phospholipids, which help maintain the acrosomal part in sperm and may cause changes in sperm morphology (Zhao, et al., 2016).

Lead and cadmium have been identified as toxins from smoking that can affect reproductive disorders (Harlev, et al., 2015). The study showed that among all the toxic substances for cadmium, it can be established that it is significantly toxic in the analysis of antioxidant recovery, in terms of sperm parameters and seminal markers, compared to the content of other contained metals. Concerning the level of cadmium concentration in sperm parameters in all groups of subjects, a high positive correlation of Cd with ROS was reported in the state of a person with a sterile smoker, while the results were lower for the toxic effect (Ranganathan, et al., 2018). In vivo experiments, which were carried out on mice as

animal models exposed to cigarette smoke, leading to damage to testicular cells, which was also accompanied by confirmation of histopathological studies of the testes of mice, which showed that in the lower Leydig cells and are damaged in the presence of increased content of cadmium (Jahan, et al., 2014).

The overall effect of smoking on male fertility may be due to a combination of increased oxidative stress, DNA damage, and cell apoptosis, which may not only explain decreased sperm quality, but also impaired spermatogenesis. maturation of sperm and sperm. Factors that may contribute to this effect in male smokers include the presence of nicotine and its metabolites, cotinine, benzo (a) pyrene, and cadmium levels (Dai, et al., 2015). For example, a meta-analysis of 13,317 men found that smoking was associated with higher mean testosterone levels, which could be explained by inhibition of testosterone breakdown by cotinine (Zhao et al., 2016).

Based on the results of this study on the parameters of semen analysis obtained the results of no meaningful difference between light smokers (the amount of cigarette consumption 1-10 cigarettes per day) and heavy smokers (the amount of cigarette consumption 11-20 cigarettes per day). While there has not been a significant potential link between smoking and infertility incidence in men, some of the available evidence on this study of male smoking and fertility may support recommendations for a person to quit smoking habit and reduce exposure to tobacco smoke among couples trying to conceive.

CONCLUSION

This study showed that there was no significant comparison of semen (concentration, motility, morphology of spermatozoa, liquefaction, pH, and volume of semen) in the male patients diagnosed with infertility who performed semen analysis tests at the andrology polyclinic of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, in the period of January to December 2017, with a total of 92 samples (50 light smokers and 42 heavy smokers). There are still not many studies that focus on comparing the number of cigarette consumption to the occurrence of infertility in men. However, in short, it can be concluded that smoking affects the results of semen analyzes in infertile men. Therefore, men with fertility difficulties should be educated to stop smoking as soon as possible in order to optimize their reproductive potential.

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