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Decrease in the Count of Spermatogonium in Male Mice (Mus Musculus) That Exposed To Cigarette Smoke

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Abstract : *There has been an increase in smoking habits in Indonesia and some other developing countries. The main component of cigarette is nicotine of 50% which is absorbed through the respiratory tract, mouth mucus, and skin. The effect of cigarette smoke can spoil sperm viability and spermatogenesis. Free radicals contained in cigarette smoke can cause oxidative stress, oxidative stress is a major factor of infertility in men. Oxidative stress is caused by an increase in ROS (Reactive Oxygen Species) which will leads to agglutination of sperm that can decrease sperm count. This study was using a completely randomized factorial design. The study subjects are 60 male mice, which were randomly divided into 5 group. The first group was the who received treatment (exposure to cigarette smoke in 0 minutes), the second group (exposure to cigarette smoke in 5 minutes), and group III (exposure to cigarette smoke in 10 minutes). The last group (exposure to cigarette smoke in 15 minutes). After 35 days of treatment, further analyzing the number of spermatogonium cells. The result of the one way Anova showed that the spermatogonium, had a statistically significant difference on some experimental groups (p<0.05). Based on LSD test showed that there was significant difference between exposed to cigarette smoke in 0, 5, 10, 15 minutes for 35 days. The conclusion of this research was that exposure to cigarette smoke can reduce the count of spermatogonium.*

Keywords – *Cigarette smoke, Spermatogonium*

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I. INTRODUCTION

Indonesia is one of the largest consumer countries in the world. According to the World Bank, cigarette consumption in Indonesia is around 6.6% of the world's total consumption. Indonesia's cigarette consumption in the last 30 years has increased sharply, from 33 billion cigarettes per year in 1970 to 230 billion cigarettes in 2006. Indonesia ranked fifth in world cigarette consumption, and ranked 7th in tobacco producers. The causes of infertility vary widely, but in men can be classified effect to disruption of sperm transport and impaired sperm function. Modern lifestyles are highly influential to the high rate of infertility associated with increased exposure to oxidative stress, such as the use of electronic devices with radiation wave emissions, high pollution levels and varied and less healthy foods [1].

Smoking can affect the quality and quantity of sperm, in cases of infertility, sperm analysis results from patients who smoke showed a concentration abnormalities, followed by morphology and motility of sperm.. Cigarette smoke which is the result of burning from cigarettes has been found to contain harmful chemicals other than nicotine, tar and nitrosamine, also found carcinogens and mutagens such as polonium, benzo (a) pyrene, dimethyl benz (a) anthracen, dimethyl nitrosamine, and napthalane. While CO2, H2O, NOx, Sox, and CO are compounds of cigarette smoke and mercury, lead, and cadmium are also found in cigarette smoke [2].

ROS (Reactive Oxygen Species) produced by cigarette smoke is one of the free radicals and can cause damage to spermatozoa DNA, resulting in increased apoptosis of this cell. When overproduction of ROS and weak antioxidant defense mechanisms, oxidative stress can occur, so it will be harmful to spermatozoa. For that needed a cleaning system that can neutralize the effects of ROS in the form of antioxidants [3].

Over-production of reactive free radicals or oxygen (ROS, reactive oxygen species) can damage sperm and ROS has been recognized as one of the causes of infertility. Free radicals are physiologically present in human sperm [4]. The onset of free radicals in the body is accompanied by an endogenous defense mechanism, by producing substances that have an anti-free radical effect called antioxidants. However, as the ROS level rises beyond the body's antioxidant defense system, oxidative stress occurs. Free radicals from cigarette gas particles also cause sperm agglutination resulting in decreased spermatogenesis of sperm (i.e., [5]).

II. EXPERIMENTAL DETAILS

This research is true experimental research using Randomized Completely Randomized Design (RALF). This research was conducted in Immunobiology laboratory of University of Mataram and examination of spermatid done in Pathology Laboratory of Faculty of Veterinary Medicine of Udayana University, with span of time around February until Maret 2018.The population of this study was male mice (Mus musculus), while the sample in this study was adult male mice Strain Balb-C as much as 60 tail.

Then 15 mice in each group were treated. The mice were inserted into a rubber tub and then covered with a plastic enclosure that had been connected to the aerator, the exposure was carried out for 0, 5, 10, 15 minutes, the smoke exposure procedure was equal to group I, II, III, and IV. After 35 days of exposure to cigarette smoke 1 cigarette per head per day,analyzed to compare the number of spermatogonia.

Preparation of microanatomical using paraffin method and staining of hematoxylin eosin. The testicular microanatomical preparation is then observed under a microscope using 10 × 40 enlarged and photographed. Observations were made on the seminiferus tubules which were cut off and taken randomly. Cell calculations start from the top left, right top, bottom and center. The parameters observed include the count of spermatid cells. All data were analyzed using ANOVA analysis and continued with LSD test.

III. RESULT AND DISCUSSION

The average number of spermatogonium cells after exposure for 5, 10, and 15 in each treatment group for 35 days, decreased, can be seen by comparing with the control group. The decrease in the number of spermatogonium cells can be illustrated in the bar chart below.

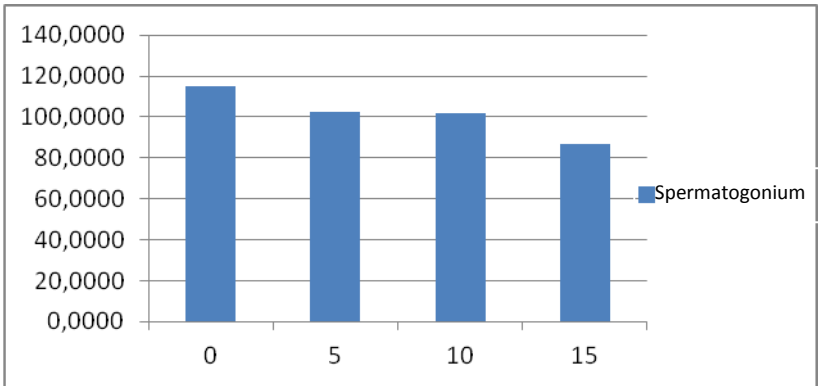
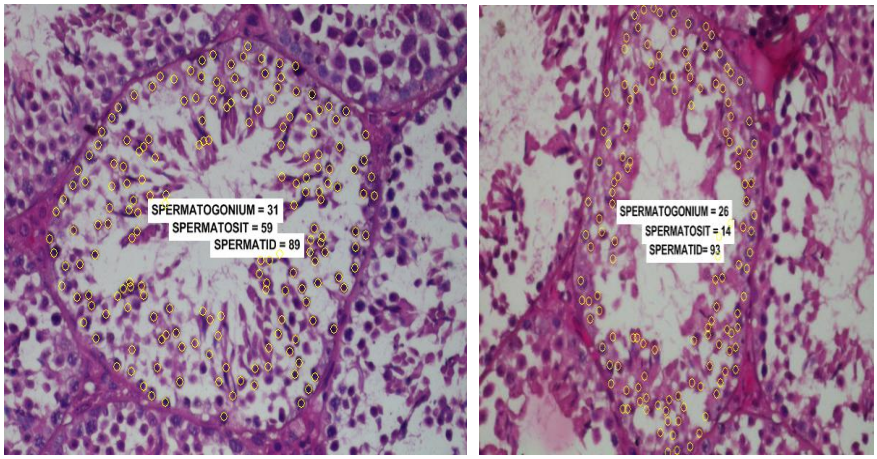
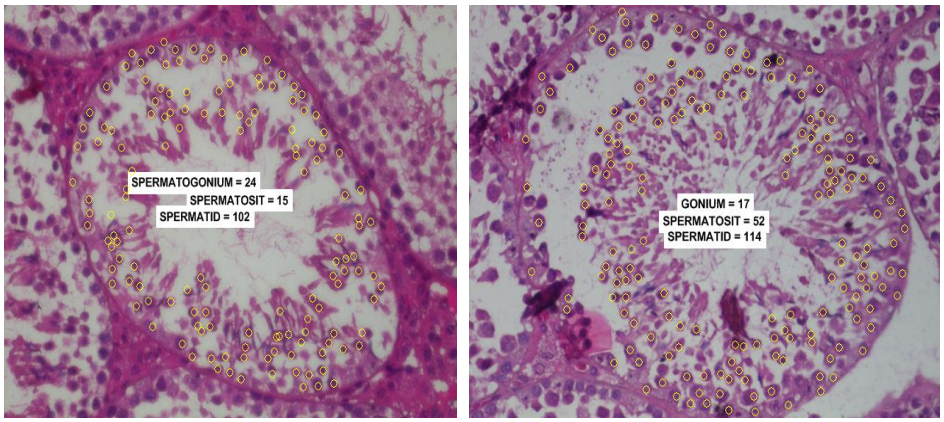


Figure 1. The stem cell spermatogonium stem diagram after exposure to cigarette smoke for 0, 5, 10, 15 minutes for 35 days.



0 minutes **5 minutes**



10 minutes 15 minutes

Figure 2 Histology of spermatogonia in Tubulus Seminiferus

The histologic features of the cigarette smoke exposure treatment group for 0, 5, 10, 15 minutes for 35 days, can be seen in Fig.2, the spermatogonium, spermatocyte, and spermatid sequence counts. the arrangement of loose and irregular spermatogenic cells, the density of spermatozoa in the tubular lumen unlike in the control group (O), spermatogenic in the treatment group appears not full, compared with the control group seen a decrease in the number of spermatogenic cells.

Table 1 The average number of spermatogonia against exposure smoke of cigarette

Smoke of Cigarette	Number of Spermatogonia
0 minutes	115.000
5 minutes	103.676
10 minutes	102.400
15 minutes	86.933

In (Table 1) it can be seen that the mean value of the sum spermatogonium control group (0 min) was 115.000 / cc, group II (5 min) with spermatogonium 103,676 / cc, group III (10 min) spermatogonium count 102.40 / cc, and group IV (15 min) was 86,933 / cc. it is clear that the longer the exposure to secondhand smoke decreases the number of spermatogonia and disrupts the spermatogenesis process.

Based on existing research results, 60 mice were taken as experimental animals, 15 mice in group I and 15 mice in group II, and 15 mice in group III and 15 mice in group IV. In this study, the average spermatids in groups I, II, III and IV had significant differences. This is shown in Fig.1 and it appears that groups II, III, and IV have decreased spermatogonium counts compared to group I or control. A decrease in the quality of spermatozoa is caused by oxidative stress caused by increased ROS (Reactive Oxygen Species) of cigarette smoke that will cause DNA damage and ultimately apoptosis of spermatozoa [6].

Nicotine is one of the free radicals produced by cigarettes. Nicotine by its toxic properties can decrease the number of spermatogenic cells directly through the hormonal pathway can also be through cell damage and death due to the effects of nicotine given are pro-oxidant. Pro-oxidants that enter the body cause an imbalance between pro-oxidants and antioxidants resulting in oxidative stress. Oxidative stress due to nicotine exposure will cause apoptosis in spermatogenesis cells in semi-niferus tubules by increasing the BAX ratio, this BAX protein will suppress BCL-2 activity in the mitochondrial membrane resulting in changes in membrane permeability of the mitochondria, this change results in the release of cytokrom- c to the cytosol, in the cytokol-c cytosol will activate Apaf-1 which will activate this caspase cascade and the active kaspase will result in DNA-se, then the active DNA-se penetrates the nuclear membrane and damages the DNA, so that the cell DNA is damaged or fragmented and eventually cells experience death or apoptosis. The apoptosis will cause germ cells to become atresia and their numbers decrease (i.e., [7]).

The results of the study for the control group or group that was not given exposure to cigarette smoke, obtained the number of sperm-togenik cells are different, because each mice have genetic material composing spermatogenesis different. Theoretically, primordial germ cells male mice appear around 8 days of pregnancy, with only 100 counts being the beginning of millions of spermatozoa to be produced and still in the extra gonad region. Day 9 and 10 of pregnancy are partially degenerated and some are proliferated and even move on the 11^{th} and 12^{th} days into the genetal area, at that time the average number is about 5000, but each number is different [8].

IV. CONCLUSION

Free radicals contained in cigarette smoke can cause oxidative stress, oxidative stress is a major factor of infertility in men. Oxidative stress is caused by an increase in ROS (Reactive Oxygen Species) which will leads to agglutination of sperm that can decrease sperm count. The minimum administration time (5 minutes) has shown a decrease in the number of spermatogonium cells in male mice (Mus musculus). A very significant difference in effect for primary spermatogenesis cells is 5 to 15 minutes of treatment.

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