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The Effect of Consuming Avocado (*Persea americana*) on Mice (*Mus musculus*) Sperm Quality

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Abstract

The nutrients such as protein and vitamin are proven to improve the sperm quality. One nutrient rich fruit is avocado round green variety, which contains vitamins A, C and E higher than other varieties. This study aimed to determine the effect of consuming avocado on the mice sperm quality. This experimental study was using a Completely Randomized Design with four treatments, each with eight replications. Avocado dosage treatments were control, 75 % w/v, 100% w/v, and 133% w/v , with the number of mice were 32 age four weeks. Avocado was given three times a day, each 0.5 mL for six weeks besides the main food. The observed response was the sperm quality including number, motility and morphology. Sperm was taken from the epididymis after the mice were killed by cervical dislocation method. Sperm was made a suspension using 0.9% NaCl solution and a smear preparation to observe the sperm morphology while staining with basic stain crystal violet. The sperm quality was observed by Neubauer's counting rooms through a multimedia microscope. Data was analyzed using Kurskal Wallis test for the sperm number and Anova test for motility and morphological responses. The result showed that avocado had a significant influence for reproductive health, particularly for increasing spermatozoa quality, including concentration, motility and morphology of sperm. The higher dose of avocado given the higher quantity and quality of sperm resulted.

Keywords : Avocado, lipids, mice, sperm, quality, vitamins

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1. Introduction

The male infertility is intended as a condition for men who are unable to impregnate their partners for at least one year after intercourse without using a protector [1]. This is caused by abnormalities in several parameters including volume, pH, viscosity of semen and the sperm quality [2]. Specifically, the sperm quality often used to assess whether a person's sperm normal or not are the amount / concentration, motility and morphology. The sperm is normal if the concentration is > 15 million / mL, progressive (forward and fast) sperm are > 32% and has a normal head, neck and tail morphology > 4% [3]. The concentration abnormality and the sperm motility cause sperm unable to fertilize the ovum, so that a person becomes infertile [4] .

The condition is caused by several factors such as ranging from lifestyle, diet or dietary habit, and age [5] [6] to genetic, especially genes found in the AZF region in the long arm of Y chromosome [7]. One effort to improve infertility is by providing balanced input of nutrients such as from medicinal plant [8]. One type of food that functions for reproductive health is fruits. Avocado (*Persea americana*) is a fruit has the potential to improve reproductive health because it contains protein, vitamins and minerals that can improve the sperm quality.

Avocado (*Persea americana*) is commonly consumed by people as beverage mix. Some people also often consume it simultaneously with milk or other sweeteners. This habit leads to misperception about the benefit of avocado for health. People assume that avocado contains high fat which can increase body weight. Otherwise, the pure avocado's fruit contains a lot of beneficial components such as vitamins A, B, C, E, K which are very high and B6, thiamine, riboflavin, niacin, as well as dietary fiber, potassium, folate [9][10]. United States Department of Agriculture presented the nutritional composition in 100 g of fruit flesh consisted of protein 1.96 g, carbohydrate 8.64 g, total fat 15.4 g, vitamin C 8.8 mg, Vitamin E 1.97 mg, vitamin

K 21 mg. Avocado is also dominant with unsaturated fatty acid content, namely monounsaturation of 9.8 g and polyunsaturation of 1.82 g of saturated fatty acids 2.13 g [11] in addition to ascorbic acid 9, 37 mg [12]. Previous studies have shown that vitamin intake can affect sperm, which help increase production. Lack of vitamins A, B, C and E in the body can reduce the number and quality of sperm [13]. Recovery of infertility in men can also be done by administering vitamin E at a dose of 100 mg / kg / day which has an effect on increasing testicular weight, sperm count, sperm motility, and estrogen production, but also increasing the survival and development of sperm [14]. The protein content in avocado is also beneficial for sperm. Protein functions to protect the sperm plasma membrane during spermatogenesis in the testes producing spermatozoa with normal morphology [15]. Avocado also contains high fatty acids, especially unsaturated fatty acid groups. Unsaturated fatty acids are useful for improving the sperm quality [16].

The avocado of green round variety has higher vitamin A, C and E content compared to other avocado varieties. In 100 gr of round green avocado flesh contains vitamin E 79.45 mg, vitamin A 4121 mg and vitamin C 71.02 mg. While in 100 grams of avocado long green varieties contain vitamin E 40.24, vitamin A 832 mg and vitamin C 49.69 mg [17]. Based on the nutritional values in each variety, we used round green varieties. This research is expected to add data on the fruit potential for health, especially fertility. The purpose of this study is to determine the effect of consumption of avocados (*Persea americana*) on the sperm quality in mice *(Mus musculus)*.

2. Materials and Methods

This research is an experimental study, using a completely randomized design (CRD) with 4 treatments each with 6 replications. The concentration of avocado doses used were control (P1), 75 % w/v (P2), 100% w/v (P3), and 133% w/v (P4). Avocado used is ripe avocado picked from trees with the slippery fruit skin characteristic. The Avocado pulp is separated, dissolved with aquadest. Determination of concentration based on LD_{50} test result. The used animal in this study is male mice (*M. musculus*) aged 4 weeks and an average body weight of 10 - 11 grams, healthy, and active as many as 24 individuals. Mice were kept in cages made of plastic tubs measuring 35 cm × 20 cm × 17 cm that were given a base of sawdust and given a wire cap. Each cage contained 4 animals and placed in the same place. Before being treated, all mice were acclimatized for a week and avocado filtered first before it given. Each concentration of avocado was given in each group of mice for 6 weeks orally using 0.5 ml Sonde 3 times a day while for it control not awarded avocado. In addition to treatment with avocado, all mice were fed the pellet and drunk aquadest *ed libitum*.

After the treatment, all mice were killed using the cervical dislocation method and the epididymis was taken. The epididymis was cut into small pieces using scissors and then placed in a petri dish containing 1 mL of 0.9% physiological NaCl and stirred with a stirring rod to form a suspension of sperm. The sperm observation included concentration, motility, and morphology of sperm using a microscope. The sperm concentration was calculated using the Neubauer counting chamber. One drop of sperm suspension (10 µL) was dropped in the Neubarer counting chamber, then covered with a microscope slide. After the preparation has spread, the observation was carried out with an enlargement of 40 × 10 times. The sperm concentration (million / mL) was calculated from the dilution factor × number of observations × 0.05 × 10^6. The sperm motility was observed by means of 10 µL suspensions of sperm dripped on microscope slide, covered it with the other slide and observed with magnification 40 × 10 times. The movement categories observed are: a) progressive, that was sperm moving actively or rapidly either linearly or in large circles, b) non-progressive, i.e. sperm swimming in a small circle, or only moving flagella, and c) immortality (IM), i.e., sperm do not move. The sperm motility was calculated by dividing the number of spermatozoa category a + b by category a + b + c multiplied by 100%. Before observing the morphology of sperm, a smear preparation was made by dripping one drop of sperm suspension on the slide and allowed to dry. Next the slide was immersed in absolute methanol for 5 minutes, rinsed with distilled water and dried. The slide continued to soak in safranin solution for 5 minutes and 3 times immersion in phosphate buffer solution and in violet crystal solution for 5 minutes. After rinsing with running and dry water, sperm were observed under a microscope at 1,000 times magnification and 100 sperm were observed.

The normal sperm has hook-like head, tapered and curved, not circular, and a long, straight tail. Abnormal sperm was assessed from the shape of the head, neck and tail. Referring to [3] before, some abnormalities in the head shapes are double, amorphous, micro, round, while abnormal tail includes short, double, broken, angular, and looped tails. Some abnormal sperms that found in neck shapes such as asymmetrical enter the head of sperm, thick, and sharp curved. The percentage of normal sperm count was calculated from the division of normal-form sperm with all observed sperm multiplied by 100%. The sperm concentration data is analysed using the Kurskal Wallis test while motility and morphological data used the Anova test.

3. Results and Discussion

The observation result of mice sperm concentration after consuming avocados were shown in Figure 1. Based on the average sperm concentration showed that the

more concentrated the avocado given higher sperm concentration. The highest concentration of sperm (288.75 million / mL) lied in the 133% w/v avocado treatment, while the lowest concentration was carried out in the control group (58.53 million / mL). This condition was reinforced by the result of Kuskal Wallis test showed that there were significant differences ($p < 0.05$) between the treatment and control groups. Furthermore, Duncan's further test results (Table 1) showed that the 75 % w/v and 100% w/v treatments were not different but different from the 133% w/v treatment.

The same thing also appears to the effect of feeding avocado on sperm motility as it was in Figure. 2. The higher avoidance of the avocados dose the higher average percentage of sperm motion. Avocado doses of 133% w/v have the average percentage of the highest progressive motility (95.63%) compared to other doses. In contrast, the control group had the average percentage of the most progressive lowest motility (86.13%).

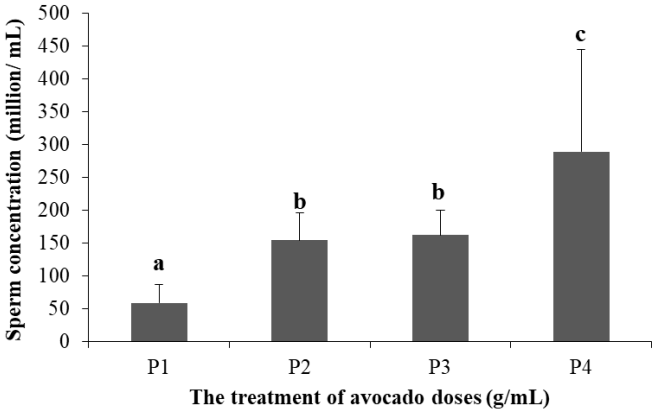

Figure 1. The average of sperm concentration after consuming different doses of avocado. P1= control; P2= 75 % w/v; P3= 100% w/v; and P4= 133% w/v.

The Anova test result for the average motility of sperm after avocado administration showed a significant difference ($p < 0.05$) compared to the control group. While Duncan's further test result showed that the treatment between 100% w/v and 133% w/v was not different, but it was different from the treatment of 75% w/v.

The sperm morphology result as an effect of avocado administration was presented in Figure 3. Like the observations of the two previous indicators (spermatozoa concentration and motility), the 133% w/v treatment had the highest average percentage of normal sperm morphology while the control treatment was average lowest average.

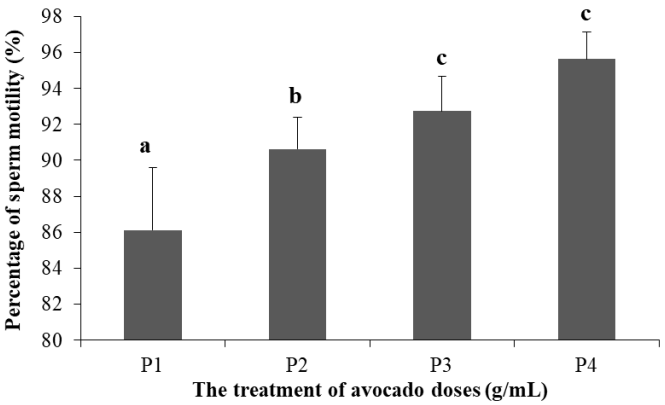

Figure 2. The percentage of sperm motility after consuming different doses of avocado .P1= control; P2= 75 % w/v; P3= 100% w/v; and P4= 133% w/v.

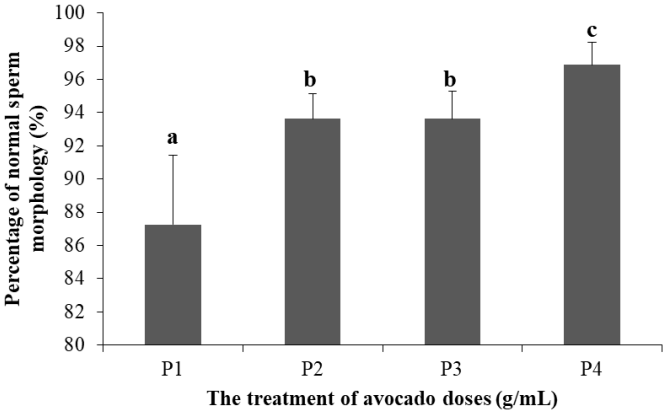

Figure. 3. The percentage of normal sperm morphology after consuming different doses of avocado. P1= control; P2= 75 % w/v; P3= 100% w/v; and P4= 133% w/v.

Anova test result also showed a significant difference between avocado treatments compared to control. Duncan's further test result showed the same condition as the sperm concentration indicator, in which 75% w/v treatment did not differ from 100% w/v treatment but differed from 133% w/v. These observations indicated that the density of the avocado dose given affects the sperm quality.

In addition to normal sperm, abnormal observation was also found in sperm as shown in Figure 4. The normal sperm had a head with a tapered end, and a long and straight tail. The abnormal sperm had the small or rounded head, while the short tail was bent or curled. The observa-

tion found sperm with abnormal morphological form of curled tail, bent neck and spiral forming tail.

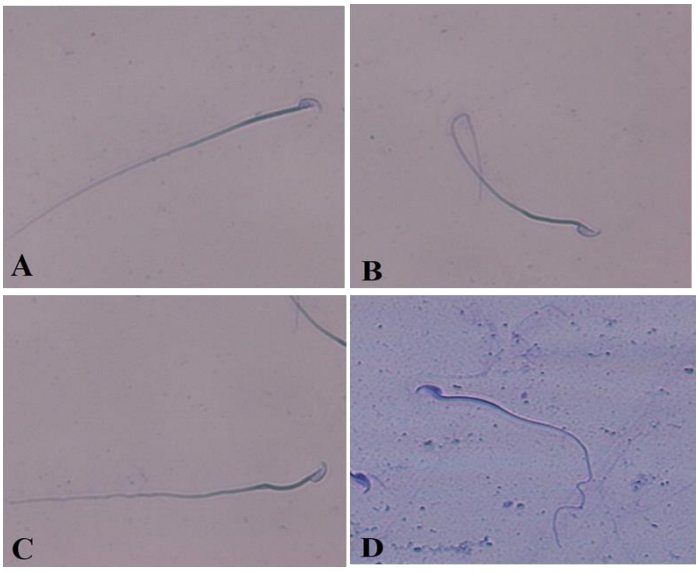

Figure 4. Morphology of sperm *M.musculus* after administration of avocado, magnification 100×. A) Normal sperm, B) Abnormal sperm curled tail, C) bent neck sperm, D) and spiral tail sperm.

The data analysis result showed that avocado administration affected all sperm indicators, namely concentration, motility, and morphology. These were because avocados contained components that function on each indicators. Sperm concentration was the number of sperm produced by the testes and refined within the epididymis through a series of processes that occurred during spermatogenesis. This process used a hypothalamic-pituitary-testicular pathway involving hormones that played a role in testicular development and spermatogenesis. The hypothalamus secreted the hormone GnRH (Gonadotropin-Releasing-Hormone) which triggered the pituitary to release the hormones FSH (Follicle Stimulating Hormone) and LH (Lutenizing Hormone). Both of these hormones worked synergistically in order to produce mature sperm so that they can fertilize the ovum. FSH played a role in the division of sertoli cells to produce ABP (Androgen Binding Protein) which helped transport testosterone into the epididymis during the sperm maturation process. While LH functioned to influence changes and maturation of Leidig cells to produce testosterone [18].

Secretion hormone was influenced by adequate nutrition; hormone secretion can change following nutritional status. Low nutritional status caused stunted puberty growth which resulted in sterility [3]. This was consistent with the study result that the control group had the lowest sperm concentration compared to the treatment (Figure 1). Meanwhile, in the treatment group, the avocado dose 133% w/v resulted the highest sperm concentration.

This condition was supported by the nutrient content in avocado, such as protein, carbohydrates, fat, and vitamins C and E. Protein also determined the sperm concentration on insect growth (ants) [19]. Protein reduction during larval development resulted in a reduction in the number of sperm produced. Even the ratio of protein and carbohydrate intake affected the number of sperm on cockroaches [20], which showed an improvement in the number of sperm along with the ratio of protein: carbohydrate intake to a maximum of 1: 2.

Besides having a role in quantity term, protein was also related to motility of sperm. Protein was composed of amino acid sequences. In mammals, amino acids were involved in spermatogenesis, namely sperm maturation and motility. During the process involved various enzymes formed from protein as raw material. Sperm motility occurred because of the energy generated from metabolism in the mitochondria that was supported by enzymes. Kinase protein is an enzyme for activating signal transducer. Protein also functions as a structure the constituent part of the sperm tail fiber so as to maintain its strength and elasticity [21].

In contrast to protein and carbohydrates, the fat content in avocado was more dominant and in the form of unsaturated fatty acids (unsaturation). The sperm contained unsaturated fatty acids which are dominated by PUFA (Poly Unsaturated Fatty Acid), while PUFA is an important constituent of phospholipid membrane cells. Thus the existence of PUFA maintained cell membrane integrity increases testicular development and spermatogenesis, motility and sperm viability. Apart from being part of the structure, PUFA is a precursor in the formation of prostaglandins. Prostaglandins play a direct role in the contractile tissue of the testis and epididymis, causing an increase in the rate of sperm discharge from the epididymis to the ductus deferen [22].

PUFA content in sperm membranes can be influenced by nutrients as the result of previous study [23][24]. Even fatty acid supplement diet in general could improve semen quality [25]. This study result showed that the administration of avocados with a predominant fat composition increased sperm motility (Figure 2). The sperm movements observed in this study were in the progressive category, i.e. fast and advanced. In contrast to the concentration of sperm, motility at an avocado dose of 100% w/v did not show a significant difference with the highest dose of 133% w/v. This was because after obtaining a complete structure, the motility of sperm was more determined by the energy from the mitochondria that were at the base of the tail.

The avocado contained vitamins that useful for fertility, namely vitamins C and E. This vitamin is an antioxidant that removed the effect of free radical by releasing an electron that would bind to free radical so that it become neutral [26]. Thus, this vitamin protected the damage sperm membrane caused by ROS. Various studies have

been carried out using vitamin C and E as well as a combination of both. The study result showed that giving vitamin C increases the number and motility of sperm [27], even consuming vitamin C 250 mg twice a day for three month improved sperm motility and morphology the infertile men after varicocele surgery [28]. While in teratozoospermia men, vitamin C, also known as ascorbic acid, increased motility, viability, acrosome reactions and DNA integrity [29]. Giving ascorbic acid in male mice induced with cyclophosphamide, improved testes from oxidative stress. In line with this research vitamin C did not only increased sperm consentration and morphology but also testosteron serum in gentamicin-induced male mice.

The vitamin C included exogenous antioxidant and vitamin E simultaneously is mainly obtained from foods such as almond, avocado and sweet potato. Vitamin E itself functioned to prevent lipid peroxidation and stimulated other anti-oxidant function [30][31]. Lately, research trend has not only used individual vitamin but combined both. As a non-enzymatic antioxidant, the vitamin was able to compensate for the sperm cytoplasmic enzyme destruction, so that it was proven to be able to protect the concentration, motility and morphology of sperm. The combination of vitamin C and E prevented the formation of free radicals that vitamin C reduced vitamin E radicals namely tocopheroxyl radicals again become trocopherol [32][33].

The other research result showed a positive effect on the combination of both vitamin C and E including [34] which proved the addition of vitamin E (1 g / day) and vitamin C (1 g / day) for two months reduced DNA destruction of sperm significantly. The combination of vitamin C, E and CoQ10 intake effectively improved sperm concentration, motility, morphology and also DNA integrity of sperm [35].

The combination effect of vitamin was supported by research which proved the combination of vitamin E (400 mg / day) and clomiphene citrate (25 mg / day) given for 6 months on idiopathic OAT (Oligo Asthenoteratozoospermia) increased the concentration and motility of sperm better than the effects of each vitamin [36] . Even the other researcher added a significant increase in sperm concentration and motility not only in OAT patients but also non OAT too [37]. In male infertile astenoteratozoospermia, the combination of vitamin C and E reduced sperm that were degraded and significantly increased the concentration, motility and morphology of sperm [38]. Furthermore, the combination of Vitamin C and E could improve the quality of sperm samples exposed to cigarette smoke [39].

The observation result on the sperm morphology carried out the treatment group had a significant effect (Figure 3). This condition was the same as the observation result on sperm concentration. In the control group had the lowest average percentage of normal sperm that is 87.25% compared to the treatment group. The 75% w/v and 100% w/v treatment group did not show any difference while the 133% w/v treatment group had the highest morphological percentage of normal sperm that was 96.88 %. This sperm morphology data showed the dominance of normal sperm morphology compared to abnormal morphology. The normal morphology of the mice's sperm was marked by the head with a tapered hook, the neck connected to the head and the tail was long and straight. Abnormalities in all three parts were called sperm with abnormal morphology or teratozoospermia. Some of the abnormalities found in this study included abnormalities of the bent neck and tail curling and forming a spiral (Figure 4).

The sperm were produced in seminiferous tubules and have enhanced morphology in the epididymis. Morphological abnormalities that occurred in the process of the seminiferous tubules were called primary disorders, while abnormalities during refinement in the epididymis were called secondary abnormalities. During the refinement process in the epididymis changed in the sperm structure from the head to the tail. This time quality nutrition is needed such as protein [40]. Protein protected the plasma membrane of sperm during spermatogenesis in the testes to produce normal morphology sperm [41]. As explained earlier, avocado contains 1.96 g protein, although it’s not as much as 15.4 g fatty acids. This was consistent with the research result that avocado administration had no significant effect on sperm morphology, but administration with 2 mL dose resulted in better morphology of normal sperm [42]. This condition indicated the optimum dose to produce normal morphology of sperm. Vitamin E is also able to influence morphological form because vitamin captured free radical and prevented chain reaction, so they could avoid per oxidative destruction that affected the morphology and fertility of sperm [43].

4. Conclusion

The avocado fruit *(Persea americana)* plays important role for reproductive health, particularly for increasing spermatozoa quality, including concentration, motility and morphology of sperm. The higher dose given, the higher quantity and quality of sperm resulted. This research result also disproved that avocado contains high fat which can increase body weight, except consumed it with milk or other sweeteners simultaneously.

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