

## THE EFFECT OF PHYSICAL STRESS ON THE QUALITY OF MOUSE SPERM (*MUS MUSCULUS L.*) ADULT MALES

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### ABSTRACT

Physical stress is one cause that can affect sperm quality. Vibration exposure as a form of physical stress that can stimulate the hormonal system so that it can disturb the process of spermatogenesis which consequently can decrease quality of sperm. This was a true experimental research with post test only control group design was done in the Integrated Laboratory of the Faculty of Medicine Halu Oleo University. Samples was 28 mice (*Mus musculus L.*) adult male, it were divided into 4 groups: control group (K), the treatment group 7 minutes 30 second (P1), 15 minutes (P2) and 30 minutes (P3) with the vibrations of 50 Hz for 21 days. Sperm quality include motility, viability, concentration and morphology. Data were analyzed descriptively test and Fisher's test. The results of this study indicated the significant differences ( $p < 0,05$ ) to quality of spermatozoa of adult mice. The conclusion of this study was vibration exposure at 50 Hz in the duration of 7 minutes 30 seconds, 15 minutes and 30 minutes for 21 days can decreased quality of sperm. It was suggested for further research can examined the levels of testosterone and histology on Leydig cells and Sertoli cells of the testis.

**Keywords:** *vibration, hormonal, sperm quality, male mice*

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### INTRODUCTION

Reproductive health is a very sensitive thing for everyone who has grown up. But not everyone pays attention to their reproductive health (Zielinski et al., 1991). Though reproductive health can affect a person's quality of life in terms of fertility or fertilization (Vinturache et al., 2015). One of the causes of infertility comes from male factors. Male factors contribute 40% to the incidence of infertility, which includes abnormalities in sperm number, motility and morphology (Hestiantoro, 2011). The comparison of environmental factors and occupational hazards to the occurrence of infertility in men is not yet known but it is suspected to be the cause of declining human sperm quality in industrialized countries (CDC, 2014). According to the Indonesian Association of Reproductive Endocrinology and Fertility (HIFERI) and the Indonesian Society of Fertility in Vitro (PERFITRI) in the book Consensus for Infertility Management (2013), states that excessive stress can be associated with infertility, but adequate research results have not been obtained. Types of stress in physical form are temperature, noise, vibration and radiation (Fu et al., 2020).

Vibrations can interfere with the quality of a person's health in the world of work. According to Wibowo et al (2011), one of the vibrations that has an impact on one's health is whole body vibration. One of the effects of vibration is that vibration can affect sperm quality. These vibrations will affect the hormonal system in the process of spermatogenesis (Ermiza, 2012). As for research conducted by Penkov & Tzvetkov (1999), vibration can reduce sperm count, spermatogenesis process, testicular size, and others. But it is different from the research conducted by Bosco et al (2000), the study said that the hormonal response to giving whole body vibration (*Whole Body Vibration*) in men can increase the concentration of the hormone testosterone, where the hormone is one of the hormones that can affect sperm quality.

This is one of the debates in the world of health, because there has not been much research on giving physical stress in the form of vibrations to sperm quality microscopically (Das & Agrawal, 2011). Based on the description above, the author wants to continue and prove that giving physical stress in the form of vibrations to male mice can affect sperm quality.

## **METHOD**

This research is a type of *true* experimental research with a *post test only control* group design. The sample was mice (*Mus musculus L.*) adult males aged 2 – 3 months with a body weight of 21-30 grams. Mice were divided into 4 groups, namely the control group (K), the treatment group 7 minutes 30 seconds (P1), 15 minutes (P2) and 30 minutes (P3).

### **Adaptation of experimental animals**

Experimental animals were adapted in the Integrated Laboratory of the Faculty of Medicine, Halu Oleo University for seven days with food and drink feeding. Food given is 8 g per day and drinks 8 ml per day for each mouse. These experimental animals were placed in cages where each cage consisted of 10 adult male mice.

### **Conduct of research**

#### **a. Treatment provision**

Giving vibrations by placing each group of mice onto a vibrating table consisting of a container measuring 30 x 18 x 17 cm placed on the top of the vibrating machine of 50 Hz within 7 minutes 30 seconds (P1); 15 minutes (P2) and 30 minutes (P3) per day are given for 21 days.

#### **b. Mouse surgery**

After 21 days of treatment, these mice were anesthetized locally with 0.1 ml of lidocaine subcutaneously around the testicular organs then the mice will be surgically processed, the testicles and epididymis. Both organs are placed in a petri dish containing 0.9% NaCl. Furthermore, the epididymis cauda is cut small until smooth while the cauda is pressed slowly until the secretion or epididymis fluid comes out and stirred so that a homogeneous spermatozoa suspension is obtained. The obtained suspension is analyzed microscopically at a temperature of 37°C, and the specimen is immediately examined no later than 60 minutes postoperatively. Mice that have been dissected will be euthanized by cervical dislocation method by applying pressure to the posterior part of the base of the skull bone and spinal cord so that the mice can die immediately

#### **c. Examination of spermatozoa motility**

A sperm suspension of 10 µl is placed on the glass of the object and then closed using a *glass cover*. Then it is observed under a microscope with a magnification of 10 x 40. The examination is carried out by observing in one field of view, then estimating the ratio in percent, between spermatozoa motility advanced good, less good, and not good. Motility categories are as follows (1). Spermatozoa with good motility i.e. moving straight forward, agile, active, and fast (%); (2) Spermatozoa with poor motility, namely slow motion (%); (3) Spermatozoa are not motile (%) Motility of spermatozoa is calculated based on categories 0 (dead), 1 (immobile), 2 (slow-moving), 3 (fast-moving). The percentage of motile spermatozoa is determined by adding the 2.3 categories divided by the number of 0.1, 2.3 multiplied by 100%.

Criterion:

- Normal values: good motility  $\geq 50\%$
- Abnormal value: good motility  $\leq 50\%$

**d. Spermatozoa viability examination**

The suspension of spermatozoa is pipetted as much as 10  $\mu\text{l}$ , then placed on the object glass, then added 10  $\mu\text{l}$  of 1% Eosin Y solution, then covered with a *glass cover* and observed under a microscope with a magnification of 400 times. Living spermatozoa are colorless, while dead ones are red, yield in percentage form. The calculation of the percentage of viability of living spermatozoa is done by means of the number of dead sperm cells divided by the number of observed cells multiplied by 100%.

Criterion:

- Normal values:  $\geq 75\%$  of living sperm
- Abnormal values:  $< 75\%$  of living sperm

**e. Examination of the amount of concentration of spermatozoa**

First, sperm must be diluted before examination in the counting chamber. Dilution is carried out according to the number of sperm in the field of view. By using a leukocyte thoma pipette, for dilution 20 times sucked sperm to the mark of 0.5, then sucked Eosin Y solution (dissolved in 100 ml of aquades) until the mark of 11. Then shake the pipette vertically so that the mixture becomes homogeneous, then shake the pipette according to the number eight (shake several times until the mixture is completely homogeneous). For the *Hemocytometer Improved Neubauer examiner*, the first droplet is removed. Then drip in the counting chamber, wait a few minutes until all the counting chambers are filled with mixed sperm. Examination using microscope magnification 400 times or 450 times. If in this middlebox obtained N (number of spermatozoa) then the concentration is  $N \times 200,000 / \text{ml}$  (if the dilution is 20 times), and  $N \times 100,000 / \text{ml}$  (if the dilution is 10 times).

Criterion:

- Normal value:  $\geq 20$  million/ml
- Abnormal value:  $< 20$  million/ml

**f. Morphological examination of spermatozoa**

A morphological examination can be observed in smear preparations by dripping one drop of 10  $\mu\text{l}$  of sperm suspension on a *glass object* slightly to the edge. After the creation of a sperm smear, the preparation is dried in the air for 5 minutes. Then the preparation is fixed with 96% methanol for 10 minutes and dried again. Then dripped 5% Giemsa solution let stand for 30 minutes, then rinsed with distilled water. Left to dry and observed morphological abnormalities that occur with a microscope with a magnification of 1000 times. The result of observations is expressed as a percentage.

Criterion:

- Normal value :  $\geq 30 \%$
- Abnormal value:  $< 30 \%$

## **RESULTS AND DISCUSSION**

Based on the results of research that has been conducted at the Integrated Laboratory of the Faculty of Medicine, Halu Oleo University, univariate analysis was obtained which included sperm analysis based on parameters, namely motility, viability, number of concentrations and sperm morphology in mice, namely the results that in the control group there were 7 samples of normal sperm motility (100%), normal sperm viability of 5 samples (71.4%), The number of normal sperm concentrations was 7 samples (100%) and normal sperm morphology was 7 samples (100%). While in the vibration treatment group there was no motility, viability and amount of concentration and normal sperm morphology, except in the vibration treatment group for 7 minutes 30 seconds there was normal sperm morphology of 2 samples (28.6%) can be seen in table 1.

### **a. Motility of spermatozoa**

Based on the results of the analysis of the effect of vibration on sperm quality with motility parameters in the control group with all treatment groups, with the Fisher test obtained a value of  $p = 0.001$  ( $p < 0.05$ ). This shows that vibration can affect the quality of mouse sperm in terms of sperm motility parameters, can be seen in table 2.

Based on the results of observations and counting of mouse sperm motility, it was found that the percentage of sperm motility in mice was found after giving longer vibrations. This result is in accordance with Ermiza (2012) which states that exposure to vibrations can increase stress in mice so that it can cause process obstacles at the hypothalamus level and cause hormonal disorders resulting in failure of Leydig cells in secreting the hormone testosterone. As a result, there is a disruption of the process of sperm maturation in the epididymis, especially disturbances in the process of glycolysis. According to Munandar (2016), this glycolysis process will produce energy in the form of Adenosine Tri Phosphat (ATP). ATP is used by sperm as an energy source so that it can remain motile and at the same time to maintain its vitality. The energy source used in sperm motility is Adenosine Tri Phosphat (ATP). If the energy produced from the glycolysis process is small, sperm motility will decrease.

### **b. Viability of spermatozoa**

Based on the results of the analysis of the effect of vibration on sperm quality with viability parameters in the control group with all treatment groups, with the Fisher test obtained a value of  $p = 0.021$  ( $p < 0.05$ ). This shows that vibration can affect the quality of mouse sperm in terms of sperm viability parameters, can be seen in table 3.

Based on the results of viability analysis, it was found that in the control group there were only 5 normal samples (71.4%) and 2 abnormal samples (28.6%). It is suspected that the control group is influenced by various factors, one of which is the feed consumed in mice that contain fat composition that exceeds the threshold of a maximum of 7-8% fat so that if consumed excessively it will cause hyperlipidemia. According to Andri (2007), states that the natural composition of feed commonly given to mice raised for experiment and reproduction contains 20-24% crude protein and 5% fat. This is in accordance with research conducted by Pramudito (2009) which says that hyperlipidemia in wistar rats can worsen sperm quality. The increase in cholesterol can lead to increased production of oxygen radicals (ROS) and lipid peroxidation in tissues (Betteridge, 2000). An increase in ROS is proportional to an increase in LDL concentrations in hyperlipidemic patients, inversely proportional to HDL concentrations.

This will spur the onset of oxidative stress that causes DNA mutations in sperm so that sperm cell death will occur as a body response.

Based on the observations from the vibration exposure treatment group to mice, it was found that there was no normal viability of mouse sperm and based on the results of viability calculations, it was found that the longer the vibration was given, the lower the percentage of viability. This is thought to be because the provision of physical stress in the form of vibrations can cause a decrease in the FSH hormone which plays a role in the function of Sertoli cells, causing disruption of metabolic processes that result in death in sperm (Monga et al., 1999). According to Munandar (2016), this is because Sertoli cells have a function as a regulator, support, and protector for developing sperm.

#### **c. Amount of Spermatozoa Concentration**

Based on the results of the analysis of the effect of vibration on sperm quality with motility parameters in the control group with all treatment groups, with the *Fisher* test obtained a value of  $p = 0.001$  ( $p < 0.05$ ). This shows that vibration can affect the quality of mouse sperm in terms of sperm motility parameters, can be seen in table 4.

Based on the results of observations of the number of sperm concentrations studied, it shows that no normal amount of concentration is found and based on the results of calculating the amount of mouse sperm concentration, it is found that the longer it is exposed to eating vibrations, the amount of sperm concentration will decrease. These results are in accordance with research conducted by Penkov & Tzvetkov (1999) which states that vibration can reduce sperm count. According to Harahap & Erris (2014) stated that the amount of sperm concentration produced depends on the process of spermatogenesis inside the seminiferous tubules. In samples that experienced a decrease in the amount of sperm concentration allegedly due to the effects of stress caused by exposure to vibrations, thus activating hormonal responses that cause a decrease in the hormones LH, FSH and testosterone according to the statements of Corwin (2009), Ermiza (2012) and Harahap and Erris (2014). This is thought to cause the process of spermatogenesis to be disrupted, as a result of which the number of spermatogonium produced has decreased.

#### **d. Morphology of spermatozoa**

Based on the results of the analysis of the effect of vibration on sperm quality with morphological parameters with the Fisher test in the control group with the treatment group given vibration for 7 minutes 30 seconds, a value of  $p = 0.021$  ( $p < 0.05$ ) was obtained. While the results of the analysis of the effect of vibration on sperm quality with motility parameters in the control group with the treatment group were exposed for 15 minutes and 30 minutes, with the Fisher test obtained a value of  $p = 0.001$  ( $p < 0.05$ ). Both of these things show that vibration can affect the quality of mouse sperm in terms of sperm morphological parameters, can be seen in table 5.

Based on the results of the morphological analysis of mouse sperm, it was found that all samples in the control group had normal sperm morphology (100%). While in the treatment group that had normal sperm morphology, only in the vibration exposure group for 7 minutes 30 seconds as many as 2 samples (28.6%). This is suspected of the body's response to vibration in the form of homeostasis according to the statement of the theory of General Adaptation Syndrome (GAS) by Selye (1950), allegedly mouse samples are in stage 2 which is the

resistance stage, where this stage says that the pituitary continues to secrete Adrenocorticotrophic Hormone (ACTH), which then stimulates the adrenal cortex to secrete glucocorticoids, which are important for resistance to stress.

Based on the results of analysis in the vibration exposure group for 15 minutes and 30 minutes, it was found that there was no normal sperm morphology and the longer the exposure was given, the percentage of normal morphology would decrease. It is suspected that there are various disorders of the process of spermatogenesis before the formation of the head, neck and tail, especially in the process of spermiogenesis in the epididymis organs as a result of vibrations. In the epididymis, sperm undergo a series of morphological, functional, size, shape and ultrastructural changes. Changes in sperm shape are caused by a decrease in testosterone in accordance with the statements of Ermiza (2012), and Erris and Harahap (2014) who say that this disorder can be caused by hormones, free radicals and chemicals. Functionally, the epididymis depends on testosterone in the process of these changes, so that if testosterone levels decrease, it will cause abnormal sperm morphology.

**Table 1.** Results of Sperm Quality Analysis of Adult Male Mice

Group	Sperm Quality															
	Motility				Viability				Concentration				Morphology			
	N	%	T	%	N	%	T	%	N	%	T	%	N	%	T	%
K	7	100	0	0	5	71,4	2	28,6	7	100	0	0	7	100	0	0
P1	0	0	7	100	0	0	7	100	0	0	7	100	2	28,6	5	71,4
P2	0	0	7	100	0	0	7	100	0	0	7	100	0	0	7	100
P3	0	0	7	100	0	0	7	100	0	0	7	100	0	0	7	100

**Table 2.** Relationship of Vibration to Sperm Motility in Control Group and Treatment Group

Group		Motility						P Value
		Normal		Abnormal		Total		
		N	%	n	%	N	%	
Group	K	7	100	0	0	7	100	0,001
	P1	0	0	7	100	7	100	
Group	K	7	100	0	0	7	100	0,001
	P2	0	0	7	100	7	100	
Group	K	7	100	0	0	7	100	0,001
	P3	0	0	7	100	7	100	

**Table 3.** Vibrational Relationship to Mouse Sperm Viability in Control Group and Treatment Group

		Viability						P Value
		Normal		Abnormal		Total		
		N	%	N	%	n	%	
Group	K	5	71,4	2	28,6	7	100	0,021
	P1	0	0	7	100	7	100	
Group	K	5	71,4	2	28,6	7	100	0,021
	P2	0	0	7	100	7	100	
Group	K	5	71,4	2	28,6	7	100	0,021
	P3	0	0	7	100	7	100	

**Table 4.** Relationship of Vibration to Total Sperm Concentration of Mice in the Control Group and the Treatment Group

		Konsentasi						P Value
		Normal		Abnormal		Total		
		N	%	N	%	n	%	
Group	K	7	100	0	0	7	100	0,001
	P1	0	0	7	100	7	100	
Group	K	7	100	0	0	7	100	0,001
	P2	0	0	7	100	7	100	
Group	K	7	100	0	0	7	100	0,001
	P3	0	0	7	100	7	100	

**Table 5.** Vibrational Relationship to Mouse Sperm Morphology in Control Group and Treatment Group 7 Minutes 30 Seconds

		Morphology						P Value
		Normal		Abnormal		Total		
		N	%	N	%	n	%	
Group	K	7	100	0	0	7	100	0,021
	P1	2	28,6	5	71,4	7	100	
Group	K	7	100	0	0	7	100	0,001
	P2	0	0	7	100	7	100	
Group	K	7	100	0	0	7	100	0,001
	P3	0	0	7	100	7	100	

## CONCLUSION

Based on the results of data analysis and discussion of this study, it can be concluded that physical stress in the form of vibrations of 50 Hz directly with a duration of 7 minutes 30 seconds, 15 minutes, and 30 minutes for 21 days can affect the quality of mouse sperm (*Mus musculus L.*) adult males with a p-value of < 0.05; This suggests that the longer the exposure

to vibration, the lower the motility, viability, amount of sperm concentration and can also increase sperm morphological abnormalities.

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