



---

## The Effect of Noise to Morphology of Rats (*Mus Musculus*) Spermatozoa

Khori Halimah <sup>✉</sup>, Sri Ratna Rahayu, Ari Yuniastuti

Universitas Negeri Semarang, Indonesia

---

### Article Info

#### Article History:

Accepted 19 January 2020

Approved 03 August 2020

Published 23 December 2020

#### Keywords:

Noise, Morphology of Spermatozoa, Rice Milling

### Abstract

Infertility is a serious problem in health and social issue. Exposure to noise is one of the causes of physical and psychological stress that can activate central and peripheral responses in the endocrine system and autonomic nerves as a form of adaptation, so as to reduce the normal percentage of spermatozoa morphology. This study aimed to determine the effect of noise in rice milling on spermatozoa morphology. This study was conducted with laboratory experimental research methods with post-test only control group design. The rats were divided into 2 groups randomly, namely the control group with noise exposure +60 dB (KK) and the treatment group that received noise exposure  $\pm$  90 dB (KP) in UD Berkah Tani Grinding Mill for 35 days. On the 36<sup>th</sup> day, the rats were terminated and spermatozoa were taken to giemsa staining, then observe under a microscope. The data normality were analyzed using Shapiro Wilk and homogeneity were tested using Levene test. The results showed that there were a significant in the spermatozoa morphology between the control and treatment group ( $p < 0.05$ ). It was indicate that there was a difference of noise exposure for 35 days.

---

<sup>✉</sup> Correspondence Address:

Gedung A, Kampus Pascasarjana, Jl. Kelud Utara III, Semarang

E-mail: [Khorihalimah5@gmail.com](mailto:Khorihalimah5@gmail.com)

**p-ISSN 2528-5998**

**e-ISSN 2540-7945**

## INTRODUCTION

Infertility is a health problem in the last thirty years throughout the world (Mahat et al., 2016). In the United Kingdom and the United States, an estimated 6% and 10% infertility rates occur respectively. In Nigeria and parts of sub-Saharan Africa including the Republic of Sudan and Cameroon, infertility can exceed 30% (Sharma, 2017). Infertility itself can be interpreted as the inability to reach conception in a 12-months period for a partner, even though unexpected sexual relations are regular and adequate (Sharma, 2017). A man is said to be infertile if he cannot impregnate his partner after one year of unprotected sexual intercourse. This is an important medical and social problem in the world, 15% of infertile couples and 40% are infertile due to male infertility (Mahat et al., 2016). According to Sharma (2017) infertility has become an unpleasant problem, which is not only limited to these countries, but it also an incident worldwide.

According to Ghavi et al. (2017), men with infertility have a risk of experiencing depression, low self-confidence in sexual activities and low levels of health. Joja et al. (2015) examined that men with infertile will experience self-esteem disturbances and social disability, which in turn will cause a decrease in responsibility towards his wife and family. Attention to psychological needs and rehabilitation in infertile couples can help them improve their mental health and quality of life (Ghavi et al., 2017). According to Mahat et al. (2010), spermatogenesis begins at puberty with primitive germ cells, namely spermatogonia, a large cell diameter about 12  $\mu\text{m}$ . spermatogonia which is primitive germinativum cells located next to the seminiferous tubular lamina, develop into primary spermatocytes. The process begins in the puberty period. Primary spermatocytes undergo meiotic division, so their chromosomes are reduced. This two-stage process, the cells divide into secondary spermatocytes and then into spermatids, which contain a number of haploid chromosomes (23). Spermatids develop into spermatozoa (sperm). The number of spermatids formed from a spermatogonia is 512.

Through the process of regular spermatogenesis in humans it takes about 74 days to form a mature sperm from germinativum cells (Ganong et al., 2017).

The process of spermatogenesis begins with spermatogonia as relatively small primitive germ cell with diameter of about 12  $\mu\text{m}$  and which is usually located near the epithelial basal lamina. These cells will undergo mitosis at the time of maturation of the genital system which will produce a new generation that can continue to divide as stem cells called spermatogonia type A or can differentiate into progenitor cells called type B spermatogonia (Ramgir & Ushasree, 2015).

The first Meiosis arises from smaller secondary spermatocyte cells with only 23 chromosomes (22 + X or 22 + Y). The reduction in the number from 46 to 23 was accompanied by a reduction in the amount of DNA per cell which was originally 4N to 2N. Secondary spermatocytes will divide again and produce spermatids containing 23 chromosomes. Spermatids are easily recognized because they have a small size (diameter 7-8  $\mu\text{m}$ ), have nucleus with a solid chromatin region and are located in the seminiferous tubules near the lumen. Between the first and second meiotic division process there is no S-phase (DNA synthesis) so the amount of DNA per cell is reduced by half during this second division which produce in haploid (1N) cells. The process of meiosis will produce cells with a number of haploid chromosomes, and with fertilization the cell will regain a normal diploid number (Ramgir & Ushasree, 2015). The process of spermatogenesis will continue to become spermiogenesis, which is the final stage of spermatozoa production. Spermatids will turn into spermatozoa, which are cells that have the function of delivering male DNA to the ovum. When spermiogenesis occurs there is no cell division. Spermiogenesis is a fairly complex development process that includes the formation of acrosomes, compaction and elongation of the nucleus, formation of flagellum, and loss of most cytoplasm. Once released mature spermatozoa are transported within seminiferous tubules lumen.

According to Mahat et al. (2016) some causes that affect fertility in men include age, obesity, smoking, alcohol, stress, work, scrotal temperature, and urinary tract infections. While the risk factors for male infertility are environmental, psychological and genetic factors. According to Ramgir & Ushasree (2015), the main environmental factors that affect infertility are temperature, radiation noise and electromagnetic noise. The definition of noise is an unwanted sound that comes from natural activities such as human speech, and man-made sounds such as machine sounds (Reni & Atris, 2016). Noise can affect us both psychologically and physiologically. Noise can be measured with a sound level meter that measures noise between 30-130 dB and a frequency of 20-20,000 Hz. (Gabriel, 2014).

According to Gabriel (2014), there are 2 noise sources: the moving noise source and the immovable noise source. The examples for moving noise source are motorized vehicles, trains, airplanes or helicopters. While examples of the immovable noise source are wood companies (rice mills), rice milling, discotheques, power plants, weaving factories, offices (typewriter sounds) (Gabriel, 2014).

In the study of Jalali et al. (2012) and Umar et al. (2015), increased exposure to noise and temperature can increase stress levels in the body. Noise and temperature stress is a form of physical and psychological stress that can activate the central and peripheral responses in the endocrine system. Workers who are constantly exposed to noise will experience a state of increasing stress which causes nervous and hormonal disorders in the body.

According to Harahap & Erris (2014) and Umar et al. (2015), noise and temperature stress can reduce the quality of spermatozoa morphology so that it results in decreased fertility that occurs in men. Workers who are constantly exposed to noise and high temperatures will experience increasing stress (Soom, 2018).

According to Ramgir & Ushasree (2015), stress due to noise and temperature will stimulate the brain's Paraventricular Nucleus (PVN) to secrete Corticotrophin Releasing Hormone

(CRH) and Arginine Vasopressin (AVP), where the hormone will increase the secretion of Adeno Corticotropin Hormone (ACTH) which will cause a decrease in hormone levels produced by the hypothalamus, namely Gonadotropin Releasing Hormone (GnRH). GnRH levels affect the production of FSH and LH in the pituitary. Where LH will stimulate Leydig cells to produce testosterone and FSH stimulate Sertoli cells to maintain the process of spermatogenesis in the testes. Testosterone and FSH will work on the Sertoli cells will produce a variety of proteins, differentiation and cell metabolism that will maintain normal spermatogenesis.

Spermatozoa motility examination is performed to determine whether there are abnormalities in the spermatozoa tail structure or not. If a lot of motile spermatozoa live then there is an abnormal structure of the tail of spermatozoa, especially in its flagellar structure. Meanwhile, according to (Ramgir & Ushasree, 2015), testosterone and FSH will work on the Sertoli cells will produce various proteins, differentiation and cell metabolism that will maintain normal spermatogenesis. Where fertility in a man can be seen from the quality and quantity of normal spermatozoa which include sperm count, motility, morphology and ejaculate volume (Sukmaningsih et al., 2009).

Harahap & Erris (2014) research results on differences in noise intensity between 65 dB, 85 dB and 105 dB intensity on sperm in white rats (*Rattus norvegicus*) get results that the higher the level of noise intensity that is given will reduce the hormone testosterone then decreased the quantity and quality of spermatozoa including the morphology of spermatozoa. Research on the effect of noise on the environment with an intensity of more than 55 dB every night will experience a significant increase in infertility (Min et al, 2016). Other studies suggest that changes in temperature in the climate due to global warming can affect infertility (Soom, 2018). The results of Umar et al. (2015) stated that the treatment of male wistar rats given a temperature exposure of 40 degrees Celsius had an abnormal morphological average of 18.3% after being given mangosteen peel extract.

The selection of rat (*Mus Musculus*) as experimental animals because rat is one of the animals most often used in research because it is considered quite economical and efficient because it is easily maintained. This animal does not require a large place, a short pregnancy time and has many births (Putri et al., 2015). In addition this animal has a structure and function of organs that are similar to humans and has a response that is also similar to the material or agents that are tested. The process of spermatogenesis of rat is basically the same as that of other mammals. One seminiferous epithelial cycle of  $207 \pm 6$  hours, and 4 cycles. Production of mature spermatozoa from spermatogonia cells lasts 5 weeks in rat. Testes and especially mature spermatozoa, are the richest sources of hyaluronidase, and this enzyme effectively dissolves cumulus cells around the mature ovum at the time of fertilization. Each spermatozoa carries enough enzymes to clear the way through the cumulus cells to the ovum gel matrix. Cement hyaluronic acid materials tend to merge into cumulus cell granulosa cells, so that the sperm head can be supplied with abundant enzymes. Characteristics of normal sperm that has a head shape like a fishing hook and a long straight tail, while abnormal sperm have an irregular head shape, can be shaped like a banana, or irregular (amorphous), or too crooked, and the tail is not straight even not tailed, or there is only a tail without a head. Sukmaningsih et al. (2009) said that rat (*Mus musculus*) need time to cycle spermatogenesis for 35.5 days after taking 4 cycles of seminiferous epithelium. At one time the epithelial cycle of seminiferus in rat was  $207 \pm 6$  hours.

Based on the description above, some previous studies have conducted research on laboratory animals in laboratories using tools as a source of noise and temperature. Previous research has never been done directly in the industrial workplace, it is necessary to research the effect of noise exposure and temperature on workers who are exposed to noise in their work. In the research to be conducted, the object of the research was in the form of rat that were given noise exposure to the rice mill because of the

difficulty in getting respondents who were willing to be examined for spermatozoa.

## METHODS

The sample used in this study was 20 rats, divided into two groups randomly. They placed in a cage according to their respective groups and the study design as well. Control Group which is rat exposed to noise, and Treatment Group which is rat exposed to noise from saw mills with a sound intensity level of  $\pm 90$  dB and temperatures above  $35^{\circ}\text{C}$  (Bramasti, 20012). On the 36<sup>th</sup> day, rat in each group will be killed by dislokatio cervicalis method. Then sperm is taken by cutting the cauda epididymis to the ampulla vas deferens about 1.5 - 2 cm. Hold the ductus deferen with tweezers then massage with a spatula and hold the spermatozoa on a petri dish filled with 1 ml of a 0.86% physiological NaCl solution. Retrieval time must be as fast as possible and morphological examination is carried out immediately.

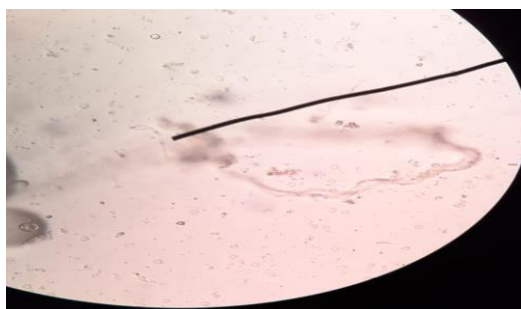
Spermatozoa were collected on day 36, rat in each group would be killed by cervical dislocation method. Then the sperm is taken by cutting the cauda epididymis up to the ampulla vas deferens about 1.5 - 2 cm and then sorted by scapel and placed in a petri dish that has been filled with 0.25 ml of Natrium Chloride physiological solution 0.86 % 0.9 gram NaCl powder mixed with 100 ml of aquabidest. In the process of taking it must be as fast as possible and immediately carried out morphological examination.

To examine the morphology of spermatozoa, the sperm are taken from petri dish using a pipette and place the sperm drip on the glass object. A prepartate made on the glass object, then dry. Give them a methanol for 15 minutes then stain with giemsa for 15 minutes, letting it air dry. Then give the immersion oil to increase the optical resolving power of microscope. It observed under microscope with 400x magnification in the field of view until 100 sperm cells were obtained. The sperm morphology including the head, neck, cytoplasm to the tail of spermatozoa

The validity and reliability test of the instrument in the form of the instrument will be calibrated at Sultan Agung Islamic University Integrated Laboratory. All data obtained are then analyzed and seen whether or not normal data using the normality test using Shapiro Wilk and homogeneity testing with the Levene test. Followed by an unpaired T test with a significance value ( $p < 0.05$ ). Statistical tests were performed with the SPSS program on a computer.

## RESULTS AND DISCUSSION

Observation of morphology of spermatozoa using a microscope with 1000x magnification using Giemsa staining, supported is spermatozoa with the characteristics of normal spermatozoa that contain shapes such as using fishing rods and long hooks, while abnormal sperm can use regular settings, can be used as a substitute, can be used as a transporter, delivery that can be done (amorphous), or too bent, and the tail is not straight even without a tail, or only the tail is headless (Figure 1 and 2).



**Figure 1.** Normal Sperm Morphology at Rat Control



**Figure 2.** Abnormal Sperm Morphology at Rat Control

Data were examined for normality to find out data distribution evenly by using the Shapiro-Wilk test. Both groups showed that the initial data distribution was not normal but the data was transformed to obtain an average percentage of spermatozoa morphology in all groups with normal distribution with a P value  $> 0.05$ . Then homogeneity test with Levene test was performed. Based on the homogeneity test results obtained the value of  $P < 0.05$ . This shows that the morphological data of spermatozoa in all groups was normal and homogeneous.

Because of the normal distribution of data and homogeneous data variations, an unpaired T test was obtained with a price of  $p < 0.05$ . The results showed that between the control group and the treatment group there were significant differences in the morphology of spermatozoa with a value of  $p = 0,000$  ( $p < 0.05$ ). This shows that there is a difference of noise exposure for 35 days. In the statistical test of unpaired T-test, it was found that there was a difference between the control group (KK), the group without exposure to noise with the treatment group (KP) and the treatment group obtained  $p = < 0.05$  so that there were evident differences in the morphology of spermatozoa with lower morphological mean, these results are in accordance with research conducted by Harahap & Erris (2014).

Workers who are constantly exposed to noise and high temperatures will experience a state of stress that continues to increase Soom (2018). According to Adewoyin et al. (2017),

Stress due to noise and temperature will stimulate the brain's Paraventricular Nucleus (PVN) to secrete Corticotrophin Releasing Hormone (CRH) and Arginine Vasopressin (AVP), where the hormone will increase the secretion of Adrenocorticotrophic hormone (ACTH) which will cause a decrease in hormone levels which is produced by the hypothalamus, Gonadotropin Releasing Hormone (GnRH). GnRH levels affect the production of FSH and LH in the pituitary. Where LH will stimulate Leydig cells to produce testosterone and FSH stimulate Sertoli cells to maintain the process of spermatogenesis in the testes. LH serves to stimulate Leydig cells to produce testosterone and maintain the morphology of testosterone to remain high in the testes. Testosterone and FSH will work on the Sertoli cells will produce a variety of proteins, differentiation and cell metabolism that will maintain normal spermatogenesis.

LH functions to stimulate Leydig cells to produce testosterone and maintain the morphology of testosterone to remain high in the seminiferous tubules in the testis. According to Adewoyin et al. (2017), testosterone and FSH will work on Sertoli cells will produce various proteins, differentiation and cell metabolism that will maintain normal spermatogenesis.

Hormonal system in the form of testosterone secretion, FSH and LH is what influences the formation of normal spermatozoa morphology. So that if there is a disruption in the production of the hormone spermatogenesis, the morphology of spermatozoa will be affected.

## CONCLUSION

There is a difference between the control group and the treatment group. It can be concluded that there is an effect of noise on rice milling on spermatozoa morphology with an  $p = 0,000$  ( $p < 0.05$ ). There is a difference between the control group and the treatment group.

This research is a preliminary/ pre-clinical study that can be continued with the object of research to humans as workers who are exposed to noise and temperature when doing their work. Further research needs to be done on the use of

personal protective equipment that is more effective in reducing noise exposure and temperature in workers, as an effort to prevent interference that occurs in the process of spermatogenesis that can lead to infertility.

## REFERENCES

- Adewoyin, Malik. (2017). Male Infertility : The Effect of Natural Antioxidants and Phytocompounds on Oxidative Stress. *Disease*, 5(9): 1–26.
- Bonato, M., Charlie, K. C., & Irek A. M. (2012). Effect of Temperature and PH on the Motility and Viability of Ostrich Sperm. *Animal Reproduction Science*, 133 : 123– 128.
- Bramasti, R. 2012. *Kamus fisika*. Surakarta: Aksara Sinergi Media.
- Gabriel Zamani, W. (2014). Identifikasi Bahaya Kecelakaan Unit Spinning I Menggunakan Metode Hirarc Di PT. Sinar Pantja Djaja. *Unnes Journal Of Public Health* ,3(1): 1–9.
- Ganong Min., Kyoung-bok., & Jin-young M. (2017). Exposure to Environmental Noise and Risk for Male Infertility : A Population-Based Cohort Study . *Environmental Pollution* 226: 118–24.
- Ghavi, F., Leili M., Saeed A., & Masomeh G. J. (2017). Male Infertility and Its Impact on Women's Sexual Behaviors: Need Attention to Psychological Problem as a Psychological Rehabilitation. *Iranian Rehabilitation Journal*. 15(2): 87–94.
- Harahap, I., Erris. (2014). Pengaruh Kebisingan Terhadap Kuantitas Dan Kualitas Spermatozoa Tikus Putih (Rattus Norvegicus) Jantan Dewasa. *Media Badan Penelitian dan Penelitian Kesehatan*, 24 (3): 123–28.
- Jalali, M & Khodabakhsh, K. (2012). Effect of Noise Stress on Count, Progressive and Non- Progressive Sperm Motility, Body and Genital Organ Weights of Adult Male Rats. *Joundishapour University of Medical Science Journal*, 5(1): 1–4.

- Joja, O. D. D & Paun, D. (2015). Psychological Aspects of Male Infertility . An Overview. *Social and Behavioral Sciences* 187: 359–63.
- Mahat, M., & Roshan, K. (2016). Biochemistry & Analytical Biochemistry Risk Factors and Causes of Male Infertility. *Biochemistry & Analytical Biochemistry*, 5(2): 1002–71.
- Min Nadri, F, A., Khavanin, Z., Mazaheri., & Soleimanian. (2016). Effect of Noise Pollution on Male Fertility ( Review ). *JOHE*, 5(1): 53–62.
- Putri, T. U., I B Ardhana P., & Edi L. (2015). Perancangan Fitur Aplikasi Pada Perangkat Mobile Untuk Pengukuran Bising Lingkungan Dan Posisi. *J. Oto. Ktrl. Inst (J.Auto.Ctrl.Inst)*, 7(2): 89–96.
- Ramgir N J., & Ushasree, B. (2015). Effect of Everyday Noise on Hearing in Rural and Urban Population: A Comparative Study. *Narayana Medical Journal* , (2): 79–82.
- Reni, H. & Atris, K S. (2016). Hubungan Kebisingan Mesin Tromol Dengan Stres Pekerja Di Kabupaten Bone *Universitas Negeri Semarang Jurnal Journal of Public Health*, 5(44).
- Sharma, Asha. (2017). Male Infertility, Evidences, Risk Factors, Causes, Diagnosis and Management in Causes and Risk Factors of Infertility. *Annals of Clinical and Laboratory Reserch*, 5(3): 1–10.
- Soom, Ann V. (2018). Theriogenology Heat Stress Responses in Spermatozoa : Mechanisms and Consequences for Cattle Fertility. *Theriogenology Elsevier Journal*, 113: 102–12.
- Sukmaningsih. (2009). Gangguan Spermatogenesis Setelah Pemberian Monosodium Glutamat Pada Mencit (*Mus Musculus L.*) *Jurnal Biologi*,(2): 49–52.
- Umar, Saiful Hi, Edwin De Queljoe, And Lydia Tendeau. (2015). Pengaruh Pemberian Ekstrak Kulit Buah Manggis (*Garcinia Mangostana L.*) terhadap Kualitas Spermatozoa Wistar Jantan (*Rattus Norvegicus*) yang Diberi Paparan Suhu Panas. *Jurnal e-Biomedik (eBM)*,3(2): 670–75.