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The Impact of Aging on the Quality of Sperm in White Rats (Rattus Norvegicus) Wistar Strain

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Abstract

Due to the reproductive aging process, the testes, epididymis, and other reproductive organs gradually lose all their physiological capabilities. This study aimed to determine the effect of aging on the sperm quality of Wistar rats (Rattus Norvegicus). The research design is quantitative and descriptive. This study used white male rats (Rattus norvegicus) of the Wistar strain aged 19–20 months. The number of samples was 18 individuals, with a purposive sampling technique following the inclusion and exclusion criteria. Mice were put to sleep on the seventh day, then the cauda epididymis and testes were separated, and a media container was used to accommodate them. The spermatozoa produced were then examined for their motility, morphology, and viability. The results showed that the range, mean, and standard deviation of progressive motility of spermatozoa was 5.83%, 217% ± 1.87%, normal morphology was 39.17%, 61.6% ± 9.57%, and spermatozoa viability was 19.50%, 74.6 ± 5.83%. There was a progressive decrease in motility, but morphology was expected, and spermatozoa viability was within normal limits. This study concludes that aging affects spermatozoa motility, but the morphology and viability of spermatozoa are still within normal limits. Future studies should do genetic analysis to determine how hereditary factors affect the quality of sperm in aged white rats and compare the results to those of young rats to identify changes in sperm quality.

Keywords: Aging, Morphology, Viability, Motility, Spermatozoa.

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1. INTRODUCTION

The majority of chronic illnesses and functional problems are significantly increased by aging, which causes a decrease in regenerative potential and molecular and cellular decline so that the organism becomes weak and susceptible to disease and death (Carmona & Michan, 2016; Wagner *et al.*, 2016; Hernandez-Segura et al., 2018).

The testes, epididymis, and other reproductive organs gradually lose their physiological abilities due to the reproductive aging process (Luceri *et al.*, 2018). With age, oxidative stress increases, sperm motility decreases, normal morphology decreases, sperm count decreases, and DNA fragmentation increases (Nago *et al.*, 2021). Reactive oxygen species (ROS) are significant in various male infertility issues (Asadi *et al.*, 2021). Oxidative stress results from an imbalance between the body's ability to fight off the adverse effects of free radicals, also known as reactive oxygen species (ROS), and their creation (Torres-Arce *et al.*, 2021). ROS production, which affects aging biomarkers, plays a significant role in the age-related loss of male fertility (Baker & Sabanegh, 2015). According to the research, aged animals produced more free radicals than their younger counterparts and had lower antioxidant activity in their spermatozoa (Sabeti *et al.*, 2016).

According to Henkel, Sandhu, and Agarwal (2018), oxidative stress is closely linked to various pathologies, such as aging and male infertility. Based on the research done by Pino et al., (2020), males over the age of 50 were significantly more likely to exhibit abnormalities in semen volume, sperm concentration, and sperm DNA fragmentation; males aged 41 and older were more likely to have lower sperm concentration levels; males aged 31 and older were more likely to have decreased sperm motility; when sperm concentration remained constant, more sperm volume and motility anomalies were seen as the age increased; when the volume of sperm was held constant, an increase in sperm concentration and motility was observed as anomalies.

ROS can be formed in the body (endogenous ROS), and a small part is the result of exposure from outside the body (exogenous ROS), namely reactive oxygen originating from environmental pollutants, radiation, bacterial, fungal, and viral infections (Parwata, 2015; Conti *et al.*, 2016; Panel, Ghaleh, & Morin, 2018). One of the hypotheses of the concept of aging is the theory of oxidative stress.

During normal respiration, oxygen will be reduced by adding four electrons to become water (H20). Small amounts of harmful chemicals, including anion peroxide (O2-), hydrogen peroxide (H2O2), and hydroxyl radicals (OH), are created as a result of the oxidative enzymes that cause these changes in the cells (Bisht *et al.*, 2017; Luceri *et al.*, 2018). The OH- radical will produce fat and then become a new radical, LO- or LOO-.

Polyunsaturated fatty acids (PUFAs) predominate in the phospholipids of the sperm plasma membrane. The production of lipid peroxides and aldehydes is sensitive to PUFAs, which are linked to lower sperm motility, viability, structural integrity, and metabolic activity (Evans *et al.*, 2021). This study aims to ascertain how white rats (Rattus norvegicus) of the Wistar strain of old age relate to the morphology, motility, and viability of spermatozoa. The selection of Wistar strain rats as research subjects is because they have an average of 92% live spermatozoa, which is higher than the percentage of live spermatozoa in Sprague-Dawley (90.7%) (Simbolon et al., 2013). This study aimed to determine the effect of aging on the sperm quality of Wistar rats (Rattus norvegicus).

2. RESEARCH METHOD

This study used a descriptive design. Wistar strain white rats (Rattus Norvegicus) aged 19-20 months as the research sample, equivalent to 45-46 years in humans (Sengupta, 2013),

with body weight 200-250 grams, in good health, and not physically disabled. The time for conducting research is from January to March 2020 at the Udayana University Integrated Histology Laboratory.

White rats (Rattus norvegicus) male Wistar strain, with bodyweight 200-250 grams, age 19-20 months, a total of 18 rats were put into cages. Rats were kept in cages measuring 40 cm x 15 cm x 10 cm, one cage for two rats. Rats were fed BRI CP511B pellet feed produced by PT. Charoen Pokphand as much as ± 12-20 grams per day and drink ad libitum. The acclimatization period of the sample was seven days, with 12 hours of light phase and 12 hours of darkness at a temperature of 25 ± 0.50C and humidity of 50-60% to reduce stress.

After passing the acclimatization period for seven days, euthanasia was carried out with rat termination. Rats were anesthetized first using ketamine: xylazine 75–100mg/kg: 5–10 mg/kg (10:1 ratio) IM, then euthanized by cervical dislocation method. After that, an open orchidectomy with a midline or pre-scrotal incision was carried out. The testicles were milked out of the wound. The tunica vaginalis is incised, and the spermatozoa cord is shown to identify the epididymis. The testes are housed in a media container after being cut loose from the cauda epididymis. Rat bodies burning machine.

a. Testicular Sampling and Cauda Epididymis Sampling

The method for acquiring testes and spermatozoa samples from the cauda epididymis secretion involved removing the epididymis and testes, which were subsequently placed in a Petri plate containing 0.9% NaCl solution. The proximal portion of the corpus epididymis and the vas deferens' distal portion were sliced to separate the epididymis under a surgical microscope with a 400-fold magnification. Spermatozoa from the cauda epididymis were removed as per standard procedure by inserting a medium or air tube/syringe through the vas deferens and pushing the spermatozoa out through a small incision made distal to the epididymis and suspended with 0.9% NaCl. The proximal cauda is then cut slightly with scissors. Different containers/tubes are utilized to place the testes and the suspension of spermatozoa obtained from the cauda epididymis (Pamungkas, 2012).

b. Spermatozoa Motility Examination

The examination was carried out immediately when Spermatozoa were taken from the cauda epididymis by dripping a drop of Spermatozoa on an object glass. Droplets were attempted to be the same size for each examination. Observations were employed under a microscope with a magnification of 400 times (Hook & Fisher, 2020; Bjorndahl & Brown, 2022).

1) Progressive motility (PR): Spermatozoa travel freely at any speed, either straight or in broad circles.
2) Non-progressive motility (NP): all types of Spermatozoa that do not have progressive criteria, such as swimming in small circles, tail/flagella that are difficult to move the head, or only the tail that moves.
3) Immotility (IM): not moving at all

The standard motility value is Progressive motility (PR) = 32% or PR + NP = 40%.

c. Calculating spermatozoa viability

Observation and calculation of spermatozoa viability were carried out using sperm smear preparations stained with 1% eosin and 10% nigrosine. Observations were made using a light microscope with a magnification of 400 times. Spermatozoa viability was calculated on 100 spermatozoa cells (%) with observation replication six times for each white rat. Viability can be determined by the difference in the spermatozoa cells' color. The live spermatozoa are light-colored, while the dead spermatozoa are purple (Majzoub &Sabanegh, 2017).

d. Observation of Spermatozoa Morphology

By spreading spermatozoa on an object glass, adding one drop each of Eosin 1% and Nigrosine 10%, homogenizing the mixture, and allowing it to air-dry for 5 minutes, the morphology of the spermatozoa was evaluated. They were then observed under a light microscope with a magnification of 400 times. The repetition is done six times. The typical morphology of spermatozoa is when the head and neck are intact, and the tail is straight. Spermatozoa were regarded as expected if they had a hook-shaped head, a straight neck, and a single, free tail. If the head is abnormally small or large, the neck is branched or broken, the tail is branched, coiled, and broken, and there are cytoplasmic droplets on the head, neck, or tail, the morphology is abnormal (Majzoub & Sabanegh, 2017).

Explain the chronology of research, including research design, research procedures (in the form of algorithms, Pseudocode, or other), ways of testing, data acquisition, and method of data analysis. The description of the procedure of the research must be supported by references so that the explanation can be accepted scientifically. Furthermore, adding an ethical clearance number (Costabile, 2013). This research has obtained ethical approval with number 361/EA/KEPK-BUB/2020 and used descriptive analysis to probe the data.

3. RESULTS AND DISCUSSION

A total of 18 male Wistar rats in good health, meeting the qualifying requirements of proper sex, age, and weight (200-250 grams), were included in the study. The analysis of the mean and standard deviation of the weight of the mice used was 228.11gr ± 10.53%.

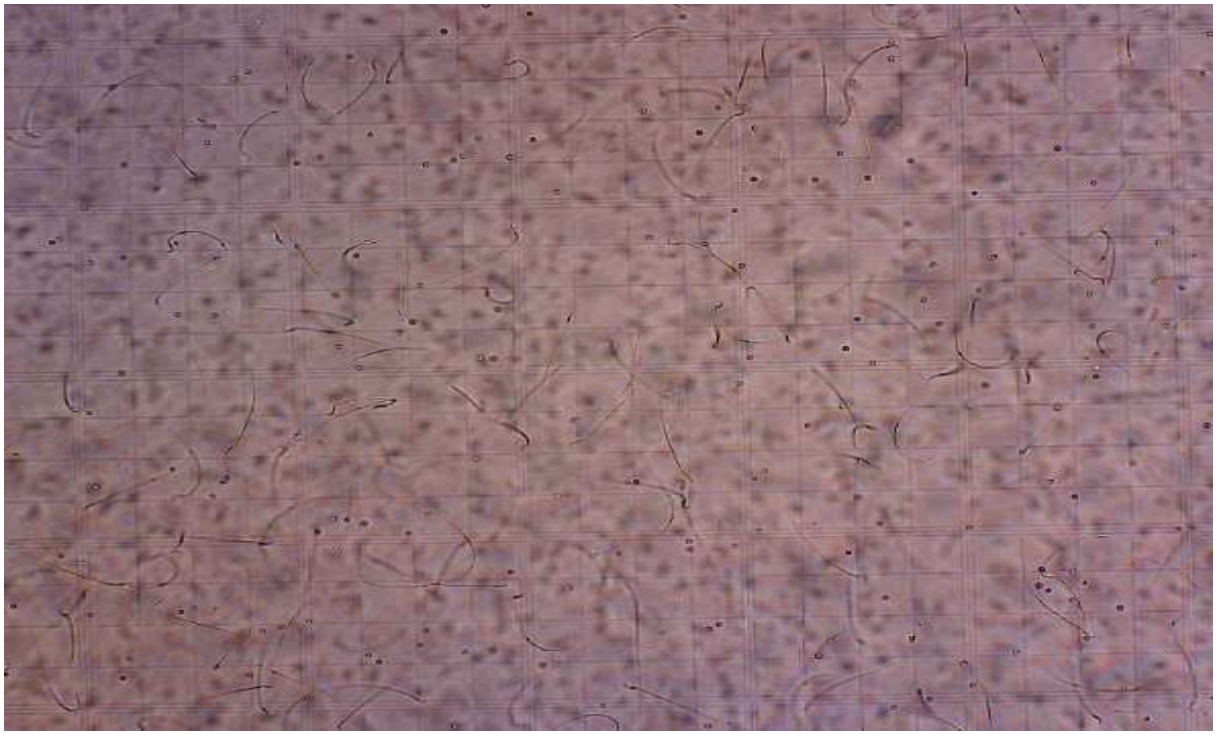

Figure 1. Calculation of progressive spermatozoa motility of white rats (Rattus norvegicus) Wistar strain with 400 times magnification

Figure 1 shows motility. Under a microscope with a magnification of 400 times, the observations focused on identifying progressive motility spermatozoa, which refers to spermatozoa exhibiting unrestricted movement in either a linear trajectory or extensive circular paths at varying velocities.

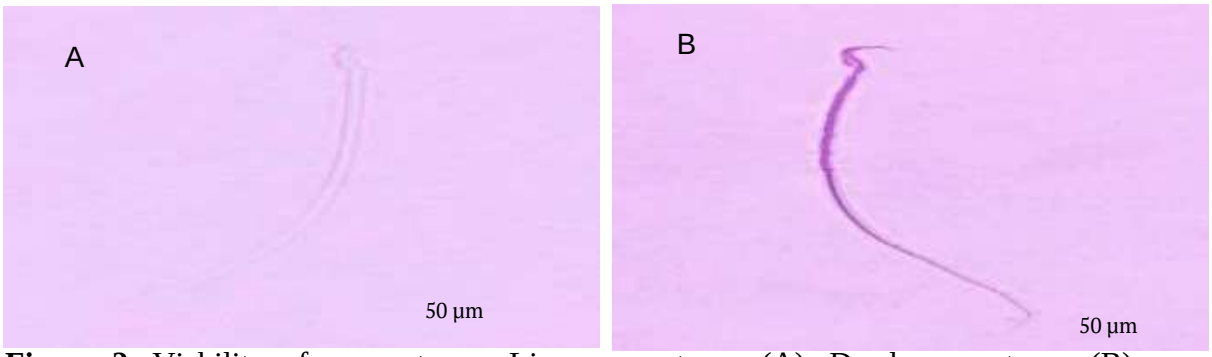

Figure 2. Viability of spermatozoa. Live spermatozoa (A). Dead spermatozoa (B) were observed under a microscope with a magnification of 400 times.

Figure 2 shows the viability of spermatozoa. The difference in the color of the spermatozoa cells can determine viability. The live spermatozoa are light-colored, while the dead spermatozoa are purple.

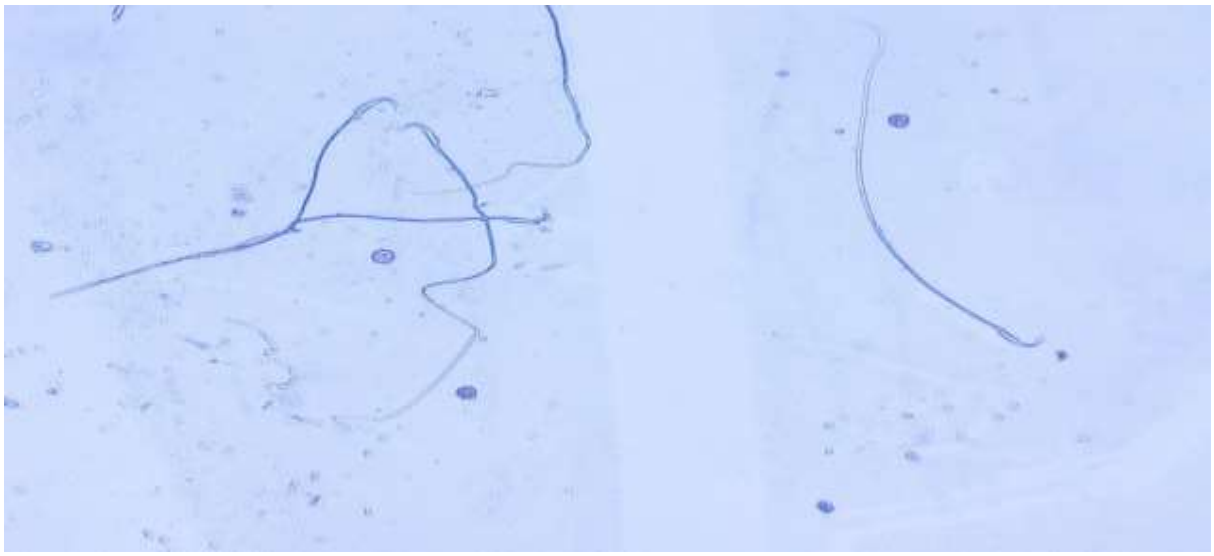

Figure 3. Spermatozoa morphology examination of white rat (Rattus norvegicus) Wistar strain with 400 times magnification.

Figure 3 shows the morphology of spermatozoa. The spermatozoa were regarded as expected if the head was curved like a hook, the neck was straight, and the tail was one with a free tip. A morphology was deemed aberrant if the head is too tiny or large, the neck is broken or branched, the tail is branched, coiled, and broken, and there are cytoplasmic droplets on the head, neck, or tail.

Table 1. Descriptive analysis of spermatozoa of white rats (Rattus norvegicus) Wistar strain

Variable	Range	Mean	SD
Progressive motility	5,83	21,7	1,87
Normal morphology	39,17	61,6	9,57
Viability spermatozoa	19,50	74,6	5,83

Table 1 shows that the range, mean, and standard deviation of progressive motility of spermatozoa is 5.83%, 21.7%±1.87%, normal morphology 39.17%, 61.6%±9.57%, and spermatozoa viability. was 19.50%, 74.6±5.83%.

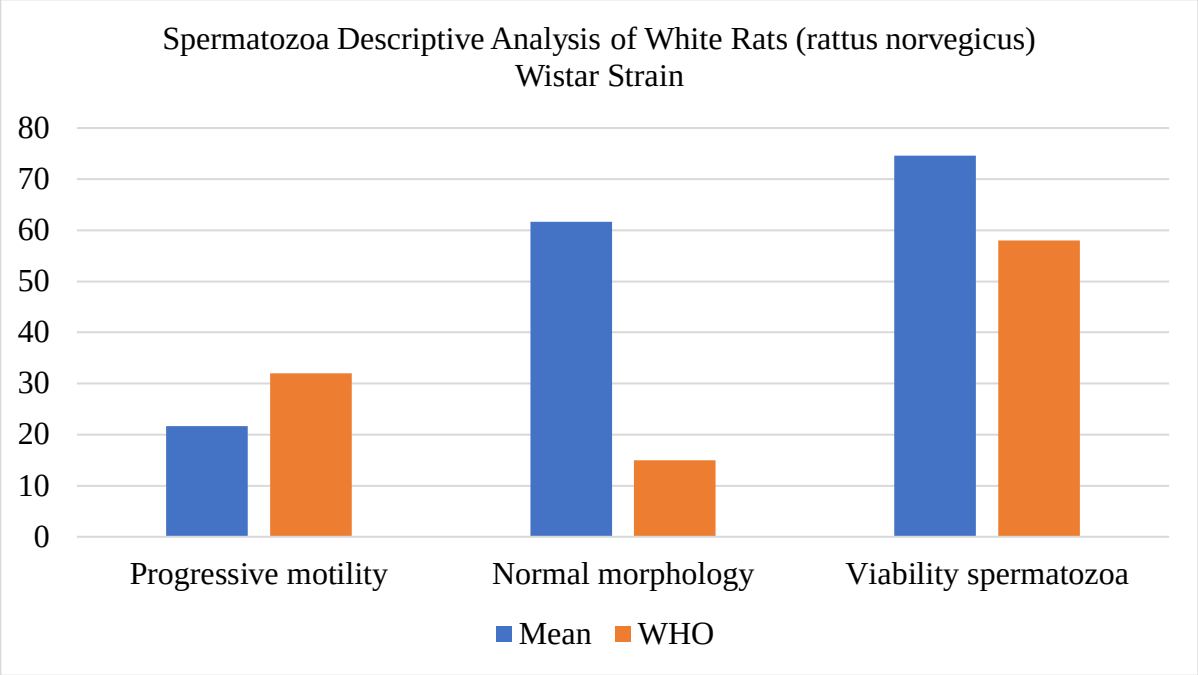

Figure 4. Spermatozoa Descriptive Analysis of White Rats (Rattus norvegicus) Wistar Strain

The criteria normal male fertility include sperm morphology showing the percentage of abnormal forms found in semen and morphological disorders if sperm shape <15% normal morphology. The standard average viability value is 58% of motile sperm are found <58% of viable sperm, then the possibility of sperm motility will decrease because there are dead sperm (necrospermia), and the average motility value is Progressive motility (PR) 32% or PR + NP = 40%.

The results showed that the range, mean, and standard deviation of progressive motility of spermatozoa was 5, 83%, 217%±1.87%, normal morphology 39, 17%, 61.6%±9.57%, and spermatozoa viability was 19, 50%, 74.6±5.83%. At the level of all organs and systems, aging in men is a natural process that can be altered by endogenous and external influences. Spermatozoa undergo genetic and epigenetic alterations as a result of the effects of aging on the reproductive system, which interfere with male reproductive function and lower sperm quality and quantity (Gunes *et al.*, 2016; Nguyen-Powanda & Robaire, 2020). Spermatozoa production in men occurs continuously, but the quantity and quality decrease with age (Costabile, 2013; Fricke & Koppik, 2019).

ROS production, which affects aging biomarkers, plays a major part in the age-related loss in male fertility (Alahmar, 2019; Wang *et al.*, 2022). The findings showed that older animals created more free radicals than younger ones and had decreased antioxidant activity in their spermatozoa (Sabeti *et al.*, 2016; Fatehi *et al.*, 2018).

The shape and number of the spermatozoa in this study, however, remained within normal bounds, and the loss in spermatozoa quality only happened in the steady decline of spermatozoa. This may be brought on by increased aging-related Mehrotra *et al.* (2013) oxidative stress, which lowers the energy generated to maximize spermatozoa motility. This result is consistent with research, which demonstrated that increased ROS production had been linked to reduced spermatozoa motility. Spermatozoa membrane lipid peroxidation affects the fluidity and mobility of spermatozoa axonemes, inactivation or reduction of membrane enzymes, structured DNA damage, and cell death (R. Dias *et al.*, 2020).

4. CONCLUSION

White rats (Rattus norvegicus) of the Wistar strain, aged 19–20 months, were used in this study, equivalent to 45-46 years of human age. The conclusion was that there was a progressive decrease in motility, but normal morphology and spermatozoa viability were still within normal limits. More research is needed to identify the effect of aging on lowering spermatozoa motility at the organelle level, such as how mitochondria operate in creating the energy needed for spermatozoa motility. Future studies should do a genetic analysis to determine how hereditary factors affect the quality of sperm in aged white rats and compare the results to those of young rats to identify changes in sperm quality.

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