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Quality of Spermatozoid Preclinical Analysis on Male Mice *Mus Musculus L*

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Abstract— One form of in vivo experimental is performed on Mus musculus mice to determine the quality of spermatozoid in animals. As many as 30% of the causes of infertility are in male factors, namely decreased sperm motility as a consequence of mitochondrial dysfunction, minimum energy production. Another cause of decreased spermatozoid quality in humans is lack of minerals, especially zinc (Zn). Zn deficiency in the long run can inhibit the formation of sperm cells. This study was conducted to determine preclinical analysis of the quality of spermatozoid male *Mus musculus* with AnadaraMan Plus pellet intervention. The results of this study is there was no difference in smell, color, and consistency in quality of sperm *Mus musculus L*, while the pH and motility of sperm increased with increasing doses of AnadaraMan Plus.

Keywords— mus musculus, spermatozoid, infertility, production.

1. Introduction

Preclinical testing is carried out with the aim of researching an ingredient that is considered to have healing subsatnces and or non-healing substances that have been circulating in the community for long time but have not been proven scientifically, safety and efficacy [15]. One form of in vivo experimental is performed on Mus musculus mice to determine the quality of spermatozoid in animals. The survey results found the failure of pregnancy in couples who have been married for one year, 40% due to infertility in men, 4% due to infertility in women, and 10% of men and women, 10% unknown causes [2]. Infertile events have a major impact on family life because in addition to causing medical problems, infertility can also cause economic and psychological problems [11] [17]. As many as 30% of the causes of infertility are in male factors, namely decreased sperm motility as a consequence of mitochondrial dysfunction, minimum energy production [13]. The quality of spermatozoid in semen is determined by the amount, motility and morphology [27]. Another cause of decreased spermatozoid quality in humans is lack of minerals, especially zinc (Zn). Zn deficiency in the long run can inhibit the formation of sperm cells [9]. Many studies has been conducted on spermatozoid in animals for example the effect of nutritional supply of green bean sprouts *Phaseolus radiatus L*. and the effect of nutritional supply of blood shell *A. granosa L*. in the form of pellets on the level of spermatozoid density of *Mus musculus L*. mice [16]. These studies found herbal medicines can affect spermatozoid density. The herbal remedy derived from *Anadara granosa* shells is the most popular in Indonesia [25] [7]. Previous studies have not provided much information about the potential of blood shell *Anadara granosa L*. and *Spirulina platensis*, especially those related to spermatozoid quality in male *Mus musculus*. Therefore, this study was conducted to determine preclinical analysis of the quality of spermatozoid male *Mus musculus* with AnadaraMan Plus pellet intervention.

2. Literature Review

2.1 Preclinical Test

According to Supardi (2014) preclinical test is a stage of research that takes place before clinical trials or testing in humans [24]. Preclinical testing has one main goal, which is to prove the efficacy and safety of natural materials that can be scientifically justified. Preclinical testing has 5 stages, namely:

1. Selection phase. The selection phase of natural materials to be studied is adjusted to their priorities.
2. Biological screening phase (biological screening). Phase to filter the presence or absence of pharmacological effects of the efficacy of natural substances under study against certain diseases that are simulated in experimental animals.
3. Pharmacodynamic research phase. Phase to examine the mechanism of action of natural materials on each biological system of organs of experimental animals (invivo or invitro).
4. Chronic toxicity testing phase. This stage examines the toxic effects of natural substances on experimental animals after repeated administration for 3 months to 2 years (sub acute and chronic toxicity).
5. Development of pharmaceutical preparations phase (formulations). At this stage natural ingredients that have been proven to be efficacious and non-toxic are developed into the most inexpensive, practical and aesthetic pharmaceutical preparations for clinical trials in humans.

2.2 AnadaraMan Plus

AnadaraMan Plus is a capsule containing extract of shellfish *Anadara granosa* and green algae *Spirulina platensis*., which is the only one food supplement without side effects [4]. *Anadara granosa* contains macro nutrients: protein, total fat and carbohydrates and micro nutrients such as minerals: Ca, Fe, Mg, P, Zn, Cu, Mn, and Se, essential amino acids and vitamins include: vitamins A, E, B complex, and C [1][6]. *Spirulina* contains macronutrient nutrients, namely: N, P, CHO, Ca, Mg, Na, and K and micronutrient nutrients, namely: Fe, Mn, Cu, Zn, B, and additional nutrients namely cyanocobalamin [19].

2.3 Mus Musculus Overview

Mus musculus L. is an experimental animal that is often used in research because it is easy to care for, does not require a large space, and has many children per birth. Mice are rodents that are fast breeding, easily maintained in large numbers, and considerable genetic variation [26]. The behavior of mice is influenced by several factors, including internal factors such as sex, age differences, hormones, pregnancy, and disease, external factors such as food, drinks, and the surrounding environment. They also live in a hidden place close to food sources and build their nests from various soft materials. Mice can survive for 1-2 years and can also reach the age of 3 years. Pregnancy takes 19-21 days while the age for mating is 8 weeks. Mating occurs when female mice undergo estrus. One parent can produce 6-15 children [21]. *Mus musculus* eat all kinds of food and want to try to eat all food is available. The food given for *Mus musculus* mice is usually in the form of unlimited pellets. Drinking water can be supplied with glass or plastic bottles, Mus musculus mice prefer high ambient temperatures, but can also live in low temperatures [21]. Male and female *Mus musculus* are difficult to distinguish. Female musculus can be recognized because of the close distance between the anal canal and the genital. *Mus musculus* male testes when sexually mature look very clear, relatively large in size and not covered by hair. Testicles can be drawn into the body.

2.4 Male Mus Musculus Reproductive System

The reproductive system of male *Mus musculus* is composed of external and internal organs. In the external organs there is a scrotum which is located in front of the anus of the mice and the penis in male mice which has the dual task of removing urine and placing semen into the female reproductive tract. The penis consists

of roots, body and free end that ends at the head of the penis. Internal organs in male mice *Mus musculus* consists of 3 parts, namely testes, epididymis, vas deferens [20]. Spermatogenesis is a process of developing spermatogenic cells which divide several times and eventually differentiate to produce spermatozoa [22]. Spermatogenic cells consist of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids which are spread in 4 to 8 layers that occupy the space between the basal lamina and tubular lumen. Spermatogenesis can be divided into 3 stages, namely the phase of poliferation, meiosis and spermiogenesis [23]. The time needed to complete each step of spermatogenic cell development is different, therefore various forms of cell combination occur from various types of germinal cell development in the seminiferous tubules [23]. Oakberg and Rugh have divided the seminiferous tubular terminal epithelium into 12 levels namely the I-XII level [10]. The time needed for spermatogenesis is 34.5-35.5 days in mice and at least 64 days in humans. After going through 4 seminiferous epithelial cycles. The duration of a seminiferous epithelial cycle is 207 ± 6 hours [14]. Spermatogenesis in mice resembles a process that occurs in humans and other animals and takes place in three stages. The spermatogenesis phase begins with the division of spermatogonia which occurs several times to produce spermatogonia types A2, A3 and A4 [12]. The next stage is meiosis which consists of two stages, namely meiosis I and meiosis II where each experiences a phase of prophase, metaphase, anaphase and telophase. Prophecy in meiosis I which includes leptotene, zigotene, pakiten, diploten, and diakinesis. Furthermore, the spermiogenesis stage is completed, which is the transformation of spermatids from a round shape into spermatozoa with the head, neck and tail. Spermiogenesis in mice consists of 16 levels which are generally classified into four phases, namely the golgi phase, the hood phase, the acrosome phase and the maturation phase [3]. Spermiation is influenced by hormonal modification, temperature, and toxic substances.

2.5 Quality of Sperm

The disruption of spermatogenesis in the seminiferous tubules results in a decrease in sperm quality, causing infertility. Quality sperm is sperm that has normal conditions and is able to fertilize an egg or ovum. Whether or not sperm quality can be determined from several aspects including number, morphology and motility [5]. Quality sperm counts are spermatozoa which have an amount of more than 20 million / ml ejaculate. Morphological abnormalities in sperm will be able to cause disruption in motility, namely the ability to move from spermatozoa [27]. The number of sperm produced by the testes is not reliable to diagnose a person's fertility or infertility. Even if the sperm count is normal but if the morphology and motility are poor, it can cause infertility. Conversely with a small number of sperm but has normal morphology and motility, it can still be fertile [5]. Motility is one of the important factors needed to support the success of fertility by spermatozoa. Spermatozoa motility is caused by flagels that slide rhythmically longitudinally between the posterior and anterior tubules of the spermatozoa that form the axonema. Energy for this movement is supplied in the form of adenosine triphosphate (ATP) which is synthesized by mitochondria in the tail body [8]. Increased abnormal morphological forms of spermatozoa can occur due to various kinds of disorders, especially in spermatogenesis, especially at the stage of spermiogenesis [5].

3. Research Method

The equipment used was a 400 times magnification microscope, improved neubauer hemasitometer, drop pipette, scale pipette, petri dish, small surgical scissors, sterofom, hand counter, stir bar, analytical balance, mouse cage, spoit, watch glass, test tube, litmus paper and sample bottles. The ingredients used are AnadaraMan Plus, mice, 70% alkolhol, absolute ethanol, aquades, 40% NaOH, 0.9% Physiological NaCl, plastic, rubber band, dispossible, latex, husk, standard feed, dot mice, ether, cotton wool, sample bottles, litmus paper. AnadaraMan Plus sample from the Center for Study and Research of the Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar. In this study, 16 male mice were used with 20-30 gram body weight and around 8-10 weeks of age. Mice were divided into 4 treatment groups and

each group contained 4 animals for normal control, while for the treatment of 4 fish in one cage. Before the research began the mice were acclimated for 7 days in laboratory conditions. Every day the mice are given standard food and drinking water. The treatment of mice Mus musculus test animals were divided into 4 groups. Group 1 control, given standard feed and drinking water, group II as AnadaraMan Plus treatment as much as 0.1 gr / grBB group III given treatment 0.2 gr / grBB AnadaraMan Plus and group IV were given 0.3 g / grBB AnadaraMan Plus treatment by mouth. Given treatment for 21 days. Mice spermatozoa is taken by surgery. Before dissection, mice sedated with ether, the abdomen was dissected and one testis taken, then spermatozoa obtained from the cauda epididymis [18]. The epididymis is cleaned with 70% alcohol, then put into a glass containing 1ml of physiological NaCl, cut into small scissors until smooth and stirred with a stirring rod, to form a suspension of spermatozoa. Spermatozoa suspensions in each test animal were placed in different sample bottles. Macroscopic examination is the initial examination through physical observation of the sample. Observations were made at laboratory temperature where color, odor, consistency and pH were assessed. Meanwhile, microscopic examination is done by using a 400x magnification microscope and then calculated the motility of how many percent of sperm that move fast forward straight, slow moving difficult to go straight, move in a stationary place using a hand counter. Data obtained from the test parameters are then processed descriptively.

4. Result and Discussion

The results of research conducted in the Biopharmaceutical Laboratory for a period of 9 weeks starting from March 24 to May 28, 2019, 16 mice sperm quality inspection macroscopically are seen in table 1 below:

Table 1. Macroscopic examination of sperm quality in *Mus musculus*

Intervention	Cement quality			
	Color	Odor	Consistency	pH
K1	White gray	Fishy	Thick	6,3
K2	White gray	Fishy	Thick	6,5
K3	White gray	Fishy	Thick	6,7
K4	White gray	Fishy	Thick	6,2
P1	White gray	Fishy	Thick	6,9
P2	White gray	Fishy	Thick	7
P3	White gray	Fishy	Thick	6,8
P4	White gray	Fishy	Thick	7,3
Q1	White gray	Fishy	Thick	7,5
Q2	White gray	Fishy	Thick	7,4
Q3	White gray	Fishy	Thick	7,4
Q4	White gray	Fishy	Thick	7,3
R1	White gray	Fishy	Thick	7,7
R2	White gray	Fishy	Thick	7,5
R3	White gray	Fishy	Thick	7,6
R4	White gray	Fishy	Thick	7,7

Information:
K: standard feed control + drinking water
P: AnadaraMan Plus dose of 0.1 gr / grBB is given
Q: AnadaraMan Plus dose of 0.2 gr / grBB is given
R: AnadaraMan Plus was given a dose of 0.3 gr / grBB

The results show that the color, odor, and consistency for groups K. P, Q and Rare grayish white, characteristic odor and thick. For the pH range group K is 6.2-6.7, group P is 6.8 - 7.3, group Q is 7.3-7.5 and group R is 7.5-7.7. Sperm motility is one of the important factors that determine the success of the

fertilization process. Motility shows that the results of the average group K are fast movement 37.5%, slow movement 50%, no movement 12.5%, the average group P is fast movement 66.75%, slow movement 23.75% no movement 9.5 %, the mean of the Q group fast movement 76.75% slow movement 17%, no movement 6.25%, the average group R fast movement 87.5%, slow movement 9.5%, no movement 3%. The data shows that there is no difference in the results of macroscopic observations of color, odor and consistency except in the pH test it appears that the pH increasing as the dose of AnadaraMan Plus increased. Normal pH of sperm is 7-8, which will affect sperm motility. Normal pH values are more than 7.2. A pH higher than 8.0 should be suspected of infection while lower than 7.0 with azoospermia, there may be a disgension of the vas deferens, seminal vesica, or epididymis. The results of the sperm motility percentage of Mus musculus L. mice, normal motility with fast movements is more than 50%. The results of the control group that had the fastest motility of sperm motility in mice was only 37.75%. An increase in fast moving sperm that is 87.5% occurs in the administration of AnadaraMan Plus dose of 0.3 gr / grBB while in the administration of AnadaraMan Plus dose of 0.1 gr / grBB and 0.2 gr / grBB respectively 66.75 % and 76.75%. In the control treatment where the sperm moved quickly only 37.75%. This shows AnadaraMan Plus with a dose of 0.3 gr / grBB seen sperm movement faster than the doses of only 0.1 gr / grBB and 0.2 gr / grBB.

5. Conclusion

Based on the results of this study, the examination of the quality of sperm *Mus musculus L,* showed there was no difference in the smell, color, and consistency, while the pH and motility of sperm increased with increasing doses of AnadaraMan Plus**.** Further research is needed for more varied doses.

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