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Quantity and quality of wistar and Sprague-Dawley rat spermatozoa

Syafruddin Ilyas*

***Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia**

Abstract : This study aims to quantity analysis (quantity) as well as quality (morphology, motility and viability) of spermatozoa from cauda epididymis in Wistar and Sprague-Dawley strains. The calculation of a hundred male rats (Rattus norvegicus) is obtained from power statistical analysis with software (G * Power version 3.1.9.2). Each group consisted of 50 Wistar strains and 50 Sprague-Dawley strains aged four months. At the age of three months all mice were adapted for 30 days inside the cage and fed CP551 (Pt. Charoen Pokphand Indonesia Tbk, Medan) and drinks ad libitum. After the adaptation period has been completed, each of the five Wistar and Sprague-Dawley strains are taken daily to be sacrificed and the spermatozoa from the cauda epididymis. Then made cement removal preparations to calculate the number of spermatozoa, morphology, motility and viability of spermatozoa. The preparations were observed under a microscope with 5 field of view. The data obtained were analyzed using independent T test. The results showed that there was no significant difference in the number and morphology of unstable spermatozoa ($p > 0.05$) between Wistar rat strains (230.04 ± 14.80 and 81.76 ± 6.13) and Sprague-Dawley (222.25 ± 17.01 and 79.52 ± 5.52) and the difference in mean motility and viability of spermatozoa among Wistar rats (85.44 ± 4.59 and 82.46 ± 2.85) and Sprague-Dawley (90.10 ± 3.22 and 88.68 ± 3.51) were significantly different ($p < 0.05$). It was concluded that the number of spermatozoa per mL of cement, the percentage of spermatozoa morphology, and the percentage of spermatonoia motility and viability of live spermatozoa in Wistar rats were higher than in Sprague-Dawley rats.

Keywords : amount, morphology, motility, viability, spermatozoa.

Introduction

Laboratory mice (*Rattus norvegicus*) is one of many animals used in scientific research[1]. These mice are generally used as model animals in studies in the fields of medical psychology, biology, and genetics[2]. Scientists have given rise to many specialized rat strains for experiments. Wistar and Sprague Dawley strains are the most commonly used strains in research[2]. The rat strain affects its biological characteristics[3] and the preferred feed type[4]. Selection of particular strains for a particular study can have a significant effect on the

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results of the study. For example, the proper use of mouse strains has increased the research on specific genes and the functioning of their protein products.[5]

Research on reproduction generally uses Wistar and Sprague-Dawley strains. These two strains are the result of an outbreed that has high fertilization and consistent marriage properties[5]. One of the factors used as an indicator of the quality and quality of semen is the high percentage of live spermatozoa.[6] The number of live spermatozoa in cement samples can be influenced by several factors, ie animal age, type of feed consumed, free radicals, temperature, pH, and diluent viscosity as well as individual variation.[7] According to Hartini[8] the number of spermatozoa produced depends on the process that occurs during spermatogenesis. Spermatozoa can live by being able to digest some of the substances contained in the fluid of accessories, fluid in the female genital tract and in the diluent medium.[9]

The surface of the spermatozoa is enclosed by a lipoprotein membrane.[7] When the spermatozoa cells die, their membrane permeability increases primarily in the post nuclear caps and this is the basis for spermatozoa staining that distinguishes live spermatozoa from the dead.[10] Information on the characteristics of cement, especially live spermatozoa from cauda epididymis in Sprague-Dawley and Wistar strain rats in Indonesia is still limited. It is therefore necessary to research the percentage of live epididymal spermatozoa in these two strains which can be used as additional information on the appropriate rat strains for research on the quality of spermatozoa. This study aims to determine and compare the percentage of live spermatozoa from cauda epididymis in each strain of mice. The results of this study are expected to provide information on the number of live spermatozoa from the cauda epididymis of Wistar and Sprague-Dawley strains in order to obtain the right mouse strain to be modeled animal in a study on the quality of spermatozoa.

Materials and Methods

This study used Wistar and Spraque-Dawley strains of 50 mice each, four months old, and in good health. The research took place at the Physiology Laboratory of Faculty of Mathematics and Natural Sciences, University of North Sumatra Medan. Maintenance and Mouse Adaptation

All mice used in this study came from the Department of Biology Faculty of Mathematics and Natural Sciences, University of Sumatra Utra Medan. The mice are in good health and about three months old. All mice were adapted for 30 days in animal cage Department of Biology Faculty of Mathematics and Natural Sciences, University of Suamtera Utara Medan. During the adaptation period all rats were given standard commercial feeds namely pellets (CP 551 PT Charoen Phokphan, Medan) and beverages on ad libitum.

Sperm Collections Epididymis Mice

After adaptation, every 10 rats (5 mice from each strain) of diethanasia with chloroform are inhaled, then rats are dissected for epididymal removal. The caimed left and right epididymis are slashed to remove spermatozoa. Then the cauda epididymis is placed in a petri dish that contains 1 ml of physiological NaCl and is cut into small pieces and allowed 1-2 minutes to allow the spermatozoa to come out of the epididymis and spread.

Analysis of the number and motility of spermatozoa

Analysis of the number of live spermatozoa was done based on WHO Laboratory Manual[11] by modification. The number of spermatozoa expressed in million / mL of cement and sperm motility is expressed in " % ".

Morphological Analysis, Motility and Viability of Spermatozoa

Analysis of spermatozoa with normal and living morphology was performed on the basis of the WHO Laboratory Manual[11] with modification. One drop of 2% eosin dripped on the tip of the object glass is then added 10 μL drops of rat semen, homogenized and then made preparations. The paste preparation is then fixed above the flame. The live spermatozoa were analyzed under a microscope with 40x magnification in 5 field of view to obtain 200 spermatozoa. The number of live spermatozoa is expressed in percent.

For morphological determination of spermatozoa done with the formula: percentage of normal spermatozoa morphology (%) = number of morphology normal spermatozoa / morphology of normal spermatozoa and not x 100%. To determine the percentage of live spermatozoa used the formula: Percentage of spermatozoa (%) = Number of Spermatozoa Life / Number of Spermatozoa Live and Die (200) x 100%. Living spermatozoa will not be colored by eosin dye. The dead spermatozoa will be purplish red due to damage to the plasma membrane of the spermatozoa cells.[12] After the calculation is complete, shooting from the microscope using a Canon camera (16 Mega Pixels).

Data analysis

The data of spermatozoa, morphology percentage, motility, and spermatozoa viability obtained in this study were analyzed using T test using SPSS version 23 software.

Results

The observations that have been obtained in this study can be summarized in several categories of parameters.

Number of Spermatozoa

Based on the results of research that has been done got the results as shown graph Figure 1 below.

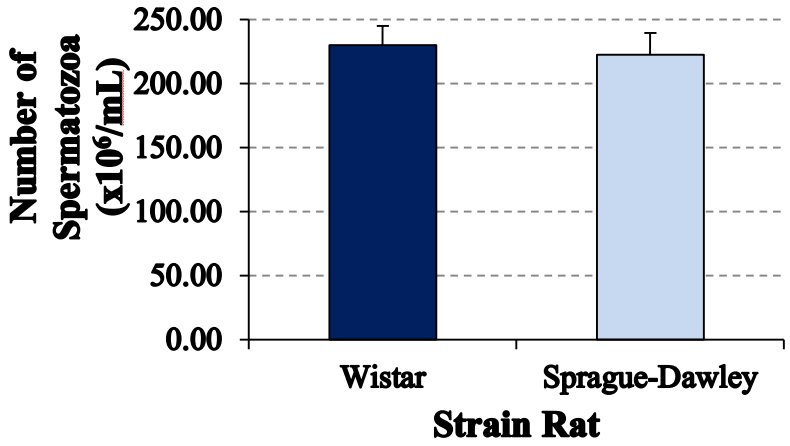
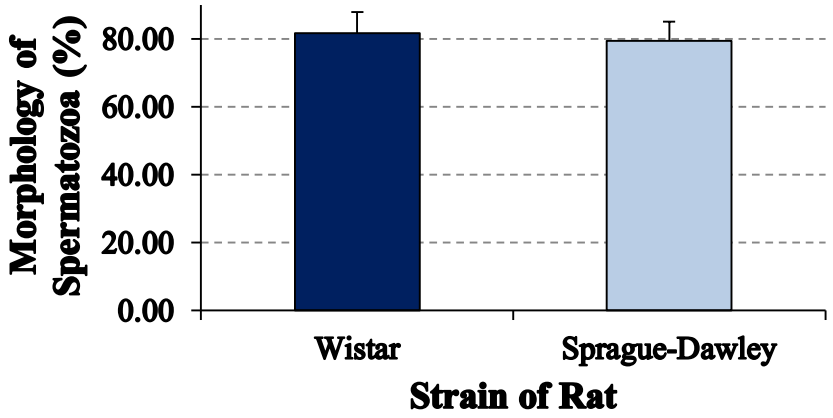


Figure 1. Number of spermatozoa of wistar and Sprague-dawley rats

Spermatozoa morphology

The results of morphological measurements of Wistar and Sprague-Dawley spermatozoa can be seen in Figure 2.



Gambar 2. Morphology of spermatozoa tikus wistar dan Sprague-dawley

Motility of Spermatozoa

Calculation of spermatozoa motility of Wistar and Spargue-Dawley mice observed in the study resulted in a marked difference ($p<0.05$), as shown in Figure 4.

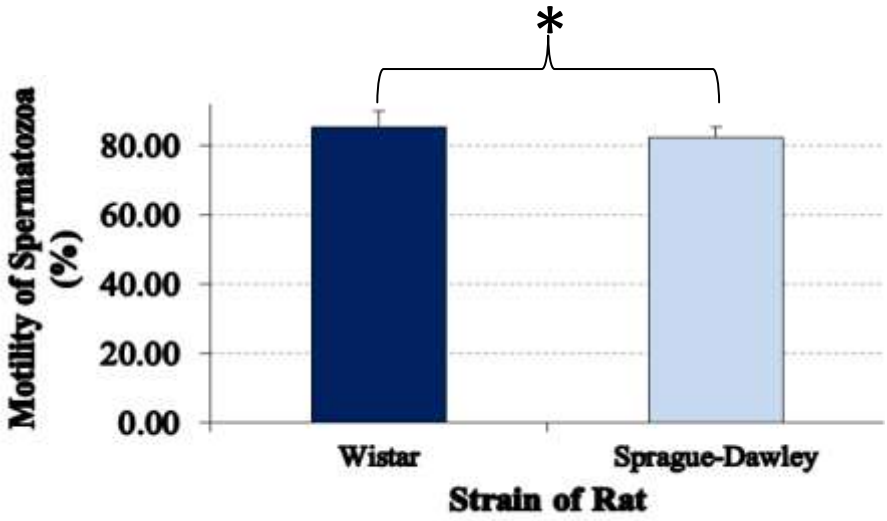


Figure 3. Motility of spermatozoa of wistar rats and Sprague-dawley

Spermatozoa Viability

Viability of spermatozoa in Wistar and Sprague-Dawley mice after observed there was a marked difference ($p<0.05$) (Figure 4).

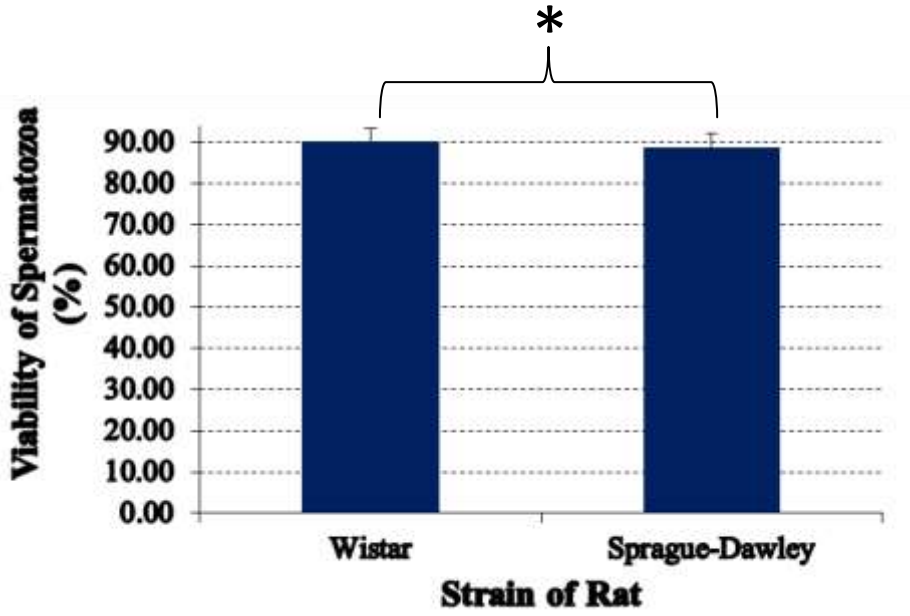


Figure 4. Vertical spermatozoa versus wistar and Sprague-dawley rats

Discussion

Life spermatozoa very dependent on the energy supply contained in the body. Outside the male genitals, spermatozoa are able to use external energy sources for their survival[13] such as fructose to be converted into lactic acid and energy with the help of the fructolysin enzyme[14]. In this study used a physiological NaCl solution that serves to maintain the viability (viability) of spermatozoa outside the body of mice. The physiological NaCl solution is classified as a commonly used extender, as it can provide buffer properties, retain cement pH at room temperature, is isotonic with cell fluid, protect spermatozoa against cold shock, and appropriate electron balance.[15] The ability of spermatozoa to live normally after exit from the testis generally only ranged from 1-2 minutes.[16] The use of a physiologic NaCl solution was able to maintain a spermatozoa life span of 20-25 minutes[17]. According to Isnaini and Suyadi[18], if cement storage is carried out with the use of physiologic NaCl solution, spermatozoa can only survive and can be used up to 60 minutes because although NaCl contains isotonic electrolytes with cell fluid but lacks energy or nutrients to maintain spermatozoa stay alive. Therefore, in this study it is important to note the time of making cement preparations to keep the quality of spermatozoa still alive. The time required from the rat epididymic spermatozoa collection, the preparation of smears until the evaluation of live spermatozoa approximately 5 minutes per sample. With the time range is not expected to affect the number of live spermatozoa mice studied.

According to Ahmed et al.,[19] and Oyeyemi et al.[20] the number of live spermatozoa and the concentration of spermatozoa cells produced by the testes determines the fertility of the male animals. Based on the results of the study it is known that the average percentage of live spermatozoa in the epididymis kenada albist strains of Wistar strand is 92% as presented in Figure4.

Conclution

It was concluded that the number of spermatozoa per mL of cement, the percentage of spermatozoa morphology, and the percentage of spermatogonia motility and viability of live spermatozoa in Wistar rats were higher than in Sprague-Dawley rats.

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