

# A Simple and Practical Method for Rat Epididymal Sperm Count (*Rattus norvegicus*)

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## Abstract

Sperm sample from epididymal source can be determined its number using minimal amount of equipment. These method will aid researcher and practitioner in sperm quality analysis to determined sperm number rapidly and practically.

**Keywords:** sperm quality, sperm number, sperm count, haemocytometer

## Introduction

Rat is the most widely used testing animal in reproductive biology for several reasons. The rat is an animal model that resembles human physiology. This animal has long been used as test animals in screening a compound to determine the pharmacological effects include mechanisms, distribution, and toxicity (Briggs & Oehme 1980). The extensive use of the rat in research has led to a relatively complete biological data (Golden 2002; White 2001).

Several methods have been developed for the assessment of male reproductive function. One of the most widely used methods is sperm quality analysis. Analysis of sperm quality can give us information about the fertility status of the male genital organ. The purpose of the analysis is to assess the sperm descriptive parameters. Usually, the parameters used to predict male fertility are the sperm number/sperm count, sperm morphology and sperm motility. The analysis may show an increase or decrease in the fertility of the animal testing.

## Methodology

### Equipments

Equipment required in rat sperm motility analysis is as follows:

- Light microscope
- Improved Neubauer Haemocytometer
- Micropipette
- Petri dish
- Surgical scissors
- Incubator
- Hand counter

### Sperm Sample preparation

Sperm samples were taken from the cauda epididymis. Cauda epididymis were separated as determined by Hamilton (1975), then placed in a petri dish, minced and incubated in 15 mL of media Biggers, Whitten, and Whittingham (BWW) (Biggers et al. 1971) for 30 minutes at 37 ° C in 5% CO<sub>2</sub> incubator to allow the sperm to swim in the medium BWW (swim-up technique).

### Sperm count procedure

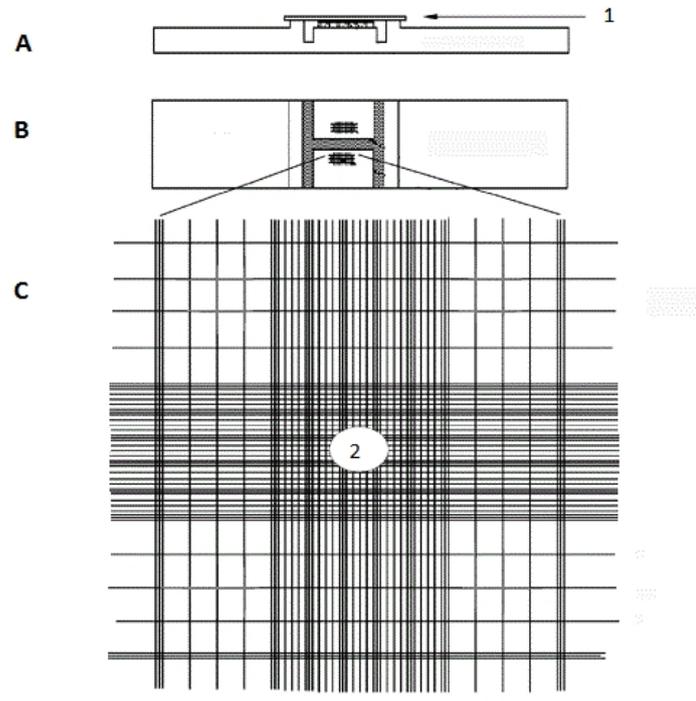
Mount the cover glass on Neubauer Improved Neubauer haemocytometer. Interference pattern (> 10 newton's rings/fringes or iridescence lines) should be seen between the glass surface of the area where the glass cover attached to the haemocytometer. Too little line/newton's rings shows that the distance between the cover glass and haemocytometer widened, therefore counting chamber volume becomes larger and the counting will be inaccurate.

A total of 10 mL sperm suspension were taken from sperm sample preparation, and then inserted into the space between the cover glass and haemocytometer. Other counting chamber also filled in the same way. Each chamber should be completely filled. Suspension inserted slowly to let the liquid evenly by capillary forces. If a chamber is overfill, it must be discarded and fill a new chamber. Removal of the superfluous chamber must not be done since this will change the sperm concentration in the chamber.

Let haemocytometer 10-15 minutes to allow the sperm settled on counting grid. Counting were done with 200x magnification using a light microscope. At counting chamber central area there are 25 large square. Each "large squares" is bounded on all sides by a triple line (figure 1). Counting performed on all of 25 large squares at each counting chamber. For sperm located on the borderline, only sperm lies on the upper or left line (triple

line) should be count as “belonging” to that square. Plot is calculated as the property of the plot. Therefore, do not

count the sperm that is located on the bottom line or the right line.



**Figure 1.** Haemocytometer. A. Side view (cover glass shown by 1). B. Top view. C. One of the counting chambers (One of the 25 large squares shown by 2).

Approximately 200 sperm in each chamber should be count to minimize standard deviation. If the number of sperm obtained from the count at one counting chamber is less than 150, made of new sperm suspension samples by reducing the volume of BWW medium.

#### **Calculation of cauda epididymal sperm number**

Firstly, the sperm number from both chamber were averaged. Secondly, the mean of counted sperm divided by volume within which they were count (volume of 25 large square= 100 nL). The sperm concentration obtained is the number of sperma per nL, which equals millions of sperm/mL (sperm/10<sup>-9</sup> L= sperm x 10<sup>6</sup>/10<sup>-3</sup> L). Thirdly, to obtain the number of sperm per cauda epididymis, sperm concentration obtained multiplied by the number of BWW medium volume used in swim up.

#### **Example of sperm number calculation**

15 mL solution of BWW media was added to epididymal sperm sample (swim-up method). Counting on one chamber produces 320, whereas in the other counting chamber is obtained 280. Both these results are summed and divided by two to get the average, obtained number 300. To obtain the concentration of sperm per nL, average of sperm number of both chamber was divided by 100, to obtain the numbers 3 x 10<sup>6</sup> sperm per mL of sperm suspension. To obtain the number of sperm per cauda epididymis, sperm concentration (3 x 10<sup>6</sup> sperm per mL) was multiplied by the volume of BWW used to swim up

(15 mL) yields: 30 x 10<sup>6</sup> sperm per cauda epididymis of rat.

#### **Discussion**

Determination of sperm number is one of the important aspects in the analysis of sperm quality. However, there are so many variations on the method in the aspects of equipment and technical details. Various different laboratories using different methods. In this study we use Improved Neubauer Haemocytometer to count sperm number. The method mostly based on the practice and experience of testing of 300 rat samples. The study were done at UIN Sunan Kalijaga Yogyakarta and Laboratory of Zoology Faculty of Science and Technology Universiti Kebangsaan Malaysia (data not shown).

Sperm count or sperm concentration of the testing species can be determined from a sample of ejaculate, epididymis, or the testes. Determination of the caudal epydidimal sperm number usually only use sperm from the cauda (Clegg et al., 2001). Difference in the sperm number after treatment of materials or drugs may give important clues about the effect of a substance on sperm production (Working, 1988).

Epididymal sperm counts are generally used in toxicology studies to assess the damage to the male reproductive system, or vice versa, to determine the therapeutic effect of a substance. Epididymal sperm count reduction indicates a reduction in daily sperm production by the testes, the transport obstacles from the testes to the

epididymis, or changes in the epididymal sperm transit time.

In general, the determination of epididymal sperm count was performed using haemocytometer (Strader et al., 1996). Determination of sperm concentration with haemocytometer is the main basis for determining whether a sample categorized as normal, and to predict whether an individual is fertile. Counting with haemocytometer has become a standard method for measuring the concentration of sperm (Freud & Carol, 1964). Compared with other methods, haemocytometer is still a better method for determining the sperm number (Kuster, 2005).

In this study, the amount of BWW volume used in a sperm sample dilution is 15 uL. However, this number can be increased or decreased to obtain suitable sperm density. In some cases, it is necessary to add 20 mL BWW solution for dilution in order to obtain the ideal sperm density to be calculated (200-400 sperm in a haemocytometer counting chamber).

Three factors need to be considered to achieve accuracy using this method. Firstly, we must ensure that the incision of the cauda cauda epididymis was consistent. Secondly, the insertion of the sample with a micropipette on each space calculation must be completely filled. Thirdly, the room temperature in the determination of sperm numbers should be the same for

the entire counting sperm samples. These factors affect the consistency of counting of the sperm number. The strict procedure will ensure the accuracy sperm count.

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