Determination of Motility and Charge Type of Mencit Sperm (Mus musculus) Through Study of Exposure Electrical Fields

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Abstract. Analysis of sperm quality is still one of the most important tools for evaluating a man's fertility. Sperm quality includes several aspects including motility and viability. Mice (Mus musculus) are usually used as experimental animals because the reproductive system is almost the same as human. The purpose of this study is to determine the motility, viability, and type of charge of sperm mice (Mus musculus) through the study of exposure electric fields in an ex vivo condition. Observations were made in the time Δt for sperm without influence and influenced by an electric field. Mice sperm is diluted with a solution of Phosphate Buffered Saline (PBS). The liquid is inserted into the hemocytometer and then placed between two iron plates connected with a DC voltage source. The distance between the two plates d = 7.0 cm. The magnitude of the voltage used is ΔV = 0 (control) and ΔV = 12.5 V. Sperm movement was observed using a fluorescence microscope then recorded using NIS Elements software. Then the movement of sperm is analyzed using Tracker software. Sperm without an electric field (ΔV = 0) has a viability of 60 minutes longer than sperm affected by an electric field (ΔV = 12.5 V) which has a viability of 30 minutes. The sperms move almost randomly in all directions without the influence of the electric field but tend to move to a positive plate when under the influence of the electric field. Based on the direction of the movement of mice sperm, the sperm is thought to be negatively charged. From the results of this study, it was found that the fast zig-zag motion had a velocity v > 37 µm/s.

1. Introduction
Sperm quality analysis is still one of the most important tools for evaluating a man's fertility. Sperm quality really determines the process of fertilization. Normal sperm has a head, middle piece, tail, and must have a healthy condition and move quickly in order to penetrate the egg cell wall. Sperm membrane charge can be used as biomarkers to identify healthy sperm for ART (Assisted Reproductive Technology). The observations showed that when semen was analysed using microelectrophoresis, 94% were NCS (Negatively charged Sperm) [1]. About (40-80) % of mice are used as laboratory animal models [2]. Mice are a common experimental animal for research because mammals and the reproductive system are almost the same as humans. The difference between human and mouse sperm is the shape of the head [3]. The ability of sperm to move properly refers to sperm motility which can be used to characterize sperm quality. Some non-moving sperms are alive but unable to swim. Therefore, one other aspect of sperm quality is sperm viability to measure the
percentage of sperm living and non-living. Sperm motility and viability are normally in the reference range of 40% - 60% [4]. Inside the body, healthy sperm moves properly through the female reproductive tract to reach the egg. Human sperm are viable for 24-48 hours after ejaculation, while mice sperms are considered normal to have viability for 6 hours [5]. Outside the body, sperm viability is shorter which depends on the environmental factors. The motility and viability of the mice sperm in this study was determined through speed and velocity distribution as a function of time.

2. Material and Methods
Observations and data collection were done in the animal physiology laboratory, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia. The materials for this research are male mice and PBS (Phosphate Buffered Saline) solution. Tools for conducting experiments are fluorescence microscopes, iron plates, power supplies, hemocytometers, petri dishes, glass decks, 1.5 ml microtubes, micropipets, yellow tips, and surgical instruments (figure 1). The static electric field is made using parallel plate capacitors connected to the DC voltage source. Two iron plates measuring 9.0 cm × 3.0 cm are connected to the power supply using a connecting cable and each plate is placed between the object's table on a microscope. The two iron plates are separated by a distance of 7.0 cm. The magnitude of voltage used in this study is ΔV′ = 12.5 volts.

![Figure 1](image1.png)
**Figure 1.** (a) Experiments using electric fields (b) a set of flouresences microscope.

![Figure 2](image2.png)
**Figure 2.** (a) Cutting mice epididymis (b) sperm suspension.

Epididymis of mice that has been cut is inserted into a 1.5 ml micro tube containing PBS solution and then chopped using scissors until the sperm is mixed into a suspension (figure 2). Sperm suspension are inserted into the hemocytometer using a micropipette and yellow tip, then the hemocytometer is closed with a deck glass. The hemocytometer is placed on a fluorescence microscope object table. By using a magnification of 20 times, sperm movements are recorded using the NIS Elements software. Recording is done at time \( t = (1, 15, 30, 45, 60, 75, 90, 105, \text{and } 120) \) minutes and each recording lasts for 60 seconds so that the movement of mice sperm can be observed as a function of time. Video recording files by NIS Elements were compressed using Handbrake.
software with the time interval between frames was \( \delta t = 0.2 \) s. The recorded video was analyzed using Tracker software to observe sperm movement by marking the position of the sperm head at a certain time. Tracker Software displays the results of sperm movement, the position \((x,y)\), which can then be known other quantities such as displacement, speed, and angle of sperm movement observed at a certain time.

Healthy sperm moves progressively in zigzag motion. The movement of sperm on a hemocytometer is a motion that is close to two-dimensional motion (figure 3). Sperm movement is tracked every \( \delta t = 0.2 \) s during the time interval \( \Delta t = t_f - t_i = 3 \) s so that there are 16 sperm position.

![Image](image1.png)

**Figure 3.** The 2-D position of an object every time interval \( \Delta t \) from \( t_i \) to \( t_f \) (a) the actual object’s trajectory, (b) the trajectory approach of objects with position \( i \) is at the origin \((0,0)\).

The movement \((\Delta \vec{r})\) of objects in the time interval from \( t_i \) to \( t_f \) is

\[
|\Delta \vec{r}| = \vec{r}_f - \vec{r}_i
\]  
(1)

An object moves in a 2-D, so the displacement \(|\Delta \vec{r}|\) of the object is

\[
|\Delta \vec{r}| = \sqrt{(x_f - x_i)^2 + (y_f - y_i)^2}
\]  
(2)

Where \( \vec{r}_f \) is the final position at \( t_f \) and \( \vec{r}_i \) is the initial position at \( t_i \). If the path of the object is not a straight line, the path traveled will be longer than the displacement value. One way to estimate the path length of sperm \((\Delta s)\) is to track the sperm head motion at each time interval \( \delta t \) from the initial position \((\vec{r}_i)\) to the final position \((\vec{r}_f)\).

\[
\Delta s = \sum_{k=1}^{15} \sqrt{(x(t_i + k\delta t) - x(t_i + (k - 1)\delta t))^2 + (y(t_i + k\delta t) - y(t_i + (k - 1)\delta t))^2}
\]  
(3)

The average velocity \( \langle \vec{v} \rangle \) is the movement of objects divided by the time taken by an object is

\[
\langle \vec{v} \rangle = \frac{\Delta \vec{r}}{\Delta t}
\]  
(4)

The ratio between the estimated path length \((\Delta s)\) and the magnitude of the displacement \(|\Delta \vec{r}|\) is

\[
n = \frac{\Delta s}{|\Delta \vec{r}|}
\]  
(5)
The average speed \( \langle v \rangle \) is the length of the object's path divided by the time taken by an object

\[
\langle v \rangle = \frac{\Delta x}{\Delta t} = \frac{n|\Delta r|}{\Delta t}
\]  

(6).

The velocity vector that will be discussed in sperm motility is to add the direction of sperm movement when \( t_i \) to \( t_f \). The direction of sperm is

\[
\theta = \tan^{-1}\left(\frac{y_f - y_i}{x_f - x_i}\right)
\]  

(7).

While in experiments using static electric field, the magnitude of the strength of the electric field between two parallel plates having a potential difference of \( \Delta V \) with the distance between the two plates \( d \) is

\[
E = \frac{\Delta V}{d}
\]  

(8).

3. Results and Discussion

3.1. Single Sperm Motion

A sperm motion video of 1 minute duration was observed at \( t_i = 0 \) s to \( t_f = 3 \) s with \( \Delta t = 0.2 \) seconds so that there were 16 positions of sperm. Figure 4 shows that the sperm move zig-zag progressive in the x-y plane. At \( \Delta V = 0 \), the path length of zig-zag movement \( \Delta s = 153.6 \) µm, the magnitude of displacement \( |\Delta r| = 91.7 \) µm, ratio \( n = 1.67 \), and direction \( \theta = 340.6^\circ \). At \( \Delta V = 12.5 \) V, the path length of zig-zag movement \( \Delta s = 168.5 \) µm, the magnitude of displacement \( |\Delta r| = 83.0 \) µm, ratio \( n = 2.03 \), and direction \( \theta = 269.8^\circ \). The average magnitude at \( \Delta V = 0 \) for 16 sperm originating from 2 mice is 1.8 ± 0.1. While the average magnitude at \( \Delta V = 12.5 \) V of 8 sperm is 2.1 ± 0.1. The ratio \( n \) used in this work for \( \Delta V = 0 \) and \( \Delta V = 12.5 \) V were \( n \approx 1.8 \) and \( n \approx 2.1 \).

![Figure 4](image1.png)

(a) Two-dimensional motion trajectory of sperm (a) \( \Delta V = 0 \) V (b) \( \Delta V = 12.5 \) V.

3.2. Speed and Speed Distribution

At \( t = 0 \) second there is \( N_0 \) sperm. The sperm count for all speeds is always normalized to the same as \( N_0 \). As the observation time increases, some sperm will die (speed is zero). The curve of total sperm count \( N/N_0 \) (%) as a function of speed \( v \) (µm/s) with the observation time parameter \( t \) (minutes) can be seen in figure 5. Based on figure 5 that the peak of the curve shifts in the direction of lower speed. The more observation time increases the less the number of sperm that can survive and decreases the maximum speed. Normal sperm quality is characterized by having sperm that move progressively zig-zag and rapidly by 25%. From the results of this study it was found that the fast zig-zag motion had a speed \( v > 37 \) µm/s.
Figure 5. Graph of live sperm distribution at various observations time (a) \( \Delta V = 0 \) (b) \( \Delta V = 12.5 \) V.

| \( t \) (minutes) | \( N \) | Velocity Distribution | \( t \) (minutes) | \( N \) | Velocity Distribution |
|-------------------|--------|-----------------------|-------------------|--------|-----------------------|
| 1                 | 47     | ![Graph](image1)      | 75                | 7      | ![Graph](image2)      |
| 15                | 36     | ![Graph](image3)      | 90                | 6      | ![Graph](image4)      |
| 30                | 31     | ![Graph](image5)      | 105               | 6      | ![Graph](image6)      |
| 45                | 24     | ![Graph](image7)      | 120               | 3      | ![Graph](image8)      |
| 60                | 19     | ![Graph](image9)      |                   |        |                       |

Figure 6. Distribution of sperm velocity at \( \Delta V = 0 \).
### Figure 7. Distribution of sperm velocity at $\Delta V = 12.5$ V.

In reference to figure 6, sperm without the influence of an electric field ($E = 0$) move randomly in all directions. Increasing time, more sperm die so that the number of sperm decreases from 47 sperm to 3 sperm for 120 minutes. Related to figure 7, sperm move randomly in all directions before being given an electric field. However, when given an electric field ($\Delta V = 12.5$ V), sperm move not randomly. Sperm move towards the positive plate. The number of sperm that move to the positive plate (+) at $t = 1$ minute (62%), $t = 15$ minutes (66%), and $t = 30$ minutes (80%). Sperm count decreases from 50 sperm to 1 sperm for 75 minutes. Sperm without the influence of the electric field ($\Delta V = 0$) has a higher viability compared to sperm affected by the electric field ($\Delta V \neq 0$). Sperm without an electric field ($\Delta V = 0$) has a viability of 40% at $t = 60$ minutes, whereas sperm affected by an electric field ($\Delta V = 12.5$ V) have a viability of 40% $t = 30$ minutes.

### 4. Conclusions

The experimental results showed that the influence of static electric fields on the motility and viability of mice sperm in ex vivo conditions. The motility of the sperm of mice was observed from the movement of the sperm, namely zigzag progressive. The speed and velocity distribution of this zigzag progressive movement depends on the time of observations. Fast zigzag progressive of sperms has a velocity $v > 37$ µm/s. At the time interval $\Delta t$, sperm has a viability of 40% at $t = 60$ minutes and moves...
randomly in almost any direction without the influence of an electric field ($\Delta V = 0$). Sperm that are affected by an electric field at $\Delta V = 12.5$ V has a viability of 40% at $t = 30$ minutes and has a tendency to move towards the positive plate (+). Sperm without the influence of an electric field ($\Delta V = 0$) has a higher viability than sperm affected by an electric field ($\Delta V = 12.5$ V). Based on the direction of mice sperm movement under the influence of electric field, the mice sperm were thought to be negatively charged. There needs to be further experiments, for example using magnetic fields to confirm the charge on the sperm of mice. The influence of the static electric field affects the quality of sperm, so it is expected to know the effect of the electric field on fertilization.

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