Experimental study on the effect of a surrounding fluid on bovine sperm motility in three dimensions

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Abstract

We constructed an experimental system that can observe bovine sperm motility in three dimensions, simultaneously from the vertical and horizontal directions. The comparison of the experiments conducted using cover glass and square tubing indicates that the effect of the cover glass on the sperm motility is not negligible. Based on this result, we investigated a three-dimensional trajectory of a motile sperm in the square tubing by simultaneously observing through two high-speed cameras. In addition, we investigated the effect of different viscosities of the surrounding fluid on the sperm motility. The experimental results indicate that the increasing viscosity causes a decline in the sperm motility. Furthermore, we focus on the width of the sperm head and the flagellar shape. At low viscosity, the time variation of the head widths is relevant with the two directions and varies periodically. In contrast, at high viscosity, an almost constant value is maintained and periodical time variations are not observed for both directions. Moreover, a difference in the viscosity is observed for the tortuosity of the flagellum shape. These results suggest that the three-dimensional flagellar structure of the bovine sperm is a helix whose cross-sectional shape is an ellipse with quite a large aspect ratio. Further, the results state whether the rotation or nonrotation of the sperm is dependent on the balance between the viscous resistance acting on the sperm and the torque due to the flagellar shape. The experimental results obtained will be useful in clarifying the mechanics of sperm motility under their actual environmental conditions at high viscosity.

Key words : Sperm motility, Three-dimensional observation, Flagellar shape, Viscosity

1. Introduction

Fertilization of the mammals is achieved by the considerably long navigation of the spermatozoon in the female reproductive organs. To reach the eggs in the oviduct, the mammalian sperms swim through the slender flagellum. The periodic variation of the flagellar bending by the sliding of the microtubules contributes to sperm propulsion. There are several flagellar waveforms corresponding to different species. The motion of sea urchin sperm, which swims in the sea (external fertilization), is planar. In contrast, the motion of the mammalian sperm, which moves within the reproductive organs (internal fertilization), is considered to be nonplanar. Therefore, it is expected that the fluid environment surrounding the sperm considerably influence sperm motilities. To understand the nonplanar motion of the sperm, the three-dimensional trajectory and flagellar shape must be studied. Furthermore, as the viscosity of the oviduct mucus, where the mammalian sperms swim, is very high compared to that of water, it is necessary to investigate the effect of this viscosity on the sperm motion characteristics. Therefore, understanding the mechanism of the three-dimensional motility of the sperm corresponding to the surrounding fluid environment is extremely important.

For more than half a century, many researchers have conducted rigorous studies on the sperm motility theoretically (Gray, J., et al., 1955; Lighthill, J, 1976; Higdon, J. J. L., 1979; Phan-Thien, N., et al., 1987), experimentally (Brokaw, C. J., 1965; Phillips, D. M., 1972; Mortimer, S. T., 1997; Crenshaw, H. C., et al., 2000; Woolley, D. M., 2003;

Denissenko, P., et al., 2012), and numerically (Gillies, E. A., et al., 2009; Smith, D. J., et al., 2010; Elgeti, J., et al., 2010; Tam, D., et al., 2011; Gurarie, E., et al., 2011; Guerrero, A., et al., 2011). Regarding the bovine sperm, which is focused upon, in the present experiment, Rikmenspoel et al. (1957) indicated that sperm velocity is proportional to the frequency of the sperm rotation. Furthermore, they observed the elongation of the flagellum and suggested that bovine sperm rotates in three dimensions. Rikmenspoel (1965) distinguished between nonrotating and rotating sperm heads and concluded that sperms with nonrotating heads are unhealthy. Regarding the rotation of the sperm head, Drake (1974) clarified that a sperm head continuously rotates 360° . Further, Rikmenspoel (1984) experimentally observed the relationship between temperature and viscosity. Ishijima et al. (1992) investigated the rotational movement of a spermatozoon around its longitudinal axis in various species, including that of bovine. Friedrich et al. (2010) conducted high-precision measurements of head and flagellum motion of bull spermatozoa as they swam along circular paths near a surface. These studies considered the two-dimensional projection of the flagellar shape on the swimming plane. Recently, several studies successfully tracked the three-dimensional trajectory of the sea urchin (Corkidi, G., et al., 2008) and human spermatozoon (Su, T. W., et al., 2012). However, most of these studies investigated sperm motility in a diluted solution, and did not focus on the three-dimensional variation of the sperm head and flagellar shape. The mammalian sperm migrates in the oviduct with very high viscosity. Several experiments have been conducted on the effect of this viscosity on the motion characteristics of sperms (Katz et al. 1978; Smith et al. 2009; Kirkman-Brown et al. 2011; Hyakutake et al. 2015a). However, these studies were two-dimensional observations. There are few studies that consider three-dimensional sperm motility corresponding to the surrounding fluid environment.

Based on these studies, we constructed an experimental system, which can facilitate in observing a sperm's three-dimensional motility simultaneously from the vertical and horizontal directions. In this experiment, we selected bovine sperm with a disc-shaped head different from that of a human sperm. Therefore, it is easy to observe the rotation of the sperm head. We employed square tubing because the conventional cover glass method may affect the vertical sperm motility. The obtained experimental results will be useful in clarifying the mechanics of sperms' three-dimensional motility under their actual environmental conditions. Additionally, this study may provide useful data that will contribute to understanding sperm motility in the microfluidic sperm sorter developed for infertility treatment (Cho, B. S., et al., 2003; Schuster, T. G., et al., 2003; Hyakutake, T., et al., 2009; Matsuura, K., et al., 2012).

2. Material and method

Figure 1 shows the experimental apparatus constructed in this study. We observed sperm motion simultaneously from vertical and horizontal directions through two high-speed cameras (K-II, Kato-koken, Japan). We employed an optical microscope (IX71, Olympus, Japan) for the observation from the vertical direction, and employed a combination of a high-magnification zoom lens (MS-Z210, Asahikogaku, Japan) with a microscope (MS-200, Asahikogaku, Japan) for the observation from the horizontal direction. These two directions are irrelevant to the effect of gravity. We used Japanese cattle semen (Suzukane, Animal Genetics Japan Co., Ltd., Japan), which was cryopreserved in a liquid

Fig. 1 Experimental apparatus; Simultaneous observation from vertical and horizontal directions using two high-speed cameras.

nitrogen tank. First, we thawed it and added a Tris-citric acid-glucose solution to sustain the sperm motility and extend the observation time. Next, to facilitate observation of the sperm motion, we separated 0.5 mL bovine semen into sperm and seminal plasma by using a centrifugal separator. The 0.1 mL separated semen was diluted with a 5 mL buffer solution (Modified HTF Medium-HEPES, Nippon Oil & Energy Corporation, Japan). To change the rheological properties of the sperm solution, we fused it with polyvinylpyrrolidone (PVP). To investigate the effect of the difference in the viscosity on the bovine sperm motility, we prepared two types of viscosities: a diluted solution with viscosity of 0.0007 Pa·s, and a reagent, which fuses the diluted solution with PVP, has a viscosity is 0.02 Pa·s; this viscosity is thirty times that of water. To investigate the effect of the cover glass on the sperm motility, we first conducted two experiments. The first is the usual observation method involving the dropping of the reagent on a microscope slide and putting a cover glass on top, where the thickness of the liquid layer between the slide and cover glass is 50-100 μm . The other observation method involves filling the reagent into a square tubing with sides of 0.5 mm each (VitroCom, USA). For image analysis, we employed a particle tracking velocimetry (PTV) technique by using the DIPPO-Motion Pro (Ditect, Co., Ltd, Japan), which is a fluid analysis software, and analyzed the images of 100 frames obtained over 1 s.

Figure 2 shows the three positions (P_1 , P_2 , and P_3) obtained through the PTV analysis. From position P_1 , we obtained trajectory of sperm motion. In addition, positions P_2 and P_3 were used to obtain the width of the sperm head H . To estimate the capacity of the sperm motility, we calculated the velocity and amplitude from the sperm trajectory. Furthermore, we calculated two velocities with respect to the sperm. One is the sperm velocity V_{SP} , which was calculated by averaging the velocities determined using the change in sperm position in each pair of successive images. The other is the straight-line velocity of the sperm V_{ST} , which was calculated using the distance between the sperm positions in the first and last frames. We calculated the amplitude of the sperm trajectory A obtained according to the progress axis of the sperm trajectory obtained using the least-squares method. We obtained the flagellar shape from the images using the tracing paper, and calculated the tortuosity of the flagellum by using the MATLAB digital image-analysis software (MathWorks, USA). As shown in Fig. 2, the tortuosity is defined as L/D , where L is length of the flagellum, and D is the straight distance between the root and tip of the flagellum.

Fig. 2 Outline of bovine sperm and motion trajectory. From position P_1 , we obtained trajectory of sperm motion. From positions P_2 and P_3 , we obtained the width of the sperm head H . V_{SP} and V_{ST} are the sperm and straight-line velocities, respectively. L is length of the flagellum, and D is the straight distance between the root and tip of the flagellum. The amplitude of the sperm trajectory A was obtained according to the progress axis of the sperm trajectory obtained using the least-squares method.

3. Results

First, to investigate the effect of the cover glass on sperm motion, we compared the results obtained using the cover glass and square tubing. Figure 3(a) shows the relationship between straight-line and sperm velocities. The reagent is a diluted solution with viscosity 0.0007 Pa·s, which is similar to that of water. The observation was conducted from the

Fig. 3 (a) Relationship between straight-line and sperm velocities. (b) Relationship between the sperm velocity and amplitude of the sperm trajectory. The viscosity of the diluted solution reagent is 0.0007 Pa·s. The white and black squares indicate the results of the cover glass and square tubing, respectively.

vertical direction. The white and black squares indicate the results of the cover glass and square tubing, respectively. This figure clearly indicates that the straight-line velocity increases with the sperm velocity. Compared with the observation using cover glass, the observation using the square tubing showed that there are sperms with greater velocities. Figure 3(b) illustrates the relationship between the sperm velocity and the amplitude of the sperm trajectory. The results indicate that the increase in the amplitude increased the sperm velocity. The comparison of the two conditions shows that the number of sperms with high amplitudes in the cover glass experiment was larger than that in the square tubing experiment.

Next, we investigated three-dimensional motile behaviors of the bovine sperms in the square tubing through microscopic observation from the vertical and horizontal directions. Furthermore, we compared the sperm motions under two viscosities. Figure 4 shows motion trajectory of one sperm sample in the three-dimensional space, where reagent viscosity is 0.0007 Pa·s. The two right figures show the sperm trajectories on xy - and yz -planes, where z -axis indicates the vertical direction. The red and violet colors indicate the start and end points respectively, and the color

Fig. 4 Motion trajectory of one sperm sample in a three-dimensional space (left). The red and violet colors indicate the start and end points respectively, and the color changes as time passes. Motion trajectory on xy - and yz -planes, where z -axis indicates the vertical direction (right).

Fig. 5 (a) Relationship between straight-line and sperm velocities. (b) Relationship between the sperm velocity and amplitude of the sperm trajectory. The black and white circles indicate that the viscosities of the reagent are 0.0007 and 0.02 Pa s, respectively.

changes as time passes. The sperm is determined to move in a zigzag pattern three-dimensionally. We calculated the three-dimensional sperm and straight-line velocities of the sperm samples. Figure 5(a) shows the relationship between two sperm velocities for two viscosities: 0.0007 and 0.02 Pa·s. The results indicate that an increase in the viscosity decreased the sperm velocities, and the ratio of the straight-line velocity with the sperm velocity is almost similar. Figure 5(b) illustrates the relationship between the sperm velocity and the amplitude of the sperm trajectory for two viscosities: 0.0007 and 0.02 Pa·s. The increase in the viscosity cause the decrease in the amplitude by the increasing flow resistance. Consequently, the sperm velocity declines for high viscosity.

Next, we focused on the width of the sperm head (H in Fig. 2). Figure 6(a) and (b) show the time variations of the width of the sperm head from two directions for low and high viscosities, respectively. These results were also observed in the square tubing. The black and red lines indicate the observations from vertical and horizontal directions,

Fig. 6 Time variations of the width of the sperm head from the two directions: (a) 0.0007 Pa·s. (b) 0.02 Pa·s. The black and red lines indicate observation from vertical and horizontal directions, respectively. The observation time is 1 s.

respectively. In the case of low viscosity, the head widths in both the directions had relevance and varied periodically. The width toward the horizontal direction was large when the width toward the vertical direction was small, and vice versa. However, at high viscosity, almost constant values are maintained and the periodical time variation is not observed for both directions.

Furthermore, to investigate the correlation between the motion of the sperm head and the flagellar shape, we calculated the time variation of the tortuosity of the flagellum by analyzing the images from the observation movies. The tortuosity is defined as L/D , as shown in Fig. 2. When the tortuosity is unit, the flagellum of the sperm is straight. As the tortuosity increases, the flagellum meanders. Figure 7(a) and (b) show time variations of the tortuosity in both directions for low and high viscosities, respectively. The black and red lines indicate observations from vertical and horizontal directions, respectively. At low viscosity, the observed results indicating high correlation of the periodical variation of both directions showed relevance. The tortuosity of the horizontal direction was high for a low tortuosity of the vertical direction, and vice versa. However, at high viscosity, almost constant values are maintained, and the periodical time variation is not observed for both directions.

Fig. 7 Time variations of the tortuosity of the flagellum from the two directions. The tortuosity is defined as L/D (Fig. 2); (a) 0.0007 Pa·s. (b) 0.02 Pa·s. The black and red lines indicate observations from vertical and horizontal directions, respectively. The observation time is 1 s.

4. Discussion

In the present study, to investigate the three-dimensional sperm motility and flagellar shape, we constructed the experimental apparatus for simultaneous observation from the vertical and horizontal directions through two high-speed cameras. The experimental data indicates that the bovine sperms migrate three-dimensionally. These results will be complementary to the former valuable studies regarding the sperm trajectory (Corkidi, G., et al., 2008; Su, T. W., et al., 2012). Furthermore, we focused on the three-dimensional shapes of the sperm head and flagellum. Through image analysis, we obtained the time variation of the width of a sperm head, and clarified the correlation between the motion of sperm head and flagellar shape. Moreover, we investigated the effect of different viscosities on the flagellar shape of the sperm. The experimental results indicate that a sperm's three-dimensional motility greatly influenced the viscosity of the fluid surrounding the sperm. This is significant in understanding the mechanics of sperm motility in the actual environment with high viscosity.

It is difficult to observe three-dimensional sperm motility by using the conventional method, that is, to put a cover glass on the microscope slide. Therefore, in the present experiment, we injected the diluted semen in the squared tubing, and observed sperm motion simultaneously from the vertical and horizontal directions. First, to clarify the

Fig. 8 Outline of the sperm from two directions. When the width of the sperm head is large, the flagellar tortuosity is also large (a). On the contrary, when the width is small, the tortuosity is also small; that is, the flagellum stretches (b).

effect of the cover glass on the sperm motility, we investigated the difference between the conventional method and the square-tubing method. The results of the comparison showed that both the sperm and straight-line velocities decreased when using the cover glass. However, the amplitude of the sperm trajectory increased in the case of the cover glass compared with that in the square-tubing case. The existence of the cover glass may restrict the sperm migration in the vertical direction, and leads to two-dimensional sperm motility. Consequently, the motile sperm velocity decreases, whereas the amplitude of the trajectory increases. These results suggest that it is necessary to construct an experimental system to remove the restriction of sperm migration in a specific direction to comprehend the three-dimensional sperm motility appropriately.

In addition to the sperm motion trajectory, we investigated the time variation of the width of a sperm head. The head of the bovine sperm is shaped as a disc. When the viscosity of the reagent is low, the head width changes periodically, implying that the head rotates. Furthermore, we focused on the flagellar motion and the time variation of the flagellar tortuosity. The results of the low viscosity in Figs. 6 and 7 suggest that the head and flagellum of the sperm move in conjunction. When the width of the sperm head is large, the flagellar tortuosity is also large (Fig. 8(a)). On the contrary, when the width is small, the tortuosity is small; that is, the flagellum stretches (Fig. 8(b)). However, the tortuosity was not completely unit, that is, the flagellum did not straighten. These results suggest that the shape of the flagellum is neither a completely two-dimensional plane wave nor a helix with a circular cross-section, similar to that of bacteria. We assume that the three-dimensional structure of the flagellum is a helix with an elliptic cross-section with a large aspect ratio. The rotation of sperm head according to the flagellar beating at low viscosity is due to the torque produced by the three-dimensionality of the flagellum shape. As a result, the three-dimensional trajectory of the sperm occurs as shown in Fig. 4. These results may be important data for the modeling of three-dimensional sperm mobility in the microfluidic sperm sorter (Hyakutake, T., et al., 2009).

In this experiment, we prepared two solutions: low viscosity (0.0007 Pa·s) and high viscosity (0.02 Pa·s) solutions. The increasing viscosity increases the flow resistance, thus decreasing the sperm velocity (Rikmenspoel, 1984). Hyakutake et al. (2015a) investigated the sperm motility for various viscosities, and indicated that the increasing viscosity causes a decline in the rotation of the sperm head. In the present study, the sperm head hardly rotated at high viscosity because of the increasing flow resistance. However, the rotation of the sperm head is undesirable from the viewpoint of the energy consumption of the organism. Under actual circumstances, as the viscosity of the oviduct mucus is considerably greater than that of water, it is presumed that the sperm hardly rotates, and this nonrotating motion is considered effective for the sperm. Therefore, we considered that the rotating motion is not intrinsic for the bovine sperm.

Lai et al. (2007) reported that the viscosity of cervicovaginal mucus is in the range of 0.1–1.0 Pa·s, which is greater than the highest viscosity (0.02 Pa·s) in the present experiment. Therefore, future studies must investigate sperm motility in an environment with a greater viscosity. Further, it is important to investigate the effect of non-Newtonian

properties of mucus on sperm motility (Hyakutake, et al 2015b). Additionally, the microgrooves of the bovine cervical wall may influence sperm migration (Suarez, 2016). As we first diluted the semen to facilitate observations in the present experiment, interactions between spermatozoa were not observed. Under actual conditions, spermatozoa moves in a three-dimensional group in the oviduct. Therefore, to understand the mechanics of sperm motility in the actual environment in more detail, the effect of the interaction between spermatozoon on sperm motility may also be important.

5. Conclusions

In the present study, we investigated the effect of different viscosities of surrounding fluid on the sperm motility in three dimensions by observing from the vertical and horizontal directions simultaneously. As the experimental results suggested that the conventional method using the cover glass affects the sperm motility in a three-dimensional space, we investigated the observation method using square tubing in more detail. The experimental results indicated that the head and the flagellum of the sperm moved in conjunction, and the viscosity of the fluid surrounding the sperm greatly influences the sperm's three-dimensional motility. These results suggest that the three-dimensional flagellar structure of the bovine sperm is a helix with an elliptic cross-section shape and a very large aspect ratio. In addition, the results determined whether the rotation or nonrotation of the sperm is dependent on the balance between the viscous resistance acting on the sperm and the torque by the flagellar shape. These results will be helpful in understanding the mechanics of sperm motility under the actual environmental conditions in the oviduct mucus.

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