Effect of viscosity on motion characteristics of bovine sperm

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\textbf{Abstract}— Bovine sperm motion in environments of various viscosities is studied. We used the semen of Japanese cattle and investigated the following parameters: the sperm velocity, the straight-line velocity, and the amplitude from the observed sperm trajectory. As the viscosity increased, the motility of the sperm decreased. On the other hand, the power expended by the sperm flagellum was of the same order of magnitude over the viscosity range of 0.0007–0.0226 Pa·s. Additionally, the increase in the viscosity brought about a change in the flagellum shape and an increase in the percentage of sperm with a nonrotating head. The existence of rotation caused a change in the sperm velocity, amplitude, and frequency of the flagellum. These results suggest that bovine sperm has evolved to swim effectively in the oviduct, which is a high-viscosity environment.

\textbf{Index Terms}—bovine sperm, viscosity, nonrotating sperm, image analysis

I. INTRODUCTION

The propulsion of various sperm is caused by the active motion of the flagellum. This active force arises from the action of inner and outer arm dynein motors. It is presumed that this movement mode evolved in response to the surrounding environment of the sperm; consequently, there exist several flagellar waveforms that depend on the species. For example, in the case of the sea urchin, the sperm swims in the sea, i.e., a method of fertilization that is classified as external fertilization. Conversely, in the case of mammals, the sperm moves within the reproductive organs, a method classified as internal fertilization. Therefore, understanding the mechanism of sperm motility according to the surrounding fluid environment is extremely important.

In this study, we consider the sperm motility of mammals. As mentioned above, mammal sperm undergoes internal fertilization. When the sperm moves towards the ovary through the oviduct, it is influenced by several circumstances. The oviductal mucus is composed of various fluids, including macromolecules and gels. Mammalian sperm migrates through the oviduct, where the viscosity is quite high compared to that of water. Moreover, the sperm moves against the flow of the oviductal fluid due to tubal peristalsis. In addition, a change in the sperm motion, called hyperactivation, occurs on the way to ovary and is expected to be affected by the calcium ions in the oviduct. Therefore, we can say that the sperm motility is significantly influenced by the rheology, shear stress, and chemical composition of the surrounding fluid, which lead to fertilization in the oviduct. In the present study, we focused on the effect of the viscosity of the oviductal mucus on the sperm motility.

Many researchers have conducted rigorous studies on the motion characteristics of sperm from theoretical [1]-[4], experimental, [5]-[12] and numerical [13]-[16] standpoints. However, there are few studies that focus on the effect of the surrounding environment of the sperm, i.e., the fluid characteristics of the oviduct, on the sperm motility. Rikmenspoel et al. [17] investigated the effect of increasing viscosity on bull sperm motility by the addition of ficoll to the medium. Brokaw [18], [19] conducted a detailed observation of the movement of sea-urchin sperm and investigated the effects of increasing viscosity. Wolf et al. [20] found that the mucus in the uterine tube is a viscoelastic fluid that includes gelatinous materials and macromolecules. Lai et al. [21] measured the viscosity of fresh human cervical mucus samples as a function of the shear rate and showed that the viscosity of the mucus was greater than that of water by two or three orders of magnitude, i.e., 0.1–1.0 Pa·s. Furthermore, they indicated that the mucus is a non-Newtonian fluid. There were several experimental studies on the effect of the viscosity on the motion characteristics of sperm [22]-[25]. Therefore, it is necessary to observe sperm motility in a high-viscosity fluid and not in a dilute fluid to understand the essential mechanism of sperm motility.

Given this background, we investigated the effect of viscosity on the motion characteristics of bovine sperm. In particular, we analyzed the trajectory of the sperm motion and investigated the effect of viscosity of the surrounding fluid on the sperm velocity and the amplitude obtained from the sperm trajectory. In the present experiment, we selected bovine sperm as an example of mammal sperm. The head shape of bovine sperm is disc-like and different from that of human sperm. The change in the width of the sperm head makes it simple to observe the rotation of the head. The obtained experimental results will be useful in...
clarifying the mechanics of sperm motility in their actual environment. Furthermore, the results will provide valuable information for the reproduction industry of the animal husbandry field.

II. MATERIALS AND METHODS

We observed the sperm motion using an optical microscope (OLYMPUS IX71, Olympus, Japan), and the type of objective lens used was LUCPlanFL 40x. We obtained pictures using a CCD camera (K-II, Kato-koken, Japan), which has an image pixel size of 640 × 480. We used Japanese cattle semen (Animal Genetics Japan Co., Ltd., Japan). First, to facilitate observation, we separated the bovine semen into sperm and seminal plasma using a centrifugal separator (LT-015, TOMY Digital Biology Co., Ltd., Japan). Then, the separated sperm was diluted with a phosphate buffered solution. Next, in order to increase the viscosity of the sperm solution, we fused it with a PVP-K90 (polyvinylpyrrolidone) solution. This suspension was warmed in a water bath and placed in a glass slide, with a pool of depth 0.1 mm, and covered with a coverslip. Since there was a gap of 0.1 mm between the bottom of the pool and the coverslip, the motion of the sperm was not restricted in the vertical direction. We used a rheometer (ARES-G2, TA Instruments) to measure the viscosity of the PVP-K90 solution at a temperature of 38.5 °C. Five PVP-K90 concentrations, 0.0%, 1.0%, 2.5%, 4.0%, and 6.0%, were selected to measure the viscosity. Figure 1 shows the relationship between the viscosity and the shear rate of the reagents. The viscosity was almost constant as a function of the shear rate, which means that PVP-K90 is a Newtonian fluid. An increase in the concentration of PVP-K90 caused an increase in the viscosity. In this study, we successfully performed experiments over a viscosity range of 0.0007–0.0226 Pa·s. The viscosities of the PVP-K90 concentrations are given in Table 1. The viscosity in the actual mucus is in the range of 0.1–1.0 Pa·s, according to the measurements of Lai et al. [21]. Therefore, the viscosity used in the present experiment was lower than that in an actual environment by one order of magnitude. We focused on the effect of increasing viscosity; therefore, from the obtained experimental data, we can discuss the effect the viscosity has on the characteristics of sperm motion.

We observed the sperm motion under a microscope and obtained images at a rate of 200 fps using a high-speed camera. For image analysis, we conducted a PTV analysis using the DIPP-Motion Pro fluid analysis software (Ditect, Co., Ltd., Japan). We obtained the trajectory of the sperm motion by placing a marker on top of the sperm head in the pictures. Figure 2 shows four pictures with markers indicated by red dots. From the obtained trajectory, we calculated two velocities. One is sperm velocity, \( V_{SP} \), which was calculated by averaging the velocities of each frame. The other is straight-line velocity of the sperm, \( V_{ST} \), which was...
calculated using the distance between the sperm position in the first frame and that in the last frame, as shown in Fig. 3. Furthermore, we also calculated the amplitude of the sperm trajectory, \( A \). This was calculated on the basis of the progress axis of the sperm trajectory obtained by the least-squares method. In Fig. 4, the trajectories of the sperm motion and the progress axis for PVP concentrations of 0.0%, 1.0%, 2.5%, and 4.0% are shown. The number of sperm samples was 17, 58, 24, 19, and 24 at 0.0%, 1.0%, 2.5%, 4.0%, and 6.0%, respectively.

### III. RESULTS

Figure 5 shows the relationship between the straight-line velocity \( V_{ST} \) and the sperm velocity \( V_{SP} \) for various viscosities. From this figure, it is clear that \( V_{ST} \) increases as \( V_{SP} \) increases, demonstrating a correlation between the straight-line and sperm velocities. The correlation coefficient was 0.78. The experimental data were mostly distributed in the area above the line through the origin with the slope \( V_{SP}/V_{ST} = 3.0 \). The large slope means that the straight-line velocity is small compared to the sperm velocity. Regarding the effect of the viscosity on the two velocities, increasing viscosity caused both velocities to decrease. When the PVP-K90 concentration was 0.0%, meaning the viscosity was 0.0007 Pa·s, the sperm velocity was in the range of approximately 100–220 \( \mu \text{m/s} \). Conversely, when PVP-K90 concentration was 6.0%, meaning the viscosity was 0.023 Pa·s, the sperm velocity range decreased to approximately 40–150 \( \mu \text{m/s} \), where some sperms stayed in the same position, i.e., \( V_{ST} \) was almost 0.

Figure 6 shows the relationship between the straight-line velocity \( V_{ST} \) and the amplitude of the sperm trajectory \( A \) for various viscosities. This figure shows that there is a weak correlation between the straight-line velocity and the amplitude of the sperm trajectory. The correlation coefficient was 0.49. As the straight-line velocity decreased, the amplitude also decreased. It is clear that the increase in viscosity caused a decrease in the amplitude of the sperm trajectory.

Figure 7 summarizes the variation of the average values of \( V_{SP} \) and \( V_{ST} \) for various viscosities. The average sperm velocity at PVP 0% was approximately 200 \( \mu \text{m/s} \), whereas it was approximately 75 \( \mu \text{m/s} \) at
PVP 6%, a decrease of more than 60%. Similarly, the average straight-line velocity decreased from 40 to 16 \( \mu \text{m/s} \) as the viscosity increased.

Next, we focused on the rotation of the sperm head. We defined that the rotating head was the sperm head that rotated more than once within the observation time (2 s). In Fig. 8, the fraction of sperm with a nonrotating head for various PVP-K90 concentrations is shown. The observational results indicate that the fraction of sperm with a nonrotating head increases as viscosity increases.

We calculated the frequency of the flagellum from the obtained images. Figure 9 shows the comparison of the frequency of rotating sperm with that of nonrotating sperm for each value of viscosity. At 0.0%, there is a lack of data for nonrotating heads because the fraction of sperm with a nonrotating head is 0.0% for this viscosity. From this figure, we observe that an increase in the viscosity causes a decrease in the frequency. Additionally, it is clear that the frequency of sperm with a nonrotating head is larger than that with a rotating head for any viscosity. Figure 10 shows the comparison of the amplitude of the sperm trajectory of rotating heads with that of the sperm trajectory of nonrotating heads for each value of viscosity. An increase in viscosity leads to a decrease in the amplitude. Furthermore, nonrotating heads also cause a decrease in the amplitude.

Under the circumstance where the sperm swims, the Reynolds number is very small. Therefore, we can calculate the force acting on the sperm head using Stokes’ law. We calculated the forces acting on the sperm for various viscosities using the following equations:

\[
F_{SP} = 6\pi \mu a V_{SP},
\]  

(1)
where \( \mu \) is the viscosity of the surrounding fluid and \( a \) is the average radius of the sperm head. We calculated two types of forces according to the sperm and straightline velocities. Figure 11 compares the calculated forces for the various viscosities. From this figure, as the viscosity increased, the forces acting on the sperm also increased.

Furthermore, we investigated the effect of the surrounding viscosity on the flagellar shape. Figures 12 and 13 show six photographs of the flagellar shape at PVP-K90 concentrations of 0% and 6%, respectively. The interval of each photograph is 0.02 s. The red line indicates the flagellum. From these figures, when the PVP-K90 concentration is 0%, at which the viscosity is approximately equal to that of water, the entire flagellum from the root to the tip moved in a curved flapping motion. However, when the PVP-K90 concentration was 6%, at which the viscosity is approximately 33 times that at 0%, the curvature of the flagellum near the midpiece was very small, and primarily only the latter half of the flagellum moved.

Finally, we calculated the power expended by the sperm flagellum. The power expended by the flagellum is the sum of the power used in overcoming viscous forces and the power required for the elastic deformation. In the present study, we calculated the power by the viscous force to investigate the effect of the increasing viscosity. This power can be expressed as follows [26]:

\[
P = 0.5 b^2 l k \mu a^2 \tag{3}
\]

where \( b \) is the amplitude of the flagellar wave, \( l \) is the length of the flagellum, \( k \) is the drag coefficient of the flagellum, and \( \omega \) is the angular velocity. In the present study, we applied the frequency of the flagellum and the amplitude of the sperm trajectory. We calculated \( k \) to be 2.1 [27]. The calculated results are shown in Fig. 14. Although the dispersion according to the samples was large, the average value did not vary much with respect to the viscosity.

IV. DISCUSSION

In the present experiment, we observed sperm motion in environments with five viscosities and obtained several parameters involved in sperm motile capability. We also investigated the effect of the viscosity on the flagellar shape of the sperm. Furthermore, we investigated the effect of the viscosity on the rotation of the sperm head. The experimental results indicate that sperm motion characteristics are significantly affected by the viscosity of the surrounding environment of the sperm.

Smith et al. [23] experimentally investigated the effect of viscosity on sperm motion characteristics using methylcellulose as the fluid surrounding the sperm. Consequently, they obtained experimental results indicating that as the viscosity increases, it has little influence on the straight-line velocity. The present experimental data differ from these results. The differences between the present experiment and the experimental data by Smith et al., are the reagents used (methylcellulose vs. PVP-K90) and the species studied (bovine vs. human). Methylcellulose is generally said to be a non-Newtonian fluid. The PVP-K90 used in this experiment is clearly a Newtonian fluid, as shown in Fig. 2. Due to this difference, it is presumed that the straight-line velocity decreases with increasing viscosity only in the case when a Newtonian fluid is used. Form the sperm velocity obtained in the present experiment, we calculated the shear rate surrounding the sperm. As a result, the shear rate was approximately 20–60 s\(^{-1}\). Therefore, to understand the mechanics of sperm motility under the actual environment in more detail, it is necessary to experimentally compare the cases with Newtonian and non-Newtonian fluids.

From Fig. 11, the force acting on the sperm increased as the viscosity increased. When the viscosity was low, the sperm did not use more force than was necessary. As the viscosity increased, the sperm had to generate more force to progress forward. Hyun et al. [25] measured the sperm swimming force using optical tweezers. They indicated that the swimming force increased as the viscosity increased. This tendency is the same as that demonstrated in the present experimental results. The viscosity of Hyun’s experiment is in the range 0.001–0.015 Pa\(\cdot\)s. In the present experiment, the maximum viscosity is 0.023 Pa\(\cdot\)s (Table 1). It is worth considering whether the greater maximal viscosity causes a peak in the swimming force or not.

An increase in the viscosity caused a decrease in not only the sperm velocity, but also the amplitude of the trajectory and the frequency of the flagellum over a viscosity range of 0.0007–0.0226 Pa\(\cdot\)s. This tendency was identical to the results of Rikmenspoel et al. [17][28]. Furthermore, the power generated by the viscous force of the sperm flagellum was calculated as follows [26]:

\[
P = 0.5 b^2 l k \mu a^2 \tag{3}
\]
from the present experimental data. The effect of the viscosity on the power expended by the flagellum was small. According to the experimental results of Rikmenspoel et al. [17], the power expended by the flagellum was also of the same order over a viscosity range of 0.001–0.016 Pa·s. In the future, a quantitative comparison of the power expended by the flagellum for higher viscosities would be required.

Interestingly, we observed differences in the flagellar shape depending on the viscosity. As the viscosity increased, the flagellar shape changed from the entire flagellum flapping to only a portion of it. Rikmenspoel [28] experimentally investigated the relationship between the distance from the root of the flagellum and the amplitude of the flagellum. According to his experiment, at high viscosities, the amplitude increased monotonically as apart from the root of the flagellum, which differs from the case of low viscosities. Therefore, the observation of the present experiment showed similar results to those obtained by Rikmenspoel [28]. Furthermore, the difference with respect to the viscosity was similarly noted by Smith et al. [23]. The thickness of the flagellum is nonuniform, with the flagellum becoming thinner from the midpiece to the end piece. In the case of low viscosity, the thickness of the flagellum minimally affected its curvature, whereas it had a much larger effect on curvature as viscosity increased. The moment acting on the sperm was determined by the balance between the moment due to the viscous drag and the elastic bending moment [17]. As the viscosity increased, the viscous moment also increased. Therefore, at the tip of the flagellum where the bending rigidity is small, the curvature became large. Consequently, it was presumed that the total moment of the sperm was adjusted by the change in the flagellar shape. This supports our assertion that the sperm motion characteristics in the diluted fluid in the previous studies are different from those in the actual environment, and sperm has progressive motility in a high-viscosity environment. These results suggest that sperm bent flagellum has changed according to the surrounding fluid environment to reach the ovum as soon as possible.

Moreover, we investigated whether the sperm head rotated or not. The results indicate that the number of sperm with nonrotating heads increases as the viscosity
increases. The rotation of the sperm head is undesirable from the viewpoint of the energy consumption of the organism. When the PVP-K90 concentration is 0%, the sperms with low motilities can rotate their heads because the force acting on the sperm head is small. However, as the viscosity increased, the viscous resistance of the sperm head became larger than the force due to the torque produced by the three-dimensionality of the flagellum shape. As a result, we presumed that some sperm with low motility did not rotate and the percentage of nonrotating sperm increased.

In the present experiment, the maximum viscosity is 0.023 Pa·s (Fig. 2). However, the viscosity in mucus is in the range of 0.1–1.0 Pa·s, according to the measurements of Lai et al. [21]. Therefore, it is necessary to investigate sperm motility in an environment with a greater viscosity. Furthermore, it is also important to investigate the effect of a non-Newtonian fluid on sperm motion. For the present experimental apparatus, we conducted only two-dimensional observations. Investigating the three-dimensional motion of flagella with increasing viscosity may lead to a better understanding of the mechanics of sperm motility in the actual environment. In this study, since we diluted the semen to facilitate observations, the interaction between sperm was not observed. Actually, sperms tend to move in a group. Therefore, the effect of the interaction between sperm on sperm motility under high viscosity is also an important factor to investigate.

V. CONCLUSIONS

In this study, we experimentally observed the motion of bovine sperm in environments with various viscosities (0.0007–0.0226 Pa·s) using a microscope. First, we investigated the relationship between the sperm velocity, the straight-line velocity, and the amplitude of the sperm trajectory. As viscosity increased, the sperm velocities and amplitude decreased. Furthermore, the number of sperm with a nonrotating head increased as the viscosity increased. The rotation of the head caused a change in the frequency of the flagellum and the amplitude of the sperm trajectory. Additionally, increasing viscosity brought about a change in the flagellum shape and an increase in the force acting on the sperm. On the other hand, the effect of the viscosity on the power expended by the flagellum was small. These results agree with previously reported experimental results. These results will be helpful to understand sperm motion characteristics in the actual high-viscosity environment.

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VII. REFERENCES


