Journal of Biomechanics ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Effect of non-Newtonian fluid properties on bovine sperm motility

Toru Hyakutake [a,*], Hiroki Suzuki [b], Satoru Yamamoto [b]

[a] Faculty of Engineering, Yokohama National University, 79-5 Hodogaya, Yokohama 240-8501, Japan
[b] Graduate School of Engineering, Yokohama National University, 79-5 Hodogaya, Yokohama 240-8501, Japan

ARTICLE INFO

Article history:
Accepted 3 August 2015

Keywords:
Bovine sperm
Viscosity
Non-Newtonian fluid
Flagellum
Image analysis

ABSTRACT

The swimming process by which mammal spermatozoa progress towards an egg within the reproductive organs is important in achieving successful internal fertilization. The viscosity of oviductal mucus is more than two orders of magnitude greater than that of water, and oviductal mucus also has non-Newtonian properties. In this study, we experimentally observed sperm motion in fluids with various fluid rheological properties and investigated the influence of varying the viscosity and whether the fluid was Newtonian or non-Newtonian on the sperm motility. We selected polyvinylpyrrolidone and methylcellulose as solutes to create solutions with different rheological properties. We used the semen of Japanese cattle and investigated the following parameters: the sperm velocity, the straight-line velocity and the amplitude from the trajectory, and the beat frequency from the fragellar movement. In a Newtonian fluid environment, as the viscosity increased, the motility of the sperm decreased. However, in a non-Newtonian fluid, the straight-line velocity and beat frequency were significantly higher than in a Newtonian fluid with comparable viscosity. As a result, the linearity of the sperm movement increased. Additionally, increasing the viscosity brought about large changes in the sperm flagellar shape. At low viscosities, the entire flagellum moved in a curved flapping motion, whereas in the high-viscosity, only the tip of the flagellum flapped. These results suggest that the bovine sperm has evolved to swim toward the egg as quickly as possible in the actual oviduct fluid, which is a high-viscosity non-Newtonian fluid.

1. Introduction

The movement of a spermatozoon, the male reproductive cell, toward an egg, the female reproductive cell, enables fertilization. The swimming process toward an egg within the reproductive organs is important for the mammal spermatozoa to achieve successful internal fertilization. Each sperm cell has a flagellum, and the propulsion of the 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 (external fertilization). Conversely, in mammals, the sperm moves within the reproductive organs (internal fertilization). Therefore, understanding how the mechanism of sperm motility corresponds to the surrounding fluid environment is extremely important.

In this study, we consider the sperm motility of mammals. As mentioned above, mammal sperm participates in internal fertilization. When the sperm moves toward 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 because of tubal peristalsis. In addition, a change in the sperm motion, called hyperactivation, may occur by a signal transduction mechanism through a medium of calcium ions on the way to the ovary. Therefore, we can say that sperm motility is significantly influenced by the rheology, shear stress, and chemical composition of the surrounding fluid. In the present study, we focused on the effect of the fluid rheological properties of the oviductal mucus on sperm motility.

Many researchers have conducted rigorous studies on the motion characteristics of sperm from theoretical (Gray and Hancock 1955; Lighthill, 1976; Higdon, 1979; Phan-Thien et al., 1987), experimental (Brokaw 1965; Phillips 1972; Mortimer et al., 1997; Crenshaw et al., 2000; Woolley 2003; Denissenko et al., 2012), and numerical (Gillies et al., 2009; Smith et al., 2010; Elgeti et al., 2010; Tam and Hosoi 2011; Gurarie et al., 2011; Guerrero et al., 2011) standpoints. Focusing on the bovine sperm used in the present experiment, Rikmenspoel and Herpen (1957) indicated that sperm velocity is proportional to the frequency of the sperm rotation. Furthermore, they observed the elongation of the

* Corresponding author. Tel./fax: +81 45 339 3882.
E-mail address: hyaku@ynu.ac.jp (T. Hyakutake).

http://dx.doi.org/10.1016/j.jbiomech.2015.08.005
0021-9290/

flagellum and suggested that bovine sperm rotates in three dimensions. Rikmenspoel (1965) distinguished between non-rotating and rotating sperm heads and concluded that sperm with nonrotating heads is unhealthy. Focusing on the rotation of the sperm head, Drake (1974) clarified that the sperm head continuously rotates 360°. 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 sperm from various species, including bovine sperm. 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. In addition, several studies recently successfully tracked the three-dimensional trajectory of the human spermatozoon (Sheng et al., 2007; Corkidi et al., 2008; Su et al., 2012). However, most of these studies investigated sperm motility in a diluted solution. Wolf et al. (1977) found that the mucus in the uterine tube is a viscoelastic fluid that contains gelatinous materials and macromolecules. Lai et al. (2007) measured the viscosity of fresh human cervical mucus samples as a function of the shear rate and showed that the viscosity of the mucus is greater than that of water by two orders of magnitude. Furthermore, they indicated that the mucus is a non-Newtonian fluid. Several studies have focused on the effect of the surrounding environment of the sperm, i.e., the fluid characteristics of the oviduct, on the sperm motility, particularly the viscosity of the surrounding fluid (Katz et al., 1978; Rikmenspoel 1984; Smith et al., 2009; Kirkman-Brown and Smith 2011). Suarez and Dai (1992) experimentally investigated mouse sperm motility in a viscoelastic fluid. Several numerical studies (Fu et al., 2007; Teran et al., 2010) have been examined on the motion of sperm in non-Newtonian fluids. However, since few experimental studies have focused on the differences between high-viscosity Newtonian and non-Newtonian fluid environments, the effect of a non-Newtonian surrounding fluid on sperm motility is not clear. Therefore, it is necessary to observe sperm motility in a high-viscosity non-Newtonian fluid to understand the essential mechanism of sperm motility.

Given this background, we experimentally investigated the effect of a non-Newtonian fluid on the motion characteristics of bovine sperm. In particular, by comparing Newtonian and non-Newtonian fluid environments, we analyzed the trajectory of the sperm motion and investigated the effect of the rheological properties of the surrounding environment on the sperm velocity and the amplitude from the sperm trajectory. Additionally, we investigated the influence of the fluid rheological properties on the flagellar shape of the observed sperm. The obtained experimental results will be useful in clarifying the mechanics of sperm motility under their actual environmental conditions. Furthermore, the results will provide valuable information for the reproduction industry of the animal husbandry field. Additionally, this study may provide useful data that will contribute to understanding sperm motility in the microfluidic sperm sorter that has been developed for infertility treatment (Cho et al., 2003; Schuster et al., 2003; Hyakutake et al., 2009; Matsuura et al., 2012).

2. Materials and methods

We observed the sperm motion using an optical microscope (OlympusIX71, Olympus, Japan) and obtained pictures using a CCD camera (K-II, Kato Koken, Japan). We used Japanese cattle semen (Suzukane, Animal Genetics Japan Co., Ltd., Japan), which was cryopreserved in a liquid nitrogen tank. First, we thawed it and added a Tris–citric acid–glucose solution, which has similar components to those of the diluted solution used when the semen was cryopreserved. This was added to prevent the loss of fertilization ability from damage to and breakdown of the sperm acrosome. As a result, we were able to sustain the sperm motility and extend the observation time. Next, to facilitate observation, we separated the bovine semen into sperm and seminal plasma using a centrifugal separator for a duration of

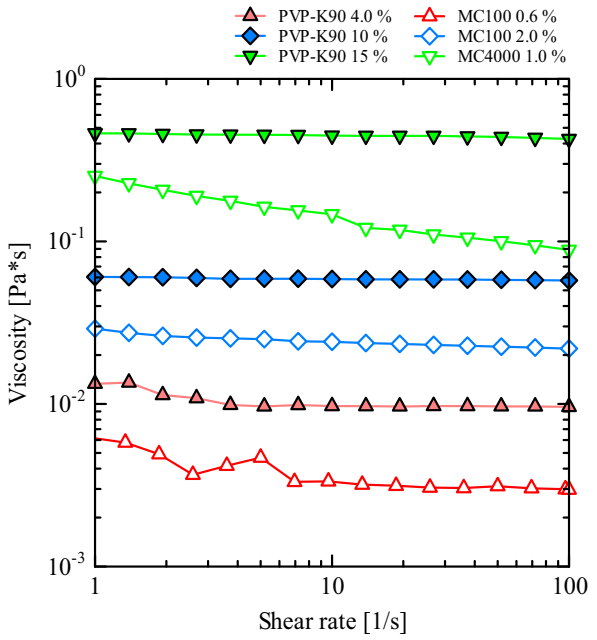

Fig. 1. Relation between the shear rate and viscosity of PVP-K90, MC100, and MC4000. The filled triangle represents PVP-K90 4.0%, the filled diamond represents PVP-K90 10%, the filled inverted triangle represents PVP-K90 20%, the open triangle represents MC100 0.6%, the open diamond represents MC100 2.0%, and the open inverted triangle represents MC4000 1.0%.

10 min. The separated sperm was then diluted with a phosphate buffered saline, where we fused it with polyvinylpyrrolidone (PVP) and methylcellulose (MC) solutions to change the rheological properties of the sperm solution. This suspension was warmed in a water bath at 38.5 °C, and the temperature of the suspension was maintained using a thermoplate during the observation. The suspension was 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 and MC solutions at a temperature of 38.5 °C. We selected PVP-K90 (Wako Pure Chemical Industries, Ltd., Japan) for the PVP solutions, and MC100 and MC4000 (Wako Pure Chemical Industries, Ltd., Japan) for the MC solutions. Three PVP-K90 concentrations, 4.0%, 10%, and 15%, were selected. Two MC100 concentrations, 0.6% and 2.0%, and one MC4000 concentration, 1.0%, were selected. Fig. 1 shows the relationship between the shear rate and the viscosity of the six solutions. The experimental data in this figure were taken from one measurement. The viscosities of the PVP-K90 solutions were almost constant as a function of the shear rate, which means that PVP-K90 is a Newtonian fluid. An increase in the PVP-K90 concentration caused an increase in the viscosity. The MC solution generally has viscoelastic properties (Amari and Nakamura, 1973). The viscosities of the MC100 solutions did not greatly change for different shear rates; therefore, we considered the non-Newtonian properties of MC100 to be weak. However, the viscosity of the MC4000 solution decreased with an increase in the shear rate, and its values were between the viscosities of the PVP-K90 solutions with concentrations of 10% and 15%. The viscosity when the shear rate was 1.0 s^{-1} was approximately three times that when shear rate was 100 s^{-1}. Therefore, we considered the MC4000 solution to have strong non-Newtonian properties.

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 employed a particle tracking velocimetry (PTV) technique analysis using the DIPP-Motion Pro fluid analysis software (Ditect Co., Ltd., Japan). We obtained the trajectory of the sperm motion by marking the sperm head in the images. At a low viscosity, the sperm flagellum was assumed to have three-dimensional movement because the sperm head rotates. Strictly speaking, we needed to observe the sperm motion in three dimensions because of the rotating sperm head. However, exact three-dimensional observation is very difficult. Therefore, we obtained the two-dimensional velocity for the observed plane in the present experiment. On the other hand, at a high viscosity, the sperm hardly rotates, so we can consider the flagellar wave plane to become parallel to the observed plane as the projected area of the sperm head becomes larger. Therefore, we extracted sperm when we observed the projected area of the sperm head to be large. From the obtained trajectory, we calculated two velocities using the MATLAB digital image analysis software (MathWorks, USA). The first 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},

Please cite this article as: Hyakutake, T., et al., Effect of non-Newtonian fluid properties on bovine sperm motility. Journal of Biomechanics (2015), http://dx.doi.org/10.1016/j.jbiomech.2015.08.005