ZOOLOGICAL SCIENCE 10: 281–285 (1993)

Effect of Osmolality on the Motility of Sperm from the Lamprey, *Lampetra japonica*

WATARU KOBAYASHI

Zoological Institute, Faculty of Science, Hokkaido University, Sapporo 060, Japan

ABSTRACT—Lamprey (*Lampetra japonica*) sperm were immotile not only in the seminal plasma but when immersed in an isotonic lamprey Ringer solution. Sperm were also found to be immotile when the semen was diluted with NaCl, KCl, or glucose solution isotonic to the Ringer solution. Sperm became motile, however, after dilution of semen with an hypotonic solution. These results suggest that motility of the lamprey sperm is probably suppressed by the osmolality of the seminal plasma and is initiated by a decrease in osmolality upon dilution into the spawning media (river water).

INTRODUCTION

Sperm of many animal species are quiescent in male reproductive organ and become motile after discharge into spawning media. It has been known that sperm of many species that show external fertilization recognize the difference between their seminal plasma and environmental media as a signal for the initiation of sperm motility [12]. Morisawa and his collaborators reported that hypotonicity induces the initiation of sperm motility in some cyprinid fishes (freshwater spawners) while hypertonicity induces sperm motility in marine teleosts [6, 7]. A change in osmotic pressure is not the sole factor for the initiation of sperm motility; the sperm of some freshwater fish show motility in isotonic media [12]. For example, salmonid sperm move after a decrease in the concentration of surrounding potassium ions even when the seminal plasma is diluted with salt solutions isotonic to it [7, 9]. Knowledge of the external factors that induce sperm motility may offer a basis for understanding the adaptation of gametes to the spawning environments.

I am interested in the fertilization of lampreys, because these animals represent one of the most ancient group of vertebrates. Information obtained from lamprey reproduction may be useful in helping us to understand the evolution of reproductive strategy in vertebrates. However, few studies have focused on the reproductive physiology of lamprey gametes. At spawning, female and male lampreys release their gametes into hypotonic river water (external fertilization). Yamamoto [15] has stated that "artificial activation of the lamprey eggs is possible in an isotonic salt solution (137 mM NaCl, 2.7 mM KCl, 2 mM $CaCl_2$, pH 7.3 with $NaHCO_3$) but fertilization is impossible in the solution". He succeeded in induction of artificial fertilization by immersing the eggs, which had been inseminated with "dry" sperm, into fresh water. These facts suggest that lamprey sperm are immotile in the Ringer's solution and initiate movement after dilution of the seminal plasma with freshwater. The present study was carried out to decide whether decrease in osmolality induces motility in lamprey sperm.

MATERIALS AND METHODS

Adult males of the river lamprey, *Lampetra japonica,* were taken in May (their spawning season) in the Ishikari river at Ebetu, Hokkaido. They were kept without feeding in constant darkness in plastic aquaria containing well water (12°C). In these conditions, they matured within 10 days. Semen was obtained by manual stripping of the animals anesthetized in 0.05% tricaine methanesulfonate (MS-222). The semen from different individuals

Accepted February 2, 1993
Received November 12, 1992

were stored in separate petri dishes at 1°C until use. Care was taken to avoid dehydration during storage. Although the semen retained a capacity for fertilizing the eggs for more than a week, the samples were used within two days after stripping.

Lamprey Ringer (LR) consisted of 137 mM NaCl, 2.9 mM KCl, and 2.1 mM $CaCl_2$ (pH 7.4 with 10 mM Hepes-NaOH) [16]. Test solutions used for the assessment of the initiation of sperm motility were 1/10 strength LR and solutions containing various concentrations of NaCl, KCl, or glucose; they were buffered with 10 mM Hepes-NaOH (pH 7.4). To examine whether external Ca^{2+} ions participate in the initiation of sperm motility, we used 1/10 strength Ca^{2+} free LR containing 13.7 mM NaCl, 2,9 mM KCl, and 10 mM EGTA (pH 7.4 with 10 mM Hepes-NaOH).

For assessment of sperm motility, two μl of the semen was quickly mixed with 198 μl of test solution in a test tube. Ten μl of the mixture was placed on a glass slide without a cover slip within 5 seconds after mixing and examined under a microscope. Sperm movement was observed under a light microscope ($\times$200) and recorded by a video recorder. Sperm represented to be motile when the sperm head showed forward movement. Percentage of moving sperm (more than 50 cells) and swimming speed of the moving sperm (n=10) were monitored by tracing the location of sperm heads on a hard copy of the frame by a video printer. Assessment of sperm motility was carried out in samples from three males. All experiments were performed in triplicate for each sample and performed at room temperature. No significant difference was recognized among these males. Data (mean$\pm$SE, n=3) from a representative fish are therefore presented in the figures. Student's *t*-test was used to determine statistically significant differences, which were considered significant when $P<0.05$.

RESULTS

Lamprey sperm were immotile in the seminal plasma. Upon dilution of semen with full strength LR, less than 1% of sperm began to move within the first few seconds. In the 1/10 strength LR, nearly all sperm immediately showed active forward movement; most of the sperm continued the movement for more than 5 minutes. Sperm initi-

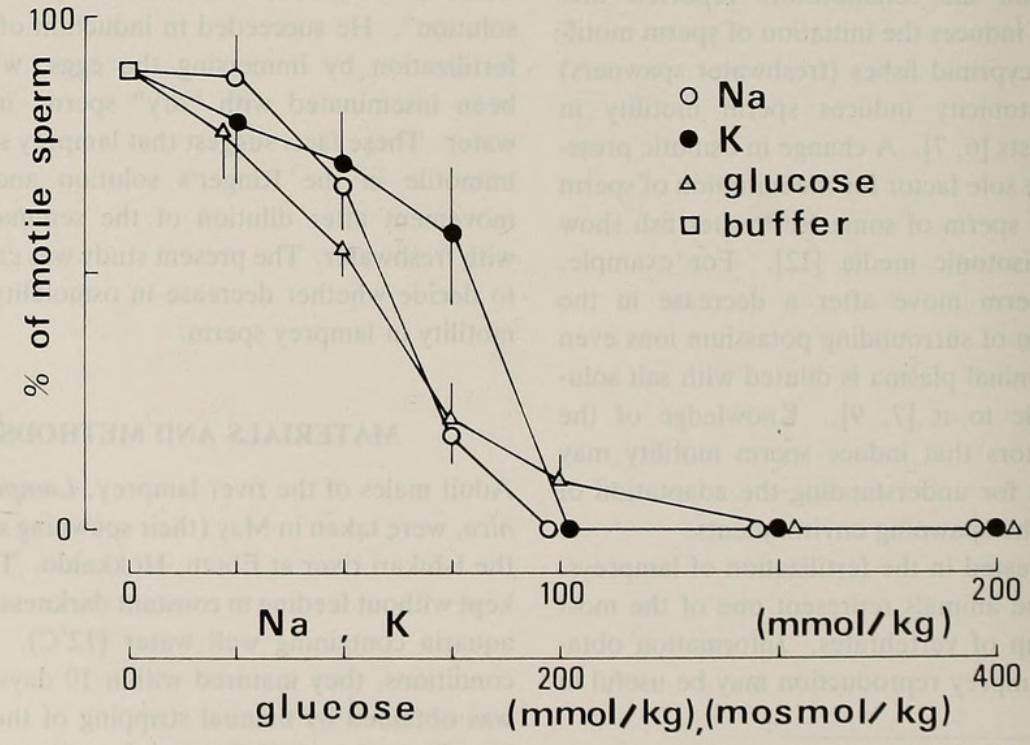

FIG. 1. Effect of NaCl, KCl or glucose on the motility of lamprey sperm. Percentage (mean$\pm$SE) of motile sperm 10 seconds after dilution of semen with the test solutions are presented.

ated similar movement in the 1/10 strength Ca^{2+} free LR. It is clear that the initiation of sperm motility does not require the presence of Ca^{2+} ions in external medium.

Figure 1 clearly shows that sperm were completely immotile in NaCl and KCl solutions at concentrations from 100 mmol/kg (200 mOsmol/kg) to 200 mmol/kg (400 mOsmol/kg). Percen-

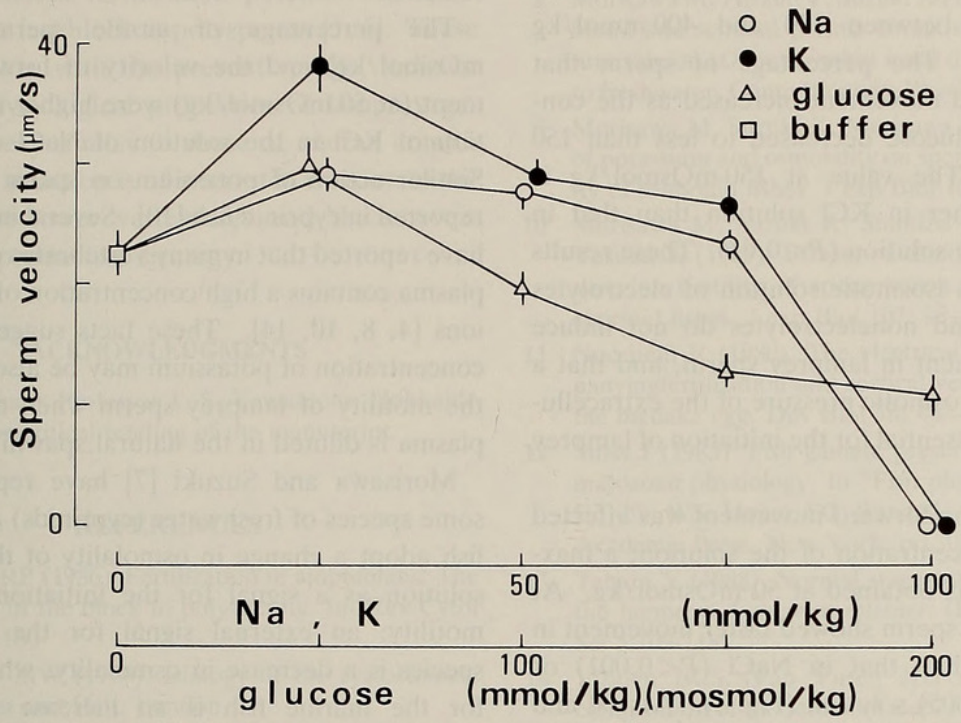

FIG. 2. Effect of NaCl, KCl or glucose on the velocity (mean ± SE) of forward movement of sperm 10 seconds after dilution of semen with test solutions.

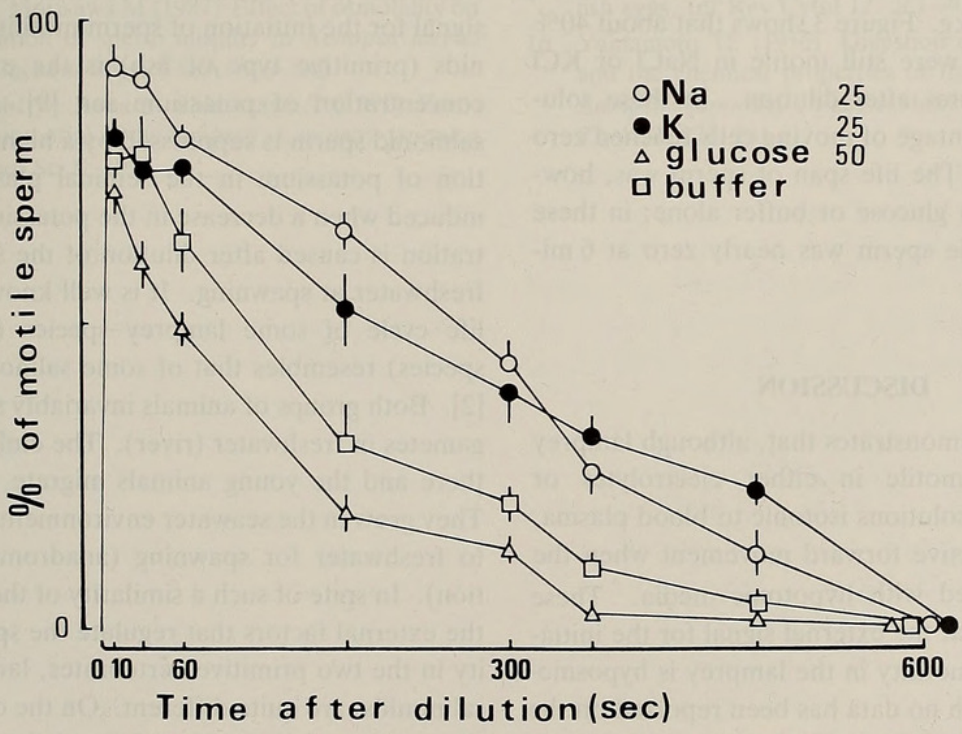

FIG. 3. Effect of the NaCl, KCl or glucose on the duration of the sperm motility. Changes in percentage of motile sperm in the test solutions (50 mOsmol/kg) and in buffer solution alone.

tage of motile cells increased as the concentration of the electrolytes decreased; a maximum value was obtained at a concentration between 0 and 25 mmol/kg (50 mOsmol/kg). A similar lack in the sperm motility in glucose solutions was observed at concentrations between 300 and 400 mmol/kg (mOsmol/kg). The percentage of sperm that showed forward movement increased as the concentration of glucose decreased to less than 150 mOsmol/kg. The value at 150 mOsmol/kg is apparently higher in KCl solution than that in NaCl or glucose solution ($P<0.01$). These results indicate that an isosmotic solution of electrolytes (NaCl, KCl) and nonelectrolytes do not induce forward movement in lamprey sperm, and that a decrease in the osmotic pressure of the extracellular medium is essential for the initiation of lamprey sperm motility.

Velocity of the forward movement was affected by osmotic concentration of the solution; a maximum speed was obtained at 50 mOsmol/kg. At this osmolality, sperm showed faster movement in KCl solution than that in NaCl ($P<0.001$) or glucose ($P<0.005$) solution (Fig. 2). At 100 and 150 mOsmol/kg, sperm velocity in NaCl or KCl solution was higher than that in glucose solution.

The percentage of motile sperm was estimated at 50 mOsmol/kg. Figure 3 shows that about 40% of total sperm were still motile in NaCl or KCl solution 5 minutes after dilution. In these solutions, the percentage of moving cells reached zero at 10 minutes. The life span of sperm was, however, short in a glucose or buffer alone; in these solutions, motile sperm was nearly zero at 6 minutes.

DISCUSSION

This study demonstrates that, although lamprey sperm are immotile in either electrolytes or nonelectrolyte solutions isotonic to blood plasma, they showed active forward movement when the semen is diluted with hypotonic media. These facts suggest that the external signal for the initiation of sperm motility in the lamprey is hyposmolality. Although no data has been reported on the osmolality of lamprey seminal plasma, it is known that in many species the osmolality of seminal plasma is nearly the same as that of blood plasma or isotonic saline solution (Ringer solution) [4, 8]. Osmolality of the seminal plasma is probably involved in the quiescence of lamprey sperm in the semen.

The percentage of motile sperm (at 150 mOsmol/kg) and the velocity of forward movement (at 50 mOsmol/kg) were higher in the solution of KCl in the solution of NaCl or glucose. Similar action of potassium on sperm motility is reported in cyprinid fish [10]. Several investigators have reported that in many vertebrates the seminal plasma contains a high concentration of potassium ions [4, 8, 10, 14]. These facts suggest that the concentration of potassium may be also related to the motility of lamprey sperm when the seminal plasma is diluted in the natural spawning.

Morisawa and Suzuki [7] have reported that some species of freshwater (cyprinids) and marine fish adopt a change in osmolality of the external solution as a signal for the initiation of sperm motility: an external signal for the freshwater species is a decrease in osmolality, while a signal for the marine fish is an increase in osmotic pressure. Amphibian sperm (newt and *Xenopus*) seem to possess a mechanism similar to the freshwater fish [3, 4, 10, 14]. In contrast, the external signal for the initiation of sperm motility in salmonids (primitive type of fish) is the extracellular concentration of potassium ion [9]: motility of salmonid sperm is suppressed by a high concentration of potassium in the seminal plasma and is induced when a decrease in the potassium concentration is caused after dilution of the semen with freshwater at spawning. It is well known that the life cycle of some lamprey species ("parasitic" species) resembles that of some salmonid species [2]. Both groups of animals invariably spawn their gametes in freshwater (river). The embryos hatch there and the young animals migrate to the sea. They grow in the seawater environment and return to freshwater for spawning (anadromous migration). In spite of such a similarity of the life cycle, the external factors that regulate the sperm motility in the two primitive vertebrates, lampreys and salmonids, are quite different. On the other hand, the pattern of the early stages of the embryonic development (cleavage) in the lamprey is very

similar to that of amphibians [13]. Electrophysiological studies of fertilization have further revealed that, unlike the case of fish eggs [11], lamprey eggs as well as anuran amphibians possess a Cl^--dependent fertilization potential that participates in a fast block to polyspermy [1, 5]. These facts, together with the present results, indicate that the physiological properties of lamprey gametes are very similar to those of anuran amphibians. This situation is of interest in relation to the evolution of lower vertebrates and the convergence of reproductive strategy.

ACKNOWLEDGMENTS

I wish to thank Professor T. S. Yamamoto, Hokkaido university, for critical reading of the manuscript.

REFERENCES

1 Elinson RP (1986) Fertilization in amphibians: The ancestry of the block to polyspermy. Int Rev Cytol 101: 59–100

2 Hardisty MW (1979) Biology of the cyclostomes. Chapman and Hall, London.

3 Hardy MP, Dent JN (1986) Regulation of motility in sperm of the red-spotted newt. J Exp Zool 240: 385–396

4 Inoda T, Morisawa M (1987) Effect of osmolality on the initiation of sperm motility in *Xenopus laevis*. Comp Biochem Physiol 88A: 539–542

5 Kobayashi W, Baba Y, Shimozawa T (1991) Fertilization potential in the lamprey, *Lampetra japonica* eggs. Zool Sci 8: 1097

6 Morisawa M (1985) Initiation mechanism of sperm motility at spawning in teleosts. Zool Sci 2: 605–615

7 Morisawa M, Suzuki K (1980) Osmolality and potassium ion: Their roles in initiation of sperm motility in teleosts. Science 210: 1145–1147

8 Morisawa M, Hirano T, Suzuki K (1979) Changes in blood and seminal plasma composition of the mature salmon (*Oncorhynchus keta*) during adaptation to freshwater. Comp Biochem Physiol 64A: 325–329

9 Morisawa M, Suzuki K, Morisawa S (1983) Effects of potassium and osmolality on spermatozoan motility of salmonid fishes. J exp Biol 107: 105–113

10 Morisawa M, Suzuki K, Shimizu H, Morisawa S, Yasuda K (1983) Effects of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes. J exp Biol 107: 95–103

11 Nuccitelli R (1980) The electrical changes accompanyingfertilization and cortical vesicle secretion in the medaka egg. Dev Biol 76: 483–498

12 Stoss J (1983) Fish gamete preservation and spermatozoan physiology. In "Fish physiology Vol 9B" Ed by WS Hoar, DJ Randall, EM Donaldson Academic Press, New York, pp 305–350

13 Tahara Y (1988) Normal stages of development in the lamprey, *Lampetra reissneri* (Dybowski). Zool Sci 5:109-118

14 Viljoen BCS, Van Vuren JHJ (1991) Physical composition of the semen of *Labeo ruddi* and *Labeo rosae* (Pisces: Cyprinidae). Comp Biochem Physiol 98A: 459–462

15 Yamamoto T (1961) Physiology of fertilization in fish eggs. Int Rev Cytol 12: 361–405

16 Yamamoto TS (1956) Digestion of egg envelopes and the chemical properties of the lamprey's egg, *Lampetra japonica*. J Fac Sci Hokkaido Univ Ser VI Zool 12: 273–281