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# ACQUISITION OF POTENTIAL FOR SPERM MOTILITY IN RAINBOW TROUT AND CHUM SALMON

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## SUMMARY

The male reproductive organ of rainbow trout and chum salmon consists of a pair of testes and sperm ducts. Spermatozoa in the distal portion of the sperm ducts exhibit full motility in the $K^+$-free medium. However, spermatozoa from the testis were almost immotile in this medium. This suggests that the spermatozoa acquire a capacity for movement during their passage from the testis along the sperm duct. In chum salmon migrating into a bay, the sperm duct was almost empty. However, after the fish have travelled upstream for 1 km to their spawning ground in the river, the spermatozoa have left the testis, moved into the sperm duct and are capable of becoming motile. Thus it is probable that the process of acquiring the ability to move occurs within a relatively short period in this simple reproductive organ. Additionally, testicular spermatozoa demembranated with Triton X-100 exhibited motility, although the motility was less than that of demembranated spermatozoa from the sperm duct, suggesting that the acquisition of motility may correspond with the development of some function of the plasma membrane.

## INTRODUCTION

Since Tournade (1913) demonstrated that mammalian spermatozoa acquire motility during transit through the epididymis from the caput to the cauda after spermiation, much effort has been devoted to understanding the mechanism of this phenomenon (Hoskins, Brandt & Acott, 1978). However, few studies have paid attention to the subject of acquisition of motility in lower vertebrate or invertebrate spermatozoa (Bedford, 1979; Depeiges & Dacheux, 1984).

Teleost spermatozoa are spermiated in the testis, transferred into the anterior portion of the sperm duct and pass to the posterior portion into a large volume of semen (Hoar, 1969; Hiroi & Yamamoto, 1968). In salmonid fishes, spermatozoa in

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the distal part of the sperm duct are able to move vigorously when they are suspended in $K^+$-free solution (Morisawa & Suzuki, 1980; Morisawa, Suzuki & Morisawa, 1983). However, there is no report concerning when or where teleost sperm become capable of movement.

In the present study, we assess the motility of sperm of salmonid fishes collected from the testis and different portions of the sperm duct and show that testis spermatozoa are almost immotile, becoming motile after leaving the testes for the sperm duct. In addition, we show that acquisition of motility occurs within a short period during the migration of chum salmon from sea to river.

## MATERIALS AND METHODS

### *Materials*

Mature 1-year-old, male rainbow trout, *Salmo gairdneri*, which were about 20 cm in length and 100–140 g in mass, were obtained from a commercial source or a prefectural fishery experimental station in Japan during the breeding season (January to February) when almost all spermatozoa are completely morphologically matured (van den Hurk, Peute & Vermeij, 1978; Oota, Yamamoto, Takano & Sakaguchi, 1965). They were transported for several hours and kept without food in a freshwater laboratory tank at 10°C. The experiments were performed within 3 days after transfer. The chum salmon, *Oncorhynchus keta*, were captured from the end of November to the beginning of December in Otsuchi Bay or Otsuchi River, Iwate Prefecture. They were transported for 30 min, transferred to a freshwater tank and kept for 0–3 days without food. In our electron microscope observations, fully-formed spermatozoa were present in the testis.

### *Partition of the male reproductive organ in rainbow trout*

In salmonid fishes, right and left sperm ducts arise near the anterior portion of each testis. As shown in Fig. 1, they extend in a posterior direction, forming a single common duct which opens at the urogenital pore. In the present experiment the fish were killed, the abdomen opened and the sperm duct was ligated into four segments with nylon thread (see Fig. 1). Portion I, the tip portion, consisting of one-third of the anterior half of the sperm duct, was slender and contained a small volume of semen; portion II, the next one-third of the sperm duct, was also slender and contained a small volume of semen; portion III, the remaining one-third of the anterior half, was thicker and included a larger volume of semen than either portion I or portion II; portion IV, the posterior half of the sperm duct, was thick and contained a large volume of semen.

### *Measurement of motility in intact and Triton X-100 extracted spermatozoa*

A small incision was made in the testis or in each portion of the sperm duct and extruded semen was harvested with a pipette and suspended directly in the medium or stored in capped test tubes on ice. All experiments were performed within 1 h after collecting the semen.

The semen was suspended in 100 mmol $l^{-1}$ NaCl solution, buffered with 10–20 mmol $l^{-1}$ Hepes–NaOH at pH 7·7, and placed on a glass slide without a cover at room temperature, 20°C. The duration of sperm motility and number of moving spermatozoa were assessed by light microscopy using dark-ground illumination. The number of moving spermatozoa was evaluated in terms of grade (−, ±, +, ++, +++, ++++): grade ++++, over 75% of spermatozoa in the microscope field of view moved vigorously; grade +++, 50–75% of spermatozoa were motile; grade ++, 25–49% of spermatozoa were motile; grade +, less than 25% of spermatozoa were motile; grade ±, very few spermatozoa were motile; grade −, all spermatozoa were immotile.

The plasma membrane of the spermatozoa was removed by mixing, on ice for 30 s, 1 volume of semen with 20 volumes of extracting medium containing (in mmol $l^{-1}$) KCl, 150; $MgCl_2$, 0·5; EDTA, 0·5; dithiothreitol (DTT), 1; Tris buffer, 2; pH 8·2 and 0·04% Triton X-100. The demembranated spermatozoa were resuspended in reactivating medium containing (in mmol $l^{-1}$) KCl, 150; $MgCl_2$, 2; $CaCl_2$, 0·04; EGTA, 2; DTT, 1; ATP, 0·2; cyclic AMP, 0·01; Tris buffer, 20; 2% polyethylene glycol; pH 8·2. Sperm movement was recorded by videomicroscopy and the percentage of moving sperm and velocity of movement were measured as described previously (Morisawa, Morisawa & De Santis, 1984).

## RESULTS

### *Motility of spermatozoa from the testis and various portions of the sperm duct in rainbow trout*

The motility of spermatozoa from the testis and sperm duct was tested in 10 rainbow trout. In two fish (experiments 5 and 9) spermatozoa from all portions of the

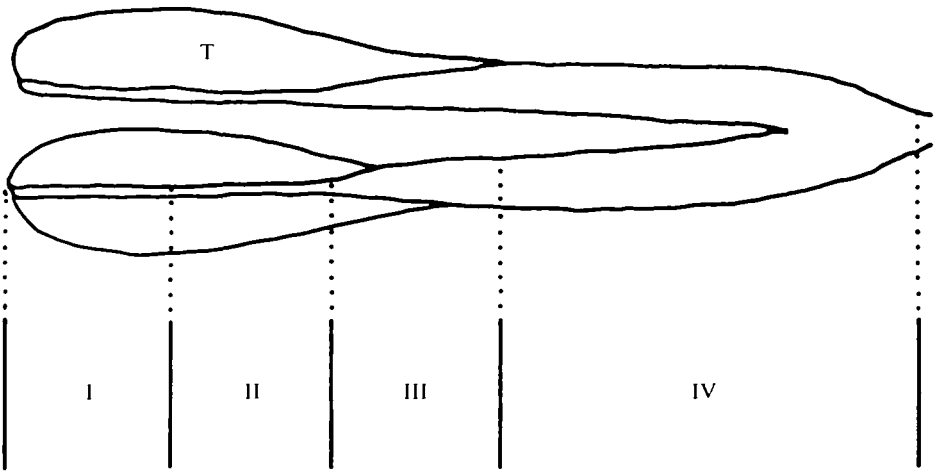


Fig. 1. Schematic drawing of the male reproductive organ of salmonid fishes. T indicates testis and I, II, III and IV indicate each portion of the sperm duct (see Materials and Methods).

Table 1. *Motility of spermatozoa from testis and various portions of the sperm duct in rainbow trout*

| Experiment no. | Testis | Sperm duct | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| 1 | ± | ± | +++ | ++++ | ++++ |
| 2 | − | − | + | ++++ | ++++ |
| 3 | − | − | ++ | +++ | +++ |
| 4 | − | − | + | ++++ | ++++ |
| 6 | − | − | − | ++ | +++ |
| 7 | − | ± | − | ++++ | ++++ |
| 8 | − | − | − | ++ | ++++ |
| 10 | − | − | − | +++ | ++++ |

For an explanation of the symbols see Materials and Methods.

male reproductive organ were immotile. The results in the other eight experiments are shown in Table 1. Almost all trout spermatozoa from the testis were completely immotile in $K^+$-free medium. However, several spermatozoa in the microscope field (<1 %) exhibited motility in one experiment (experiment 1). Spermatozoa from the anterior portion of the sperm duct (portion I) were also immotile. In portion II, spermatozoa from four of the eight trout exhibited movement, but less than grade ++++. In portion III, spermatozoa from all trout were motile; spermatozoa in four trout exhibited full motility. Almost all spermatozoa from portion IV exhibited full motility, while in two experiments (experiments 3 and 6) spermatozoa exhibited motility of grade +++. Motile spermatozoa from any portion of the sperm duct moved continuously for about 20 s.

In the preliminary experiments, seminal plasma was isolated from the semen of the trout sperm duct by centrifugation at 5000 rev. $min^{-1}$ for 10 min. When the testis spermatozoa of rainbow trout were diluted at a ratio of 1:2 in seminal plasma at room temperature, they became motile gradually. Spermatozoa exhibited maximal motility of grade ++ 1 h after dilution (average of three experiments) and then motility decreased. In contrast, almost all testis spermatozoa remained immotile for more than 2 h when they were stored undiluted in the test tube. Although the motility of testis spermatozoa diluted in seminal plasma was lower than that of spermatozoa collected from the sperm duct (see Table 1), these experiments suggest that the seminal environment may be a prerequisite for the acquisition of the potential for sperm motility.

### *Effect of potassium on motility of rainbow trout spermatozoa*

We have shown previously that rainbow trout spermatozoa from the distal portion of the sperm duct, collected by inserting a pipette through the urogenital pore, are motile in 100 mmol $l^{-1}$ NaCl solution, but immotile in a solution containing 3 mmol $l^{-1}$ KCl (Morisawa *et al.* 1983). In the present experiments, in which spermatozoa were collected from different portions of the reproductive organ,

spermatozoa from portion IV of the sperm duct were immotile at potassium concentrations greater than 1 mmol $l^{-1}$ but motile at lower potassium concentrations (Fig. 2). In contrast, spermatozoa collected from portion II were almost immotile at all potassium concentrations. All spermatozoa were quiescent in media containing more than 1·0 mmol $l^{-1}$ potassium.

*Motility of spermatozoa from testis or sperm duct in chum salmon*

As shown in Table 2, spermatozoa in the mature testes of chum salmon which had been caught in the river were almost immotile. In two of four experiments, spermatozoa were completely quiescent and a few spermatozoa were motile in two other experiments. However, in all four experiments, all spermatozoa from the sperm duct exhibited full motility. In chum salmon which had been caught in the bay, the sperm duct was almost empty. However, when the fish were transferred to

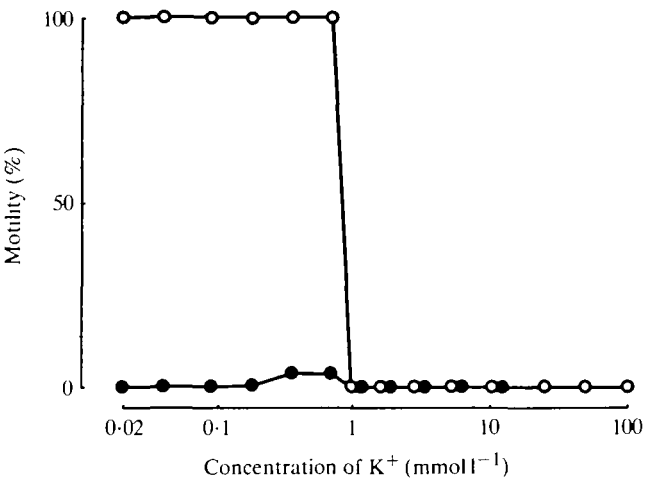


Fig. 2. The effect of potassium on the motility of rainbow trout spermatozoa from anterior and posterior portions of the sperm duct. Motility of sperm from portion II (●) and portion IV (○).

Table 2. *Motility of spermatozoa from testis or sperm duct in chum salmon*

| | Sperm motility | |
|---|---|---|
| | Testis | Sperm duct |
| Caught in the river | ± | ++++ |
| | − | ++++ |
| | ± | ++++ |
| | − | ++++ |
| Caught in the bay and kept 3 days in fresh water | ± | ++++ |
| | ± | ++++ |
| | ± | ++++ |

For an explanation of the symbols see Materials and Methods.

Table 3. *Motility of testis or sperm duct spermatozoa demembranated with Triton X-100 in chum salmon*

| | Sperm duct ($N = 3$) | Testis ($N = 3$) |
|---|---|---|
| Intact sperm motility | fully motile | almost immotile |
| Demembranated sperm | | |
| motility | 77 ± 7·5 (%) | 57 ± 6·3 (%) |
| velocity | 35·9 ± 4·78 ($\mu m\,s^{-1}$) | 36·8 ± 1·19 ($\mu m\,s^{-1}$) |

Values are mean ± S.E.

fresh water and kept for 3 days the sperm duct was filled with spermatozoa. In these freshwater-adapted fishes a few spermatozoa from the testis were motile but all spermatozoa from the sperm duct were fully motile in all experiments.

### *Motility of demembranated spermatozoa in chum salmon*

Chum salmon were caught in the bay, transferred to fresh water and kept for 2 days. In this condition, testis spermatozoa were completely quiescent in $K^+$-free, 100 mmol $l^{-1}$ NaCl solution in two of three experiments and few sperm were motile in the third experiment. Spermatozoa from the sperm duct exhibited full motility in three experiments. When testis spermatozoa were demembranated and then reactivated, 57% were motile and moved at approximately 37 $\mu m\,s^{-1}$. However, 77% of demembranated spermatozoa from the sperm duct were motile and moved at approximately 36 $\mu m\,s^{-1}$ (Table 3). There was no significant difference between the motility of demembranated spermatozoa from the testis and the sperm duct ($P > 0{\cdot}05$).

## DISCUSSION

We have demonstrated the occurrence of the acquisition of a potential for sperm motility in salmonid fishes. In these species, it is well known that $K^+$, a major constituent in the seminal plasma (Morisawa, Hirano & Suzuki, 1979; Morisawa *et al.* 1983), is the physiological factor causing quiescence of spermatozoa in the distal portion of the sperm duct and that spermatozoa begin to move when they are spawned into $K^+$-free fresh water and thus released from the suppression by $K^+$ (Morisawa & Suzuki, 1980; Morisawa *et al.* 1983). Spermatozoa collected from the distal portion of the sperm duct exhibit vigorous motility in $K^+$-free medium. In the present experiments, rainbow trout spermatozoa, collected from the testes or anterior portion of the sperm duct, are almost immotile in $K^+$-free medium. The number of motile spermatozoa gradually increases from the anterior to the posterior portion of the sperm duct, spermatozoa from the distal portion exhibiting vigorous motility (Fig. 2; Table 1). These facts suggest that trout spermatozoa, which remain

in a repressed state in the testis, acquire the ability to move in $K^+$-free conditions during transit through the sperm duct.

In chum salmon caught in the bay, the testes held a large number of spermatozoa, although the sperm duct contained only a small volume of semen and thus was almost empty (Morisawa *et al.* 1979). However, in the river salmon or freshwater-adapted fish the whole sperm duct was filled with spermatozoa. Spermatozoa from the testes of seawater, freshwater or freshwater-adapted fishes were almost immotile. In contrast, spermatozoa which had left the testis and moved into the sperm duct were fully motile (Tables 2, 3). The spawning ground of chum salmon of the Otsuchi River is less than 1 km from the bay, thus the fishes in the bay may reach their spawning ground within a short period (about 12 h) of leaving sea water (M. Iwata, personal communication; see also Morisawa *et al.* 1979). Thus it may be concluded that after leaving the testis the transit of spermatozoa through the sperm duct occurs during the short migration of fish from the bay to the river and that acquisition of sperm motility occurs within this time. Rapid acquisition of the potential for motility has been also reported in mammalian spermatozoa (Hinton, Dott & Setchell, 1979).

Although the storage test seems to show that the acquisition of sperm motility is not simply dependent on time but that the seminal environment is also important, it still remains obscure as to which external factors are indispensable for the establishment of the acquisition of sperm motility in salmonids. However, a comparison of the response to cyclic AMP of demembranated spermatozoa from the testis and the sperm duct may show which internal mechanism induces the acquisition of sperm motility. In a previous study using spermatozoa collected from the sperm duct, we showed that the motility initiation system was triggered by the cyclic AMP-dependent phosphorylation of the 15 K protein which is present on the motor apparatus, the axoneme in the flagellum (Morisawa & Okuno, 1982; Morisawa & Hayashi, 1985) and that flagellar motility is conducted by the ATP-induced sliding of microtubules (Okuno & Morisawa, 1982). In the present experiment, demembranated spermatozoa from salmon testis were motile. Although the motility of these spermatozoa was somewhat lower than the motility of demembranated spermatozoa from the sperm duct, the difference was not significant. This suggests that the trigger system for the initiation of sperm motility by cyclic AMP-dependent phosphorylation of the major motile system, i.e. dynein ATPase and tubulin, is functional in the testis spermatozoa. Thus it seems probable that acquisition of sperm motility is correlated with the development of some function of the plasma membrane.

At present, several factors such as changes in the state of the sulphhydryl group (Bedford, 1979), forward motility proteins (Hoskins *et al.* 1978), or increased intracellular pH and cyclic AMP concentration (Hoskins *et al.* 1978; Vijayaraghavan, Critchlow & Hoskins, 1985) have been proposed as factors responsible for the acquisition of sperm motility in mammals. However, it still remains unclear which mechanisms are absolutely indispensable for the acquisition of sperm motility. Salmonid fishes, in which the process of acquiring the ability to move occurs in the simple reproductive organ within a relatively short period, may be good species in which to investigate the process of the acquisition of sperm motility.

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