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# Effects of ions on the motility of fresh and demembranate spermatozoa of common carp (*Cyprinus carpio*) and paddlefish (*Polyodon spathula*)

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## Abstract

In the present study was summarize the factors activating and/or inhibiting the motility of intact and/or demembranated spermatozoa of common carp and paddlefish as teleostean and chondrostean models using methods of sperm demembranation. The movement of spermatozoa of cyprinids species, common carp, is influenced by osmotic pressure or high concentration of ions. The movements of spermatozoa of chrondrostean species, paddlefish, are under reciprocal control by the concentration of $K^+$ and $Ca^{2+}$.

## Introduction

Limited information is available on the characteristics of spermatozoa in chondrostean species, i.e. paddlefish or sturgeons, which have some phylogenetical similarities to mammalian spermatozoa. The common carp belong to teleostean fish with phylogenetically primitive spermatozoa (round head, thin flagellum, rudimentary acrosome, Kudo 1998). The duration of swimming in chondrostean fish spermatozoa is relatively long, up to 9 min (Linhart et al. 2002) in contrast to 15–50 s in freshwater teleosts (Billard et al. 1995).

The goal of the present study is to summarize the factors activating and/or inhibiting the motility of intact and/or demembranated spermatozoa of common carp (teleostean species) and paddlefish (chondrostean species) as models with developing methods of sperm demembranation.

## Materials and methods

Induced spermiation for carp and paddlefish males followed methods of Billard et al. (1995) and Linhart et al. (2002), respectively. The velocity and percentage of sperm swimming were measured under dark field microscopy with stroboscopic illumination (Linhart et al. 2002). In carp the sperm swimming was initiated and measured after mixing 1 $\mu$l of prediluted sperm 1:200 in immobilizing solution (IS, 200 mM KCl, 30 mM Tris-HCl, pH 8, Perchec et al. 1995) with 49 $\mu$l of swimming medium (SM). The SM is composed of 45 mM NaCl, 5 mM KCl and 30 mM Tris-HCl, pH 8 with 1 mg $ml^{-1}$ BSA, Sigma A-7511. Motility of paddlefish spermatozoa was measured after mixing 0.5 $\mu$l of sperm with 49 $\mu$l of SM (20 mM Tris-HCl, pH 8.2 with 1 mg $ml^{-1}$ BSA). The successive positions of the recorded sperm heads were measured from video frames using a video-recorder (SONY SVHS, SVO-9500 MDP) after sperm activation analyzed by the image analyzer Olympus Micro Image 4.0.1. for Windows.

### *Demembranation and reactivation of sperm*

One $\mu$l of diluted carp sperm 1:200 in IS was mixed at room temperature on the glass slide with 50 $\mu$l of demembranating and reactivating medium (DRM = 45 mM NaCl, 5 mM KCl, 30 mM Tris pH 8, 1 mM EGTA, 1 mM $MgCl_2$, 0.01% Triton X-100,

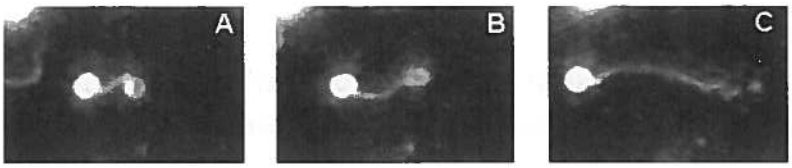


*Figure 1.* Fully demembranated sperm of common carp with coiling at 2 min after dilution in DRM (A); The same spermatozoon with coiling reactivated 1.5 min later after adding of ATP with reestablishing of half length (B) and at 7 frames (7/25 s) later full length (C) of demembranated flagellum.

1 mg $ml^{-1}$ BSA) and 2 min latter 1 mM of ATP (vanadate free) was added. Two $\mu$l of paddlefish sperm were mixed at 0 °C with 50 $\mu$l of demembranation medium (DM = 20 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 20 mM Tris HCl pH 8.2 and 0.04% Triton X-100). After 30 s, 2 $\mu$l were mixed at room temperature on the glass slide with 50 $\mu$l of reactivating medium (RM = 20 mM NaCl, 1 mM DTT, 20 mM Tris-HCl pH 8.2, 1 mM $MgCl_2$, 2 mg $ml^{-1}$ BSA and 1 mM ATP, vanadate free). The BSA was required to prevent the sperm cells from adhering to glass slides or particles. The swimming ability of spermatozoa was assessed as described above. The osmolality of the seminal fluid was measured using a Vapor Pressure Osmometer (Wescor) and expressed as mOsmol $kg^{-1}$ of $H_2O$.

## Results and discussion

### *Osmolality of seminal fluid and motility in swimming medium*

The osmolality of seminal fluid differed between carp and paddlefish with levels of 288–320 mOsmol $kg^{-1}$ and 33–63 mOsmol $kg^{-1}$, respectively. When sperm of carp and paddlefish were transferred into SM, spermatozoa were activated and displayed the following characteristics: (i) carp–10 s and 1 min after activation with velocity 110–170 and 50–100 $\mu$m $s^{-1}$, respectively and movement was maintained for to 2 min; (ii) paddlefish–10 s and 1 min after activation with velocity 100–150 and 80–140 $\mu$m $s^{-1}$, respectively and movement was maintained for to 9 min.

### *Regulation of motility by osmolality*

Fully activated spermatozoa with distilled or dechlorinated tap water were disorganized after osmotic shock, showing flagellum coiling 30 s after dilution in carp and swelling particles on the flagellum at 2–3 min after dilution in paddlefish (Linhart et al. 2002). Sperm movement in paddlefish and carp was inhibited in glucose solution of 120 and 300 mOsmol $kg^{-1}$ (Billard et al. 1995), respectively.

### *Inhibitory effect of ionic concentrations*

A low concentration of 0.5 mM KCl fully inhibited sperm movement in paddlefish, but movement was re-initiated by adding 0.25 mM of $CaCl_2$. Low concentration of $Ca^{2+}$ appeared to reverse inhibitory effect of 0.5 mM KCl in paddlefish. In common carp, inhibitory effect of low concentration of $K^+$ on sperm motility was not observed. Only the high levels of 150 mM/300 mOsmol $kg^{-1}$ of $K^+$ or other ions such as $Na^+$ inhibited sperm movement in carp (Perchec et al. 1995; Billard et al. 1995).

### *Demembranation and reactivation of sperm*

In the carp and paddlefish, the inhibitory effect of $K^+$ on the axonemes of demembranated spermatozoa in the DRM and RM solution containing ATP was examined, respectively. The presence of KCl or $K^+$ at 5–15 mM was used to inhibit demembranated flagellum in paddlefish (Linhart et al. 2002) and at 300 mM in carp (Perchec et al. unpublished). In both species the motility of demembranated sperm motility was developed without use of cAMP. Paddlefish spermatozoa required two steps of sperm incubation in DM and later in RM but carp required only a single incubation in DRM with adding ATP at 2 min after incubation in DRM. The flagellum of carp spermatozoa was disorganized rapidly in DRM solution after demembranation, showing demembranate flagellum coiling 2 min after dilution in carp (Figure 1A). The flagellum showing coiling was restored to 'normal shape flagellum' at 1.5 min later when ATP was added (Figures 1B and C).

## Conclusion

Movement of spermatozoa of cyprinids (c. carp) is influenced by osmotic pressure or high concentration of ions. The movements of spermatozoa of chrondrostean (paddlefish) are under counteracting control by the concentration of $K^+$ and $Ca^{2+}$. In both species, the flagellum with coiling or ballooning can be re-established when using high concentration of IS with $K^+$. Thanks are expressed to GACR no. 524/03/0178, MSMT CR no. 126100001, USDA 1890 CBG Program (KYX-01-11469) KSU, Frankfort, KY, USA.

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