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Motility of spermatozoa from shovelnose sturgeon and paddlefish

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The spermatozoa in the seminal plasma from shovelnose sturgeon *Scaphirhynchus platorynchus* and paddlefish *Polyodon spathula* were immotile with only a few spontaneously motile spermatozoa for 5–10 and 10–20 s, respectively. Spermatozoa of shovelnose sturgeon were observed to be 100% motile immediately after sperm dilution in 10 mM NaCl and 20 mM Tris–HCl, pH 8·5. The duration of mass progressive movement was 2–3 min; and 1 to 5% of spermatozoa remain active after 360 s ($P<0{\cdot}01$). Spermatozoa of paddlefish demonstrated the best motility 10 s after dilution in 10 mM NaCl with 20 mM Tris–HCl, pH 8·5. The duration of mass progressive movement was 2–3 min and 1 to 5% of spermatozoa remained active after 370 s ($P<0{\cdot}01$). The spermatozoa of shovelnose sturgeon and paddlefish were motile in a range of osmotic pressure from 0 to 100 mosmol kg^{-1} and 0 to 120 mosmol kg^{-1}, respectively. The best results with short-term storage of sperm from shovelnose sturgeon and paddlefish were observed in 100 mM glucose+20 mM Tris–HCl, pH 8·5 and 150 mM glucose+20 mM Tris–HCl, pH 8·5.

Key words: motility; sperm; activating solution; immobilizing solution; shovelnose sturgeon; paddlefish.

INTRODUCTION

The paddlefish *Polyodon spathula* Walbaum, 1792 and shovelnose sturgeon *Scaphirhynchus platorynchus* Rafinesque, 1820 are important species in the United States (Carlson & Bonislawsky, 1981; Mims *et al.*, 1993). The basic methods for artificial reproduction have been developed for paddlefish by Needham (1965) and Graham *et al.* (1986), and for shovelnose sturgeon by Conte *et al.* (1988) in the United States.

The spermatozoa of many species are immotile in the testes and the genital tract and are activated only after release into the external medium for a short period of motility (Scott & Baynes, 1980). Osmotic pressure, concentration of K^+ and sucrose, and seminal plasma pH lower than 7 are the main factors inhibiting sperm motility of salmonids, and the osmotic pressure seems to be the major suppressive factor in cyprinids (Linhart *et al.*, 1991). Although less information is available for sturgeons and paddlefish, total progressive motility duration in 'fresh water' at 15 to 20° C were reported for gray sturgeon *Huso huso* Linnaeus, 1758 (13 min; Ginsburg 1968), Russian sturgeon *Acipenser guldenstaedti* Brandt, 1833 (3·5 to 5 min; Drabkina 1961), sterlet *Acipenser ruthenus* Linnaeus, 1758 (5–20 min; Linhart unpublished data) and paddlefish (4·4 min; Mims, 1991). Best swimming parameters of sterlet spermatozoa were observed in 50 mM Tris–HCl, pH 8·0 (Cvetkova *et al.*, in press). In distilled

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water, motility of *Acipenser baeri* Brandt, 1869 spermatozoa lasted 2–3 min with initial beat frequency of 40–50 Hz and speed of 150–250 μm s^{-1} dropping to about 10 Hz and 50 μm s^{-1} after 2 min (Cvetkova *et al.*, in press). Spontaneous movement of paddlefish and sturgeon spermatozoa via their urine contamination were not examined (Ginsburg, 1968; Mims, 1991). Mims (1991) reported that short-term storage of paddlefish sperm was possible in 0·9% of NaCl solution with antibiotics for 8 days at 2·9° C. White sturgeon *Acipenser transmontanus*, Richardson, 1836 sperm has been stored for 14 days at 4° C (Conte *et al.*, 1988).

The objectives of the present work were to examine (1) the existence of spontaneous movement of spermatozoa, (2) level of mM glucose concentration for short-term storage of sperm *in vitro* and (3) level of osmotic concentration of NaCl and Tris in activating solution for increasing motility of spermatozoa after irradiation of sperm for induction of gynogenesis or after frozen/thawed spermatozoa.

MATERIALS AND METHODS

The experiment was carried out in April 1994 at the Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky, U.S.A. Shovelnose sturgeon from 1–3 kg were captured below Symthland Dam. Paddlefish from 7·0–12·0 kg were captured in Cumberland Lake, Kentucky. Broodfish were transported to the ponds of the Aquaculture Research Center in Kentucky. Sturgeon were selected and kept together in 3000 l tanks with a water flow rate of 12 l min^{-1}, 9·0 mg O_2 l^{-1} at controlled water temperature 17–19° C. Paddlefish were selected and held separately in circular metal tanks (3000 l) with a water flow rate of 12 l min^{-1}, 9·0 mg O_2 l^{-1} at controlled water temperature of 17 to 19° C. For male selection, maturity was investigated by abdominal compression and spermiation was detected by milt production.

SPERMIATION AND SPERM COLLECTION

Five males of paddlefish and shovelnose sturgeon were injected intraperitoneally with LHRHa analogue of des-Gly^{10}(D-Ala^6) LHRH ethylamide at doses of 0·05 mg kg^{-1} body weight. For milt collection 5 cm of Tygon tubing attached to a 10 ml plastic syringe was used; tubing was inserted into the urogenital pore and the syringe was filled with milt. Samples contaminated with faecal material were discarded. Milt was collected and stored on ice until motility analysis.

EVALUATION OF MOTILITY

Sperm motility was evaluated for percentage of motile spermatozoa and duration of progressive motility. Motility parameters were measured in the laboratory using dark field microscopy. Immediately after milt collection, spermatozoa were observed for spontaneous movement. A drop of 10 μl non-diluted milt was placed directly on a glass slide on the microscope stage and examinated under 200 ×. For further examination about 10 μl of milt was diluted in 100 μl of immobilizing solution (for shovelnose sturgeon 100 mM glucose+20 mM Tris pH 8·5 and for paddlefish 150 mM glucose+20 mM Tris pH 8·5) which inhibits motility of spermatozoa. One μl of this mixture was diluted in a 49 μl drop of activating solution previously placed on a glass slide on the microscope stage. This high dilution rate allowed individual motile and non-motile sperm heads to be seen clearly and permitted better evaluation of the percentage of actively swimming spermatozoa. The first examination of this sperm preparation was possible within a few seconds after the second sperm dilution. In some cases, the total duration of progressive

TABLE I. Concentration of NaCl and Tris, pH 8·5 and motility of spermatozoa

Males	NaCl (mM)	Tris pH 8·5 (mM)	Motility of spermatozoa	
			Mean (%)	Mean (s)
Sholvelnose sturgeon, *S. platorynchus*	0*	0	90 ± 0	166 ± 11·6[a]
	0†	0	90 ± 2·9[a]	222 ± 7·6[a]
	0	40	90 ± 0	[b]355 ± 7·0[a]
	10	20	100 ± 0	[b]360 ± 14·1[a]
	10	40	100 ± 0	[b]315 ± 21·2[a]
	20	0	95 ± 7·0[a]	[b]290 ± 14·1[a]
	20	20	93 ± 3·5[a]	205 ± 7·0[a]
	20	40	95 ± 7·0[a]	115 ± 21·2
Paddlefish, *P. spathula*	0*	0	80 ± 0	110 ± 10·0[a]
	0†	0	97 ± 8·3[a]	177 ± 15·3[a]
	0	40	90 ± 7·0[a]	[b]355 ± 21·2[a]
	10	20	100 ± 0	[b]370 ± 14·1[a]
	10	40	100 ± 0	[b]355 ± 7·0[a]
	20	0	90 ± 0	[b]227 ± 10·7[a]
	20	20	93 ± 3·5[a]	290 ± 14·1[a]
	20	40	90 ± 0	128 ± 10·7[a]

Groups with a common superscript (a) do not differ significantly ($P<0·05$). Means with a common superscript (b) were statistically different ($P<0·01$) with means of distilled water (*) and hatchery water (†).

movement of 95–99% spermatozoa including the spermatozoa activity was also measured. Observations were made at room temperature of 18–22° C.

EFFECT OF THE OSMOTIC PRESSURE ON MOTILITY OF SPERMATOZOA AND SHORT-TERM CHANGES IN THE SWIMMING ABILITY OF THE SPERM

Sperm were activated with 20–150 mosmol kg^{-1} NaCl and 10 to 150 mosmol kg^{-1} Tris–HCl, pH 8·5, to test osmotic pressure. For testing the swimming ability of spermatozoa, the samples were diluted in a solution composed of 100–400 mM glucose and 20 mM Tris–HCl pH 8·5. Final osmotic pressure ranged 220–820 mosmol kg^{-1}. Swimming ability of spermatozoa was measured. The sperm was processed immediately or was stored in 1 ml portions in 5 ml tubes on ice or in an environment chamber at +4° C for 48–72 h. Distilled water was used as the control activating solution.

DATA ANALYSIS

The means were evaluated by studies in triplicate and statistical significance was assessed using Student's *t*-test or one-way analysis of variance, followed by Tukey's multiple comparison test. Values of $P<0·05$ were considered significant.

RESULTS

MOTILITY OF SHOVELNOSE STURGEON AND PADDLEFISH

Direct observation of undiluted sperm by dark field microscopy showed that most spermatozoa in the seminal plasma of shovelnose sturgeon and paddlefish were immotile with a few spermatozoa motile for 5–10 and 10–20 s, respectively.

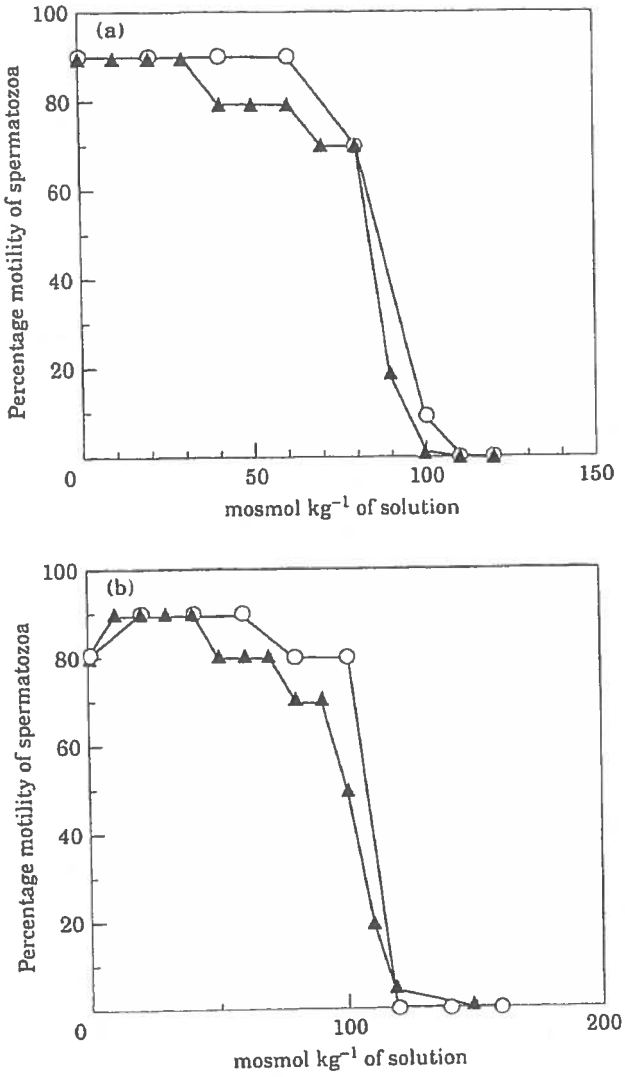

FIG. 1. The percentage motility of (a) shovelnose sturgeon *Scaphirhynchus platorynchus* and (b) paddlefish *Polyodon spathula* spermatozoa with changes in the osmotic pressure of the activation solution. ○, NaCl; ▲, Tris, pH 8·5.

After sperm dilution in 10 mM NaCl+20 mM Tris–HCl, pH 8·5 a majority of the samples from shovelnose sturgeon males had 100% motility of spermatozoa within 10 s. The duration of mass progressive movement was 2–3 min, with 1–5% of the spermatozoa remaining active after 360 s ($P<0·01$; Table I). However, a few spermatozoa exhibited motility up to 10 min. Spermatozoa from the paddlefish male samples had 100% motility within 10 s after sperm dilution in 10 mM NaCl with 20 mM Tris–HCl, pH 8·5. The duration of mass progressive movement was 2–3 min with 1–5% of spermatozoa remaining active after 370 s ($P<0·01$; Table I). However, a few spermatozoa exhibited motility for up to 12 min. Spermatozoa motility had shorter movement time in distilled water and ' hatchery water ' than in the formulated medium ($P<0·01$; Table I).

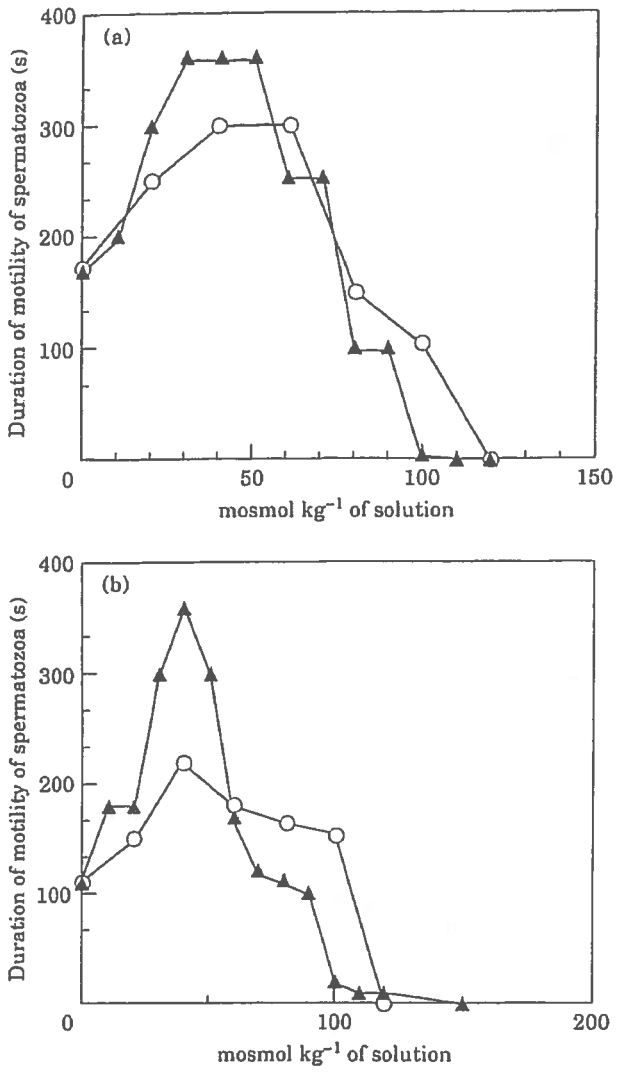

FIG. 2. The duration of (a) shovelnose sturgeon *Scaphirhynchus platorynchus* and (b) paddlefish *Polyodon spathula* motile cells with changes in the osmotic pressure of the activation solution. ○, NaCl; ▲, Tris, pH 8·5.

EFFECT OF THE OSMOTIC PRESSURE ON SPERMATOZOA MOTILITY

The spermatozoa of shovelnose sturgeon and paddlefish were motile in a range of osmotic pressures from 0–100 mosmol kg^{-1} and 0–120 mosmol kg^{-1}, respectively. There was a trend of a rapid decrease in the percentage of motile spermatozoa from 80–0% by shovelnose sturgeon with the increase of osmotic pressure from 70–100 mosmol kg^{-1}. A similar trend was observed for paddlefish spermatozoa with motility decreasing from 70–0% with increasing osmotic pressure from 100–150 mosmol kg^{-1} (Fig. 1). Duration of motility for shovelnose sturgeon increased from 160 s (Table I) in distilled water to 355 s in Tris–HCl, pH 8·5 activation solution (40 mosmol kg^{-1}; Fig. 2); and for

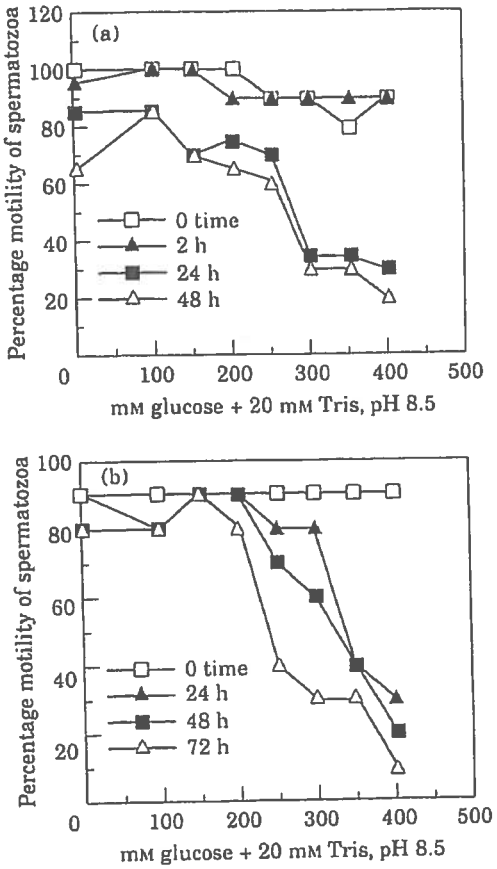

FIG. 3. Changes in the percentage of (a) paddlefish *Polyodon spathula* and (b) shovelnose sturgeon *Scaphirhynchus platorynchus* motility spermatozoa after short-term storage *in vitro* (0, 2, 24 and 48 h, +4° C) in immobilizing solution.

paddlefish from 110 s (Table I) to 355 s in Tris–HCl, pH 8·5 activation solution (30–50 mosmol kg^{-1}; Fig. 2).

SHORT-TERM CHANGES IN THE VIABILITY OF THE SPERM

After dilution in glucose solution 100–400 mM, the percentage motility of spermatozoa from both species was not affected. Percentage of spermatozoa motility from paddlefish remained at 80–90% in solutions of 0–200 mM glucose after 72 h storage of sperm (Fig. 3). Percentage motility of spermatozoa from shovelnose sturgeon remained at 65–80% in solutions of 0 and 150 mM glucose after 24 and 48 h of sperm storage. Overall, the best solutions for short-term storage of paddlefish and shovelnose sturgeon sperm were determined to be 150 mM glucose+20 mM Tris–HCl, pH 8·5 and 100 mM glucose+20 mM Tris–HCl, pH 8·5, respectively (Fig. 3).

DISCUSSION

The motility of shovelnose sturgeon and paddlefish spermatozoa was initiated by an increase in osmotic pressure as described in cyprinids (Redondo-Muller

et al., 1991) and marine species (Morisawa & Suzuki, 1980). Preliminary results from work on both species shows that 40 mosmol solution of Tris at a pH 8·5 increased duration of motility of spermatozoa cells. Drabkina (1961) reported a similar increase in duration of motility of Russian sturgeon spermatozoa using NaCl solution at 34–68 mosmol kg^{-1} (calculation by authors). For paddlefish, percentage of motile spermatozoa was highest after storage *in vitro* in 150–200 mM glucose+20 mM Tris, pH 8·5 (osmotic concentration 170–220 mosmol kg^{-1}). Mims (1991) showed good results with use of 153 mM NaCl (306 mosmol kg^{-1}) for short-term storage of spermatozoa *in vitro*.

In conclusion, this technique of using an activation solution (10 mM NaCl+20 mM Tris, pH 8·5) will be easy to apply in hatchery conditions. It is especially useful in increasing motility of spermatozoa after irradiation of sperm for induction of gynogenesis or for increasing motility of spermatozoa after freezing and thawing. These first results in developing the osmotic level for an immobilizing solution are not only important for short-term storage of sperm *in vitro* but for the first steps in dilution of spermatozoa before cryopreservation of sperm.

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