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Evaluation of Activator Solutions, Motility Duration, and Short-Term Storage of Paddlefish Spermatozoa

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Abstract

Milt from paddlefish (*Polyodon spathula*) was collected, examined for motility, and pooled. Six different solutions (dechlorinated tap water, ovarian fluid, bacteriostatic water, distilled water, 0.9% sodium chloride saline water, and milt plasma) were examined for activation and duration of spermatozoal motility. Most solutions except saline solution and milt plasma promoted activation of spermatozoa. Dechlorinated tap water was found to be the best activator with significantly longer duration of spermatozoal motility than the other tested solutions. Saline solution and milt plasma appear to be useful as carrier solutions because of their property of maintaining inactive, viable spermatozoa.

Paddlefish milt in three treatments was evaluated for chilled storage [without additives (MF); with 500 IU penicillin and 500 mg streptomycin mix in milt plasma (MP); and 0.9% saline solution (MS)]. The three treatments were stored at 2.4 ± 0.9 C in a precooled, moisture-saturated oxygen environment and observed every 24 h for 9 d. Motility of activated spermatozoa in MS (7 d) was significantly longer than MF or MP treatments (7 d). Spermatozoa in MS had the longest chilled storage time (8 d).

Paddlefish (*Polyodon spathula*) is becoming an increasingly important culture fish (Graham et al. 1986; Semmens and Shelton 1986). Basic reproductive techniques were first established by Needham (1965). Further modifications for paddlefish propagation have been incorporated using spawning techniques for white sturgeon (*Acipenser transmontanus*) (Semmens and Shelton 1986).

Several factors can adversely affect successful spawning of paddlefish and include: ages of wild-caught broodstock are not known; no external physical features are reliable for differentiating paddlefish sexes (Graham et al. 1986); and, broodstock are large (9.27 kg) and must swim continuously requiring individually large circular tanks (2.5 m diameter) kept inside the hatchery. Advance collection and storage of milt may be a convenient method of insuring good quality spermatozoa for fertilization of paddlefish ova and reduce crowding of broodstock in hatchery.

Short-term storage of chilled fish milt has been examined for many species. This procedure has been intensively studied for salmonid fishes (Barrett 1951; Buyukhatipoglu and Holtz 1978; Stoss and Holtz 1983; Terner 1986). Short-term storage of milt has also been examined for some potentially important nonsalmonid fish (Ginzberg 1968; Mollah 1985; Moore 1987). Conte et al. (1988) reported that milt of white sturgeon has been stored for 14 d at 4 C using pure oxygen. Short-term chilled storage of paddlefish milt has not been evaluated and reported. The objectives of this initial study were to examine potential spermatozoal activators and to evaluate duration of motility and short-term chill storage of paddlefish spermatozoa.

Materials and Methods

Paddlefish male brood fish (9.5 ± 1.2 kg) were caught in Cumberland Lake, Kentucky, and held in four 2.5 m circular metal tanks at Kentucky State University Aquaculture Research Center in Frankfort. Each

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tank was filled and exchanged with dechlorinated tap water at a flow rate of 24 L/min. Water temperature was 18.0 ± 0.3 C. Five fish received a single injection of luteinizing hormone releasing hormone analog (LHRHa; des-Gly^{10}, (D-Ala^{6})-LHRH Ethylamide; Sigma Chemical Company, St. Louis, Missouri) at a rate of 0.05 mg/kg of body weight to increase milt production. Tygon tubing (5 cm length) attached to a 10 cc plastic syringe was inserted into the urogenital pore to collect 20 cc of milt from each fish. Each sample was examined for at least 70% motile spermatozoa and pooled (Holtz et al. 1977).

Spermatozoal Activation and Duration of Motility

Six different solutions were used to test sperm activation and duration of motility: dechlorinated tap water; ovarian fluid; bacteriostatic water; distilled water; 0.9% sodium chloride water; and milt plasma. Solutions were analyzed for calcium, magnesium, potassium, and sodium by flame atomic absorption spectroscopy at the Department of Environmental Protection, Kentucky (Table 1).

Ovarian fluid was collected from LHRHa-injected female with a 10 cc syringe as described for milt collection. Bacteriostatic was double-distilled water with 0.9% benzyl alcohol. Milt plasma was obtained by spinning fresh milt at 600 × gravity for 15 min. All solutions were kept at 18 ± 0.3 C. Each solution was tested five times.

Intensity of spermatozoal motility was based on proportion of motile spermatozoa using a score 0 to 5 where 0 = no movement; 1 = vibratory or oscillatory movement; 2 = vibration or oscillatory movement coupled with slight forward movement; 3 = slow, mainly circular, forward movement; 4 = active forward movement, partly circular, partly in a straight line; and 5 = rapid movement in a straight line (Holtz et al. 1977). A stopwatch was used to measure the time period from initial activation to the end of mass motility. Any morphological changes of spermatozoa were recorded. Solutions were determined to be either effective spermatozoal activators or antibiotic carriers (inhibitors of spermatozoa activation) for use in the short-term chilled storage experiment.

Short-Term Preservation

The pooled milt sample was processed within 1 h into three treatments: milt only; milt with antibiotics dissolved in milt plasma; and milt with antibiotics dissolved in 0.9% sodium chloride solution. There were 10 replications per treatment. To each 1 ml of milt in the antibiotic treatment, 25 μl of milt plasma or 0.9% saline solution containing 500 IU penicillin and 500 mg streptomycin were added. From the pool 10 samples of 2 ml each were taken and placed into glass vials of internal diameter 19 mm and height 50 mm. Open vials were placed in a 10 L desiccator filled with moisture-saturated oxygen as described by Stoss and Holtz

TABLE 1. *Cation concentration (mg/L) of various solutions used for potential spermatozoal activators.*

Solutions[1]	Cation concentration			
	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)	Sodium (mg/L)
Dechlorinated tap water	33.0	11.6	2.0	18.2
Ovarian fluid	24.0	10.1	35.0	1,100.0
Bacteriostatic water	1.7	0.0	0.0	0.9
Distilled water	0.2	0.1	0.0	0.4
Sodium chloride (0.9%)	0.0	0.0	0.6	3,140.0
Milt plasma	7.8	5.5	97.3	500.0

[1] Solutions were analyzed by flame atomic absorption spectroscopy.

(1983). The desiccator was kept in the dark in a refrigerator at 2.4 ± 0.9 C. The gas in the desiccator was replaced daily by precooled, moisture-saturated oxygen.

Samples of milt were collected every 24 h from all vials. Because spermatozoa motility is the simplest and most dependable indicator of milt quality (Terner 1986), two droplets were activated, and duration of motility was measured under a light microscope (400×). The intensity of spermatozoal motility was based on the scale 0 to 5. No ova fertilization was attempted.

All data were analyzed by using the SAS ANOVA procedure (Statistical Analysis Systems 1988). Duncan's new multiple range test was used to compare means. Statements of significant differences were made based on ($P \leq 0.05$).

TABLE 2. *Duration and intensity of spermatozoal motility of paddlefish using various solutions as activators. Means followed by the same letter were not significantly different.*

Solutions	Duration[1] (min)	Score[2]
Dechlorinated tap water	4.44 ± 0.15 a	5
Ovarian fluid	2.9 ± 0.16 b	5
Bacteriostatic water	1.37 ± 0.06 c	3
Distilled water	1.27 ± 0.35 c	3
Sodium chloride (0.9%)	0.12 ± 0.02 d	1[3]
Milt plasma	0.00 ± 0.00 e	0

[1] Length of spermatozoal motility until mass mortality.

[2] Intensity of motile spermatozoa: 0 = no movement; 1 = vibratory or oscillatory movement; 2 = vibration or oscillatory movement coupled with slight forward movement; 3 = slow, mainly circular, forward movement; 4 = active forward movement, partly circular, partly in a straight line; 5 = rapid movement in a straight line (Holtz et al. 1977).

[3] Movement probably due to osmotic shock (Baynes et al. 1981).

Results and Discussion

Sperm Activation and Duration

Most solutions except milt plasma and saline solution promoted activation of spermatozoa (Table 2). Duration of spermatozoal motility was significantly longer with dechlorinated tap water than with other tested solutions. This indicated that dechlorinated tap water would be the most suitable of the tested solutions in activating and measuring duration of spermatozoal motility for the short-term storage experiment. Saline solution had significantly shorter duration of spermatozoal motility than other activating solutions. Short, vigorous activity without forward motion of spermatozoa was observed and was probably caused by osmotic shock (Scott and Baynes 1980). However, spermatozoa in saline could be activated when diluted with dechlorinated tap water. Maintenance of inactive spermatozoa allow milt plasma and saline solution to be useful as carrier solutions for antibiotics.

Spermatozoa of many fish species, when activated with ovarian fluid, have longer motility than in freshwater. However, Ginzberg (1968) indicated that sturgeon spermatozoa demonstrated no activity in ovarian fluid. Paddlefish spermatozoa motility had significantly shorter activity when activated with ovarian fluid rather than dechlorinated tap water. High levels of sodium and potassium in the ovarian fluid probably caused shorter spermatozoal activity than when in dechlorinated tap water (Table 1). Baynes et al. (1981) found with salmonids that potassium had an inhibiting effect on the initiation of motility. Turdakov (1965) had shown with some fish species that diluting the ovarian fluid would increase spermatozoal activity 2–3 times compared to spermatozoal activity in undiluted ovarian fluid.

There were obvious morphological changes with the spermatozoal flagella when activated with distilled or bacteriostatic waters. The membranes at the distal region of the flagella were observed to swell causing slow forward and circular movements. Holtz et al. (1977) reported similar morphological changes with the flagella of rainbow trout (*Oncorhynchus mykiss*) when strongly hypotonic solutions (i.e., distilled water) were

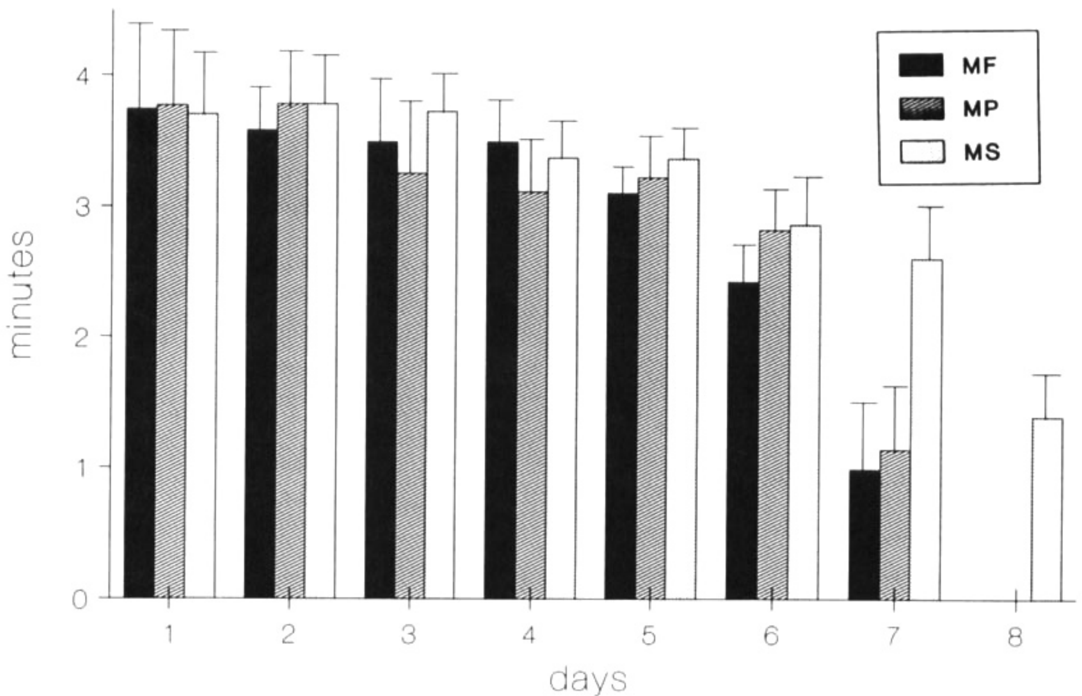

FIGURE 1. *Duration of paddlefish spermatozoal motility after storage in a chilled oxygen-rich environment was determined after activation in dechlorinated tap water. Milt was stored without antibiotics (MF), with antibiotics dissolved in milt plasma (MP), or in 0.9% sodium chloride solution (MS). Values are means (±SE) of 10 replicates.*

used as activators. The other solutions did not show any distinct morphological changes in flagella.

Short-Term Preservation

Motility of spermatozoa, activated with dechlorinated tap water, stored with antibiotics and saline (MS) was significantly longer than motility of spermatozoa stored without antibiotics (MF) or stored with antibiotics and milt plasma (MP) after 7 d under short-term chilled storage (Fig. 1). Duration of motility decreased as length of storage increased in all treatments. During the first three days in storage, differences in duration of motility of all treatments were minimal with MS showing no difference (0%) in length of motility compared to MF and MP which dropped 7% and 14%, respectively. However, differences between treatments were not statistically significant. Motility scores of 5 were recorded through 6 d for all treatments. Duration of motility of MS and MP were significantly longer than for MF. Between 6 d and 7 d there was a 60% and 67% drop in the duration of motility for MP and MF, respectively, compared to only a 9% drop with MS. Motility scores for MF and MP were 2 compared to 4 for MS. On 8 d, no motility was observed with MP and MF, whereas, motility was observed with MS. Between 7 d and 8 d duration of motility of MS had dropped 47% with an overall drop from 0 d of 62%. The MS spermatozoa still received a score of 3 indicating their potential to fertilize ova after 8 d of storage. However, ova fertilization was not proven. No motility was observed with MS spermatozoa on 9 d.

The results show that spermatozoa should be mixed with antibiotics and 0.9% saline solution in a moist oxygen-rich environment to obtain the longest chilled storage time. Spermatozoa should not be stored for

more than 8 d unless a more efficient method of storage is developed.

Many studies have shown that addition of dilute saline solution to spermatozoa of freshwater fishes can lengthen the duration of motility (Drabkina 1961; Terner and Korsh 1963; Ginzberg 1968; Holtz et al. 1977; Steyn et al. 1989). Salt (NaCl) may also have a protective effect on spermatozoa by decreasing the energy expenditure for osmotic activity during storage (Turdakov 1962). Though a small volume of saline solution was used as a carrier of antibiotics, it appears the additional sodium (78 mg/L) may have been useful in increasing the length of storage and duration of motility. Further research with various dilutions of saline and potential use of extenders could lengthen duration of motility and chilled storage period, respectively.

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