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Note

Spermiation of paddlefish (*Polyodon spathula*, Acipenseriformes) stimulated with injection of LHRH analogue and carp pituitary powder

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Abstract – The potential of carp pituitary powder (CPP) at one dose, or the luteinizing hormone-releasing hormone (LH-RH) analogue, des–Gly^{10},(D–Ala^{6})–LH-RH–*ethylamide*, at three different doses to stimulate spermiation in paddlefish (*Polyodon spathula*) was tested. Single injections of the LH-RH analogue at 0.2, 0.1, or 0.05 mg·kg^{-1} increased the number of spermatozoa per kilogram of body weight (kg^{-1} b.w.) by 4.7, 3.4, and 3.4 times respectively compared to control, but the number of spermatozoa per kilogram of body weight decreased with CPP (4 mg·kg^{-1}) by 1.7 times compared to the control. The LH-RH analogue prolonged active spermiation, with numbers of spermatozoa ranging from 7.69 to 1.19×10^{9} kg^{-1} b.w. up to 96 h after treatment. Analysis of variance showed significant influence of experimental groups on volume of sperm per male and per kilogram of body weight, and the total number of spermatozoa per kilogram of body weight, but insignificant influence on the total number of spermatozoa per male. The percentage of motile spermatozoa was not different between experimental groups for sperm collection at different times after injection. A very high positive correlation (r = 0.93) was obtained between sperm concentration and sperm transmittance measured with a spectrophotometer. This relationship was described with the following linear regression: sperm concentration ($\times 10^{9}$ mL^{-1}) = 1.3244 $X^{-0.9969}$, where X is the percentage of sperm transmittance.

hormone / spermiation / paddlefish / *Polyodon spathula* / induced breeding

1. INTRODUCTION

The volume of sperm released by sturgeon males is sometimes large (e.g. 25–200 mL for sevruga, *Acipenser stellatus*, 25–500 mL for Russian sturgeon, *Acipenser guldenstaedti*, and 800–1 000 mL for kaluga, *Huso dauricus*) (Ginsburg, 1968). In sturgeons the concentration of spermatozoa was usually reported as low compared to teleost fish (Persov, 1953; Ginsburg, 1968). In artificial propagation, the amount of sperm obtained per male is limited in paddlefish and in white sturgeon (*Acipenser transmontanus*), especially in the first period of the reproductive season, and therefore spermiation must be stimulated (Eenennaam et al., 1996; Mims, unpublished data). It has been shown that the LH-RH analogue, des–Gly^{10},(D–Ala^{6})–LH-RH–*ethylamide* at a dose of 0.05 mg·kg^{-1} in paddlefish (Mims, 1991) and carp pituitary extract (CPE) at a dose of 1.5 mg·kg^{-1} in paddlefish (Eenennaam et al., 1996) can increase sperm release after 24 h at 15–16 °C in both species.

In the present work, the potential to stimulate spermiation in male paddlefish of the LH-RH analogue (LH-RHa) at three different doses, carp pituitary powder (CPP), and control was examined. The objectives were to: 1) compare the effects of LH-RHa and

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CPP on spermiation, measured as the volume of sperm and number of spermatozoa, with a control of the percentage of sperm motility and osmolarity of sperm, and 2) compare relationship between sperm concentration and percentage of transmittance.

2. MATERIALS AND METHODS

The experiments were conducted in April 1998 at the Aquaculture Research Center, Kentucky State University (KSU, Frankfort, KY). Male paddlefish from 4.0 to 8.0 kg were captured in the Ohio River, KY. Broodfish were transported to the ponds of the Aquaculture Research Center at KSU and used within four weeks for experiments. Males were selected, held in five groups, and divided into circular metal tanks (6 000 L) with a water flow rate of 12 $L \cdot min^{-1}$, 9.0 mg $O_2 \cdot L^{-1}$, and a water temperature of 15 to 19 °C. Males were judged for maturity by abdominal compression, and spermiation was detected by sperm production. Fish were treated as follows:

– Group 1: LH-RH analogue, des–Gly^{10},(D-Ala^6)–LH-RH–*ethylamide* (Sigma Chemical Company, St. Louis, MO), injected intramuscularly at the dose of 0.05 $mg \cdot kg^{-1}$ body weight. Fish weights were 4.1, 5.7, and 5.5 kg, with an average of 5.1 ± 0.9 kg.

– Group 2: LH-RH analogue as in group 1 at the dose of 0.1 $mg \cdot kg^{-1}$. Fish weights were 6.4, 7.5, and 6.4 kg, with an average of 6.8 ± 0.6 kg.

– Group 3: LH-RH analogue as in group 1 at the dose of 0.2 $mg \cdot kg^{-1}$. Fish weights were 5.4, 7.1 and 7.46 kg, with an average of 6.6 ± 1.1 kg.

– Group 4: Carp pituitary acetone powder (CPP; Sigma Chemical Company, no. P3034) dissolved in Ringer's solution at 8 $mg \cdot mL^{-1}$ and injected intramuscularly at a dose of 4 $mg \cdot kg^{-1}$ (volume of solution injected 0.5 $mL \cdot kg^{-1}$). Fish weights were 4.7, 5.7, and 6.0 kg, with an average of 5.5 ± 0.7 kg.

– Group 5: Ringer's solution (0.5 $mL \cdot kg^{-1}$) was injected intramuscularly with no gonadotropin. Fish weights were 7.7, 6.0, and 5.0 kg, with an average of 6.23 ± 1.11 kg.

A 10-mL plastic syringe with 5 cm of Tygon tubing was used for sperm collection; the tube was inserted into the urogenital pore and the syringe was filled with milt. Sperm was collected and stored on ice in 100-mL containers until observation of motility, measurement of total volume, and sperm count with a Thoma cell (Linhart, 1991) were performed. Sperm concentration was also evaluated by measuring light transmittance; a portion of the sample was placed in a polystyrene disposable cuvette and percent transmittance at 450 nm wavelength was measured with a Milton Roy Digital Spectronic 401 spectrophotometer (Rochester, NY). The percent transmittance correlates linearly with sperm concentration when plotted on a log scale. The sperm was collected daily for 4.5 days, once every 24 h during the first two days, and then every 12 h on subsequent days. The concentration of sperm was expressed as billions of spermatozoa per milliliter of sperm. Volume of sperm per male and number of sperm per male, volume of sperm per kilogram of male body weight and number of sperm per kilogram of body weight were expressed as billions of spermatozoa per male and billions of spermatozoa per kilogram of body weight (kg^{-1} b.w.), respectively according to methods described by Linhart et al. (1995).

Sperm activity was evaluated as the percentage of motile spermatozoa in groups 1, 2, 3 and 4. Measurements of motility used dark field microscopy and Nikon microscope as described by Billard et al. (1993; 2000) and Cosson et al. (1977; 2000). Motility of spermatozoa was examined under 200× magnification immediately after mixing 0.5 µL of sperm with 49.5 µL of swimming medium (SM: 20 mM NaCl, 20 mM TRIS–HCl, pH 8.2), on a glass slide previously prepositioned on the microscope stage. The final dilution in this study was 1:100. A 5-min video recording of spermatozoa was started within 10 s after mixing in order to measure the percentage of actively swimming spermatozoa. The movements of spermatozoa were recorded at 60 $frames \cdot s^{-1}$ using a CCD video camera (SONY DXC-970MD) mounted on a dark-field microscope (NIKON Optiphot 2). The focal plane was always positioned near the glass slide surface. Spermatozoa movement was recorded using a tape recorder (SONY VHS, SVO 1520), visualized on a video monitor illuminated with a stroboscopic lamp of Strobex (Chadvick-Helmut, 9630). The stroboscopic flash illumination with adjustable frequency was set in automatic register with video frames (60 Hz) for sperm velocity measurement. The successive positions of the sperm heads were recorded 30 s after activation using a video-recorder (SONY SVHS, SVO-9500 MDP), and analyzed from video frames by micro image analysis (Version 3.0.1. for Windows with special application from the Czech Republic Olympus); the percentage of moving spermatozoa was determined by evaluation of each head position on three successive frames.

2.1. Data analysis

The data were acquired in triplicates and statistical significance was assessed using multiple analysis of variance (ANOVA, Statgraphics version 5), followed by multiple range tests (*tables I–V*). Regression analysis and correlation were obtained using Microsoft Excel 97 for establishment of the relationship in *figure 1*. Probability values < 0.05 were considered significant.

3. RESULTS

The strongest stimulation of spermiation was obtained with LH-RHa compared to CPP and control. The total volume of sperm per male and per kilogram of body weight during 4.5 days of sperm collection were significantly higher after injection of LH-RHa from 0.05 to 0.2 $mg \cdot kg^{-1}$, than of CPP or control,

respectively (*tables I–II*). The total number of spermatozoa per male and per kilogram of body weight increased after a LH-RHa dose of 0.2 mg·kg–1, then after a LH-RHa dose of 0.05 mg·kg^{-1}, or a LH-RHa dose of 0.1 mg·kg^{-1}, respectively (*tables III–IV*). The total number of spermatozoa per male and per kilogram of body weight was high on day 1 (33.62×10^9 per male and 5.46×10^9 kg^{-1} b.w.), highest on day 2 (49.29×10^9 per male and 7.69×10^9 kg^{-1} b.w.), and then slightly lower on days 3 (24.40×10^9 per male and 3.74×10^9 kg^{-1} b.w.) and 4 (7.75×10^9 per male and 1.19×10^9 kg^{-1} b.w.). The CPP induced only a small response, and was similar to Ringer's solution in the sham control treatment. The volume of sperm

Table I. Evolution of the sperm volume of paddlefish after hormonal stimulation with LH-RHa and CPP*.

Treatment	Dose (mg·kg^{-1})	Body weight (kg)	Volume of sperm collected at *n* days (mL)								
			0	1	2	2.5	3	3.5	4	4.5	Total
LH-RHa	0.05	5.1 ± 0.7^{a}	4.5 ± 1.4^{ab}	66.3 ± 17.2^{b}	85.0 ± 22.9^{a}	78.7 ± 19.0^{b}	83.7 ± 19.6^{b}	58.7 ± 18.5^{b}	74.0 ± 13.9^{b}	54.0 ± 6.1^{c}	504.8 ± 102.8^{b}
	0.1	6.8 ± 0.5^{a}	6.8 ± 5.3^{b}	81.0 ± 16.5^{b}	64.3 ± 59.0^{a}	81.0 ± 31.5^{b}	78.7 ± 16.4^{b}	87.3 ± 29.4^{b}	74.7 ± 28.2^{b}	81.0 ± 35.7^{c}	554.8 ± 188.9^{b}
	0.2	6.6 ± 0.9^{a}	5.8 ± 0.9^{ab}	66.7 ± 2.9^{b}	88.0 ± 6.2^{a}	84.3 ± 8.3^{b}	96.7 ± 4.2^{b}	72.3 ± 14.2^{b}	80.3 ± 3.2^{b}	43.3 ± 36.8^{bc}	537.5 ± 56.9^{b}
CPP	4	5.5 ± 0.6^{a}	5.8 ± 5.3^{ab}	24.3 ± 25.3^{a}	25.0 ± 26.0^{a}	18.3 ± 15.3^{a}	9.0 ± 9.6^{a}	8.0 ± 8.7^{a}	10.3 ± 4.6^{a}	10.0 ± 5.3^{ab}	110.8 ± 92.0^{a}
Control	(Ringer)	6.2 ± 1.1^{a}	0.0 ± 0.0^{a}	21.7 ± 30.6^{a}	31.7 ± 44.8^{a}	25.0 ± 35.4^{a}	28.3 ± 40.1^{a}	10.0 ± 14.1^{a}	3.3 ± 4.7^{a}	0.0 ± 0.0^{a}	120.0 ± 207.8^{a}

* Values are means ± SD. Controls were injected with a Ringer's solution. Groups with a common superscript in column do not differ significantly ($P < 0.05$).

Table II. Evolution of the sperm volume per unit of body weight of paddlefish after hormonal stimulation with LH-RHa and CPP*.

Treatment	Dose (mg·kg^{-1})	Body weight (kg)	Volume of sperm collected at *n* days (mL·kg^{-1})								
			0	1	2	2.5	3	3.5	4	4.5	Total
LH-RHa	0.05	5.1 ± 0.7^{a}	0.9 ± 0.2^{ab}	12.9 ± 1.3^{b}	16.6 ± 3.1^{b}	15.4 ± 2.2^{b}	16.4 ± 2.3^{b}	11.6 ± 3.1^{b}	14.7 ± 2.5^{b}	10.9 ± 3.0^{c}	99.3 ± 13.1^{b}
	0.1	6.8 ± 0.5^{a}	1.0 ± 0.7^{b}	11.9 ± 1.5^{b}	9.2 ± 8.1^{ab}	11.8 ± 3.5^{b}	11.6 ± 2.4^{b}	12.7 ± 3.2^{b}	10.8 ± 3.0^{b}	11.7 ± 4.1^{c}	80.8 ± 19.3^{b}
	0.2	6.6 ± 0.9^{a}	0.9 ± 0.2^{ab}	10.2 ± 1.7^{b}	13.5 ± 2.2^{ab}	12.8 ± 0.9^{b}	14.9 ± 2.9^{b}	10.9 ± 0.6^{b}	12.4 ± 2.7^{b}	6.0 ± 5.1^{bc}	81.6 ± 6.1^{b}
CPP	4	5.5 ± 0.6^{a}	1.0 ± 0.9^{b}	4.3 ± 4.4^{a}	4.5 ± 4.5^{a}	3.2 ± 2.6^{a}	1.6 ± 1.7^{a}	1.4 ± 1.5^{a}	1.8 ± 0.7^{a}	1.8 ± 0.8^{ab}	19.6 ± 15.7^{a}
Control	(Ringer)	6.2 ± 1.1^{a}	0.0 ± 0.0^{a}	2.8 ± 4.0^{a}	4.1 ± 5.8^{a}	3.2 ± 4.6^{a}	3.7 ± 5.2^{a}	1.3 ± 1.8^{a}	0.4 ± 0.6^{a}	0.0 ± 0.0^{a}	15.6 ± 27.0^{a}

* Values are means ± SD. Controls were injected with a Ringer's solution. Groups with a common superscript in column do not differ significantly ($P < 0.05$).

Table III. Evolution of the number of spermatozoa of paddlefish after hormonal stimulation with LH-RHa and CPP*.

Treatment	Dose (mg·kg^{-1})	Body weight (kg)	Number of spermatozoa collected at *n* days ($\times 10^9$)								
			0	1	2	2.5	3	3.5	4	4.5	Total
LH-RHa	0.05	5.1 ± 0.7^{a}	6.8 ± 1.3^{b}	24.3 ± 11.0^{ab}	31.5 ± 18.0^{a}	26.8 ± 18.9^{a}	13.1 ± 10.9^{ab}	4.8 ± 3.3^{ab}	4.7 ± 1.9^{b}	2.2 ± 0.5^{ab}	114.2 ± 44.5^{ab}
	0. 1	6.8 ± 0.5^{a}	6.7 ± 3.5^{b}	41.9 ± 40.6^{b}	26.4 ± 26.9^{a}	30.3 ± 19.9^{a}	15.5 ± 5.2^{ab}	9.2 ± 0.9^{bc}	4.2 ± 1.1^{b}	2.6 ± 0.5^{ab}	136.8 ± 37.7^{ab}
	0.2	6.6 ± 0.9^{a}	5.8 ± 3.1^{b}	33.6 ± 20.2^{ab}	49.3 ± 18.5^{a}	40.4 ± 29.1^{a}	24.4 ± 14.7^{b}	10.7 ± 5.3^{c}	7.7 ± 2.8^{c}	3.1 ± 3.5^{b}	175.1 ± 87.6^{b}
CPP	4	5.5 ± 0.6^{a}	5.7 ± 2.5^{b}	7.6 ± 11.9^{ab}	3.0 ± 4.3^{a}	1.8 ± 2.7^{a}	1.2 ± 1.9^{a}	0.9 ± 1.3^{a}	0.4 ± 0.4^{a}	0.4 ± 0.5^{ab}	20.8 ± 22.3^{a}
Control	(Ringer)	6.2 ± 1.1^{a}	0.0 ± 0.0^{a}	2.2 ± 3.1^{a}	32.3 ± 45.7^{a}	16.6 ± 23.5^{a}	4.5 ± 6.4^{a}	1.8 ± 2.5^{a}	0.6 ± 0.8^{a}	0.0 ± 0.0^{a}	58.0 ± 100.4^{ab}

* Values are means ± SD. Controls were injected with a Ringer's solution. Groups with a common superscript in column do not differ significantly ($P < 0.05$).

Table IV. Evolution of the number of spermatozoa per unit of body weight of paddlefish after hormonal stimulation with LH-RHa and CPP*.

Treatment	Dose (mg·kg^{-1})	Body weight (kg)	Number of spermatozoa collected at *n* days ($\times 10^9$ kg^{-1})								
			0	1	2	2.5	3	3.5	4	4.5	Total
LH-RHa	0.05	5.1 ± 0.7^{a}	1.4 ± 0.4^{b}	4.9 ± 2.3^{a}	6.1 ± 3.0^{a}	5.1 ± 3.2^{ab}	2.5 ± 1.9^{ab}	0.9 ± 0.6^{ab}	0.9 ± 0.3^{bc}	0.4 ± 0.1^{b}	22.2 ± 6.7^{bc}
	0.1	6.8 ± 0.5^{a}	1.0 ± 0.4^{b}	6.4 ± 6.4^{a}	3.9 ± 4.2^{a}	4.6 ± 3.2^{ab}	2.4 ± 0.9^{ab}	1.4 ± 0.2^{b}	0.6 ± 0.2^{b}	0.4 ± 0.0^{ab}	20.6 ± 7.1^{bc}
	0.2	6.6 ± 0.9^{a}	0.9 ± 0.5^{b}	5.5 ± 3.9^{a}	7.7 ± 3.3^{a}	6.2 ± 4.1^{b}	3.7 ± 2.1^{b}	1.6 ± 0.7^{b}	1.2 ± 0.4^{c}	0.4 ± 0.5^{b}	27.2 ± 13.8^{c}
CPP	4	5.5 ± 0.6^{a}	1.0 ± 0.3^{b}	1.3 ± 2.1^{a}	0.5 ± 0.7^{a}	0.3 ± 0.5^{a}	0.2 ± 0.3^{a}	0.2 ± 0.2^{a}	0.1 ± 0.1^{a}	0.1 ± 0.1^{ab}	3.7 ± 3.9^{a}
Control	(Ringer)	6.2 ± 1.1^{a}	0.0 ± 0.0^{a}	0.3 ± 0.4^{a}	4.2 ± 5.9^{a}	2.2 ± 3.1^{ab}	0.6 ± 0.8^{a}	0.2 ± 0.3^{a}	0.1 ± 0.1^{a}	0.0 ± 0.0^{a}	7.5 ± 13.0^{ab}

* Values are means ± SD. Controls were injected with a Ringer's solution. Groups with a common superscript in each column do not differ significantly ($P < 0.05$).

Table V. Motility of paddlefish sperm after hormonal stimulation with LH-RHa and CPP*.

Treatment	Dose ($mg \cdot kg^{-1}$)	Body weight (kg)	Motility of sperm collected at *n* days (%)							
			0	1	2	2.5	3	3.5	4	4.5
LH-RHa	0.05	5.1 ± 0.7[a]	91.7 ± 2.4[a]	99.1 ± 1.6[a]	97.2 ± 2.4[a]	78.8 ± 27.6[a]	90.9 ± 15.7[a]	100.0 ± 0.0[a]	97.5 ± 4.3[a]	91.2 ± 3.4[a]
	0.1	6.8 ± 0.5[a]	60.0 ± 31.5[a]	95.9 ± 5.0[a]	98.8 ± 1.7[a]	94.1 ± 2.0[a]	93.2 ± 6.0[a]	96.4 ± 0.6[a]	99.1 ± 1.5[a]	83.1 ± 23.8[a]
	0.2	6.6 ± 0.9[a]	59.5 ± 51.9[a]	83.4 ± 20.9[a]	73.7 ± 24.6[a]	95.8 ± 3.9[a]	95.3 ± 4.1[a]	98.2 ± 1.6[a]	92.7 ± 8.5[a]	79.1 ± 26.0[a]
CPP	4	5.5 ± 0.6[a]	64.6 ± 42.3[a]	57.2 ± 49.6[a]	94.9 ± 8.9[a]	69.9 ± 19.2[a]	60.2 ± 53.0[a]	94.4 ± 9.6[a]	99.1 ± 1.3[a]	94.4 ± 5.1[a]

* Values are means ± SD. Sperm was collected and analyzed 30 s after activation in swimming medium (20 mM NaCl, 20 mM TRIS–HCl, pH 8.2). Groups with a common superscript in each column do not differ significantly ($P < 0.05$).

production per male and per kilogram of body weight was significantly higher from day 1 to day 4.5 with the LH-RHa compared to the control, but the spermatozoa production as number of spermatozoa per male and per kilogram of body weight was slightly higher from day 3 to day 4.5 with the LH-RHa compared to the control. Analysis of variance showed significant influence of different experimental groups on volume of sperm per male ($P < 0.0041$) and per kilogram of body weight ($P < 0.0004$) and the total number of spermatozoa per kilogram of body weight ($P < 0.0355$), but insignificant influence on the total of spermatozoa per male ($P < 0.0975$).

Spermatozoa motility was regularly observed during the 4.5-day period in each experimental group. The percentage of motile spermatozoa was not significantly different between experimental groups during the period of sperm collection. The motility of sperm 30 s after spermatozoa activation was similar in all groups over the duration of the experiment with a slight decline on day 4 (*table V*). Analysis of variance showed no significant influence of the treatment ($P < 0.1168$) or of the period of sperm collection ($P < 0.0613$) on the percentage of motile spermatozoa.

In paddlefish, there was a very high positive correlation ($r = 0.93$) between the sperm concentration measured by sperm count with Thoma cell and the sperm transmittance measured by spectrophotometer, which relationship is described by the following linear regression: sperm concentration ($\times 10^9$ mL^{-1}) = $1.3244\ X^{-0.9969}$, where X is the percentage of sperm transmittance (*figure 1*).

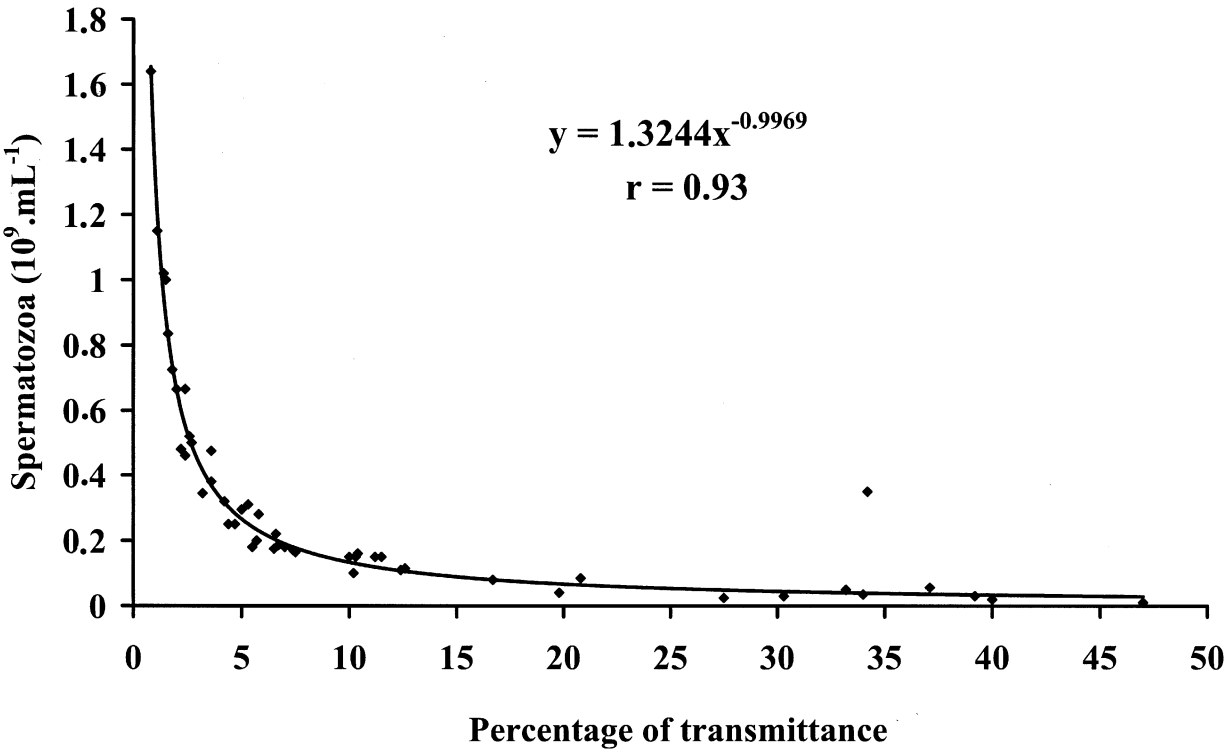

Figure 1. Relationships between sperm transmittance and sperm concentration.

4. DISCUSSION

The total number of spermatozoa collected was insignificantly higher after injection with 0.2 mg·kg^{-1} of LH-RHa (35.25×10^9 spermatozoa·kg^{-1}), compared to injection with 0.05 mg·kg^{-1} of LH-RHa (25.61×10^9 spermatozoa·kg^{-1}), as previously described by Mims (1991), Brown and Mims (1999), or compared to injection with 4 mg·kg^{-1} of CPP (4.39×10^9 spermatozoa·kg^{-1}) as reported for white sturgeons by Eenennaam et al. (1996), and compared to control group (7.53×10^9 spermatozoa·kg^{-1}). The average daily production of spermatozoa per kilogram of body weight was about 7.83×10^9 spermatozoa·day^{-1} during the 4.5 days after injection with 0.2 mg·kg^{-1} of LH-RHa, less (about 5.69×10^9 spermatozoa·day^{-1}) after injection with 0.05 mg·kg^{-1} of LH-RHa, but much less (about 0.97×10^9 spermatozoa·day^{-1}) in males injected with CPP. The CPP has no effect, or at least less effect, than in the control fish without hormonal treatment. Even though these were comprehensive results we have no explanation why the quantity of sperm was so low. The motility of sperm at 30 s after spermatozoa activation was shown to be similar in all groups with a slight decline on day 4.

The site of intraperitoneal injection behind one of the pelvic fins is convenient and induces large numbers of good quality, motile spermatozoa for up to 4.5 days. The measurement of sperm by light transmittance can be useful in estimating sperm concentration in paddlefish, in contrast to a time consuming methodology such as spermatocrit, or counting spermatozoa in a hemocytometer (Linhart, 1984). The condition of the fish used in the experiments was not adversely affected in the 4.5 days of sperm collection. Longer experiments may have a greater effect on the general condition of males.

Large volumes and numbers of spermatozoa were collected from sham-control males, which had only received injections of Ringer's solution. High quantity of sperm without hormonal treatment is usual in carp and trout (Linhart, 1991), but unusual in European catfish (*Silurus glanis*; Linhart and Billard, 1994), and also in white sturgeon and paddlefish as documented (Eenennaam et al., 1996; Mims, unpublished data). The present study was conducted during a year with unusually early warming that may have accelerated male development. The control fish were maintained in separate tanks from the treated fish and thus, no stimulation should have affected the outcome (Stacey et al., 1991).

The volume of sperm collected from paddlefish during the experiment was similar to that for sevruga and Russian sturgeon. The concentration of paddlefish spermatozoa was similar to those of sterlet or Persian sturgeon, but lower than those of Russian sturgeon, sevruga and beluga (Persov, 1953, Ginsburg, 1968). Other values, such as volume of sperm and number of spermatozoa per kilogram of body weight, have not been previously evaluated for paddlefish or sturgeon.

Hormonal injection of paddlefish increased the number of spermatozoa by about five times that of paddlefish controls. After hormonal injection, the quantity of spermatozoa per male weighing 10 kg can be increased up to 350×10^9 spermatozoa. This quantity of spermatozoa is sufficient for good fertilization of about 350 000 eggs (as calculated for sturgeons; Ginsburg, 1968) which is equivalent to about 7.5 kg of ovulated paddlefish eggs (Mims, unpublished data). In this respect, the hormonal induction of spermiation is sufficient for artificial propagation and does not need the use of intratesticular sperm.

Thus, the injection of 0.05 to 0.2 mg·kg^{-1} of LH-RHa, stimulates the production of a sufficient quantity of sperm to conduct artificial propagation of paddlefish. The total quantity of sperm available for fertilizing ovulated eggs can be increased by repeated collection at 12-h intervals over a period of 4.5 days, and storage of the undiluted seminal fluid 3–4 days at 4 °C.

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