RESEARCH CONSIDERING SPERMIATION ON *Polyodon spathula* STURGEON SPECIES TOWARDS OPTIMIZING SPAWNING BIOTECHNOLOGY AND CONSERVING BIODIVERSITY

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Abstract

The paper presents the outcome of research concerning spermiation and sperm quality on Polyodon spathula males hormonally induced by carp pituitary powder and different doses of Nerestin 5A. Sperm volume and spermatozoa density per male and per kg of body weight and sperm motility were appraised. The potential of carp pituitary powder (CPP) and of Nerestin 5A synthetic hormone, at three different doses to stimulate spermiation in paddlefish (Polyodon spathula) was tested. Single injection of the Nerestin 5A at 0.05, 0.1 or 0.15 ml/kg increased the number of spermatozoa per kilogram of body weight by 3.2, 3.2 and 4.5 times compared to the control, but the number of the spermatozoa per kilogram of body weight decreased with CPP (4 mg/kg) by 1.5 times compared to the control. The Nerestin 5A prolonged active spermiation, with numbers of spermatozoa ranging from 6.98 to 1.19 x 10^9 kg^{-1} of body weight up to 96 hours after treatment. Analysis of variance showed significant influence of experimental batches 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 batches for sperm collection at different times after injection.

Key words: hormone, spermiation, *Polyodon spathula*, induced breeding

INTRODUCTION

Polyodon spathula (*Acipenseriformes – Polyodontidae*) sturgeon is a native of North America. Due to the exceptive characteristics: rapid growth rate in optimal conditions, adaptability to grown in captivity, economic diet (is a plankton feeder) plus the quality of meet and eggs very similar to other sturgeon, but due to the drastic decline of sturgeon populations from natural waters, the species is in attention of specialists from around the world and its aquaculture is growing [5]. Acclimatization of species in Romania was initiated and carried out inside of Fish Culture Research and Development Station Nucet. Thus, from 1992 to 2000 were imported from USA, between 2000 and 10000 of embryonated eggs and larvae aged 4 – 6 days. In 2002, when the first batch reaching sexual maturity, was successfully achieved the first artificial spawning under ecological and technological conditions from Romania.

The experiments achieved during 2002 – 2010 revealed that as a result of hormonal stimulation of spermiation, paddlefish males can give sperm for a period of 4 – 6 consecutive days. This allows their use for fertilization of eggs obtained from several batches of females, increasing the economic efficiency of reproductive processes by injecting a small number of males. Volume and quality of sperm decisively act on the success of artificial spawning. In the first part of spawning season, paddlefish males give a small amount of sperm and therefore require hormonal induction of spermiation [2].

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In the present work, are presented the results of spermiation induction on paddlefish males with Nerestin 5A in three different doses, carp pituitary powder (CPP) compared to the control injected with saline solution. The effects of induction with Nerestin 5A and CPP on spermiation were studied by measuring the volume of sperm and number of spermatozoa and assessment of sperm motility.

MATERIAL AND METHODS

Experiments were conducted at Fish Culture Research and Development Station Nucet, April 2011. Batches of paddlefish male breeders weighting from 8325 to 12535 kg were reared in ponds of Nucet experimental base.

The males were selected by the characters of sexual dimorphism, the presence of bridal buttons, the appearance of genital pore, and the amount of maturation was assessed by slightly pressing the abdomen respectively. The selected males were divided in five experimental groups and held in hatchery, in 4000 l tarp tanks, with a water flow rate of 15 l/min., 9.0 mg O_2/l and a water temperature of 14 to 16 ^{0}C. After stocking breeders in tanks, they are continuously overseen, and water temperature is measured hourly.

The hormonal induction of males was achieved as follow:

- Group 1: Nerestin 5A injected intramuscularly at the dose of 0.05 ml/kg body weight. Fish weights were 8.3; 10.4 and 11.52 kg with an average of 10.08 ± 0.9 kg.

- Group 2: Nerestin 5A at the dose of 0.1 ml/kg. Fish weights were 8.5; 9.1 and 10.3 kg, with an average of 9.3 ± 0.6 kg.

- Group 3: Nerestin 5A at the dose of 0.2 ml/kg. Fish weights were 8.3; 11.7 and 12.13 kg, with an average of 10.71 ± 1.1 kg.

- Group 4: carp pituitary powder dissolved in normal saline solution of 8 mg/ml and injected intramuscularly at the dose of 4 mg/kg (volume of solution injected 0.5 ml/kg). Fish weights were 10.2; 12.1 and 11.39 kg, with an average of 11.230 ± 0.7 kg.

- Group 5: the normal saline solution (0.5 ml/kg) was injected intramuscularly. Fish weights were 9.4; 10.2 and 11.39 kg, with an average of 10.330 ± 1.11 kg.

Dried plastic syringes at the end with 10 cm tube made of infusion cannula were used for sperm collection. The male was contention out of the tank, well dried, the cannula inserted into the genital pore and gently pulling the piston to absorb sperm until filed the syringe. In average, from a male was harvested about 80 – 100 ml of sperm. This technique of sperm sampling leads to achieving a considerable volume of semen without being contaminated by faeces or water.

Assessing sperm motility was achieved with a simple microscope (ML - 4 IOR tip). The suitable optical combination for this type control is eyeglass 10X and lens 20X or 40X. A blade greed (Burker) was used. The smear was read extemporaneously according to conventional methods known in the practice of artificial spawning after semen has been enabled by technological water.

Sperm concentration was determined using the haematocrite method. This method of determining sperm concentration it's an implementation in semiology of haematological method for numbering blood elements, this technique being standard practice. The principle of the method consists in counting on the microscope of spermatozoa from squares of haematocrite vessel and assessment of spermatozoa concentration from the semen.

Sperm was harvested 4 days, every 24 hours. Sperm concentration 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) [3].

Data analyses

The data were acquired in triplicates and statistical significance was assessed using Microsoft Excel '97. Probability values < 0.05 were considered significant.

RESULTS AND DISCUSSIONS

The strongest stimulation of spermiation was observed with Nerestin 5A compared to CPP and control. The total volume of sperm per male and per kilogram of body weight during 4 days of sperm collection were significantly higher after injection of Nerestin 5A from 0.05 to 0,15 ml/kg, then of CPP or control, respectively (Tables 1–2). The total number of spermatozoa per male and per kilogram of body weight increased after a Nerestin 5A dose of 0.15 ml/kg then after a dose of 0.05 ml/kg or a dose of 0.1 mg/kg (Tables 3–4). The total number of spermatozoa per male and per kilogram of body weight was high on first day (46.3 ± 12.4 x10^9 per male and 4.8 ± 5.4 x 10^9 kg $^{-1}$ b.w.), highest on second day (49.4 ± 16.2 x 10^9 per male and 4.9 ± 4.0 x 10^9 kg $^{-1}$ b.w.), and then slightly lower on third day (29.0 ± 4.6 x 10^9 per male and 2.7 ± 2.1 x 10^9 kg $^{-1}$ b.w.) and the fourth day (9.1 ± 2.5 x 10^9 per male and 0.6 ± 0.2 x 10^9 kg $^{-1}$ b.w.). The CPP induced only a small response, and was similar to the sham controlled treatment.

Table 1 Evolution of the sperm volume of paddlefish after hormonal stimulation with Nerestin 5A and CPP*

Treatment	Dose (mg/kg)	B.W. (kg)	Volume of sperm collected at n days (ml)					
			0	1	2	3	4	Total
Nerestin 5 A	0.05	10.08	8.0± 2.3	96.7±15.3	117.9±16.7	105.8±14.1	111.8±15.9	432.2±24.7
	0.1	9.32	8.4± 4.7	96.0±14.0	110.0±11.8	98.8±10.5	95.0±11.2	408.2±38.1
	0.15	10.71	8.5± 0.3	103.8±16.1	138.1±21.3	108.2±24.2	121.0±21.7	479,6±44.6
CPP	4	11.23	10.1±3.4	46.0±9.1	47.2±9.7	20.2±4.9	19,1±5.8	142.6±14.9
Control	Saline solution	10.33	0.0 ± 0.0	15.5±5.3	31.0±7.5	16.5±2.7	4.1±1.9	67.1±10.2

* Average value ± SD

Table 2 Evolution of the sperm volume per unit of body weight of paddlefish after hormonal stimulation with Nerestin 5 A and CPP*

Treatment	Dose (mg/kg)	B.W. (kg)	Volume of sperm collected at n days (ml·kg^{-})					
			0	1	2	3	4	Total
Nerestin 5 A	0.05	10.08	0.8 ± 0.3	9.6 ±1.7	11.7± 3.9	10.5±2.3	11.1±2.1	43.7±7.7
	0.1	9.32	0.9 ± 0.6	10.3±1.3	11.8± 7.9	10.6±2.4	10.2±2.9	43.8±7.9
	0.15	10.71	0.8 ± 0.5	9.7± 1.5	12.9±2.8	10.1±3.1	11.3±2.1	44.8±9.3
CPP	4	11.23	0.9± 0.9	4.1 ±3.9	4.2 ±4.6	1.8± 1.4	1.7 ±0.6	12.7±2.3
Control	Saline solution	10.33	0.0± 0.0	1.5 ±3.7	3.0 ±4.8	1.6± 4.8	0.4 ±0.6	6.5±1.9

* Average value ± SD

Table 3 Evolution of spermatozoa of paddlefish after hormonal stimulation with Nerestin 5A and CPP*

Treatment	Dose (mg/kg)	B.W. (kg)	Number of spermatozoa collected at n days (× 10^9)					
			0	1	2	3	4	Total
Nerestin 5A	0.05	10.08	14.1±6.4	46.3±12.4	49.4±16.2	25.2± 2.4	9.1±2.5	144.1±15.8
	0.1	9.32	9.3± 0.8	44.7±11.5	36.3±11.1	22.3±3.8	5.6±1.2	118.2±11.4
	0.15	10.71	9.6±1.1	42.8±12.1	43.9±15.7	29.0±4.6	9.6±2.4	134.9±13.6
CPP	4	11.23	11.2±7.8	13.4±9.1	12.3±8.2	5.6±2.2	4.5±1.1	47.0±6.4
Control	Saline solution	10.33	10.3±0.7	2.0± 0.4	9.3±3.2	2.0±0.2	1.0±0.1	14.3±3.2

* Average value ± SD

Table 4 Evolution of the number of spermatozoa per unit of body weight of paddlefish after hormonal stimulation with Nerestin 5A and CPP*

Treatment	Dose (mg/kg)	B.W. (kg)	Number of spermatozoa collected at n days (× 10^9 kg^{-1})					
			0	1	2	3	4	Total
Nerestin 5A	0.05	10.08	1.4 ± 0.4	4.6± 2.8	4.9±4.0	2.5± 1.9	0.9± 0.3	14.3±2.3
	0.1	9.32	1.0 ± 0.4	4.8± 5.4	3.9 ± 4.7	2.4 ± 0.9	0.6± 0.2	12.1±1.8
	0.15	10.71	0.9 ± 0.5	4.0± 2.8	4.1 ± 3.1	2.7 ± 2.1	0.9± 0.4	12.6±2.0
CPP	4	11.23	1.0±0.4	1.2±2.7	1.1 ± 0.9	0.5 ± 0.3	0.4± 0.1	4.2± 0.4
Control	Saline solution	10.33	0.0 ± 0.0	0.2 ± 0.6	0.9± 4.8	0.2 ± 0.8	0.1± 0.1	1.4± 0.1

* Average value ± SD

The volume of sperm produced per male and per kilogram of body weight was significantly higher from day 1 to day 4 with the Nerestin 5A 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 1 to day 2 with the Nerestin 5A, compared to the control. Analysis of variance showed significant influence of different experimental groups on volume of sperm per male ($P < 0.00041$) 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 days 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 in day 4 (Table 5). Analyses of variance show significant influence of the treatment ($P < 0.1168$) or of the period of sperm collection ($P < 0.0613$) on the percentage of motile spermatozoa.

Table 5 Motility of paddlefish sperm after hormonal stimulation with Nerestin 5A and CPP*

Treatment	Dose (mg/kg)	B.W. (kg)	Motility of sperm harvested at n days (%)					
			0	1	2	3	4	Total
Nerestin 5A	0.05	10.08	91.7 ± 2.4	99.1± 1.6	97.2± 2.4	91.0 ±8.6	93.2± 4.7	94.4± 4.9
	0.1	9.32	60.0 ± 1.5	93.1± 5.0	97.9± 1.7	93.7± 5.8	95.4 ± 0.5	88.0± 3.7
	0.15	10.71	51.5 ± 1.9	80.1 ± 0.9	70.7 ± 4.6	95.3 ± 4.1	92.7 ± 8.5	78.0± 3.2
CPP	4	11.23	64.6± 42.3	57.2 ± 9.6	94.9 ± 8.9	60.2± 53.0	89.1 ± 7.6	73.2± 2.8

* Average value ± SD. Sperm was collected and tested at 30 s after sampling. Activation was done with technological water

CONCLUSIONS

The number of spermatozoa collected was significantly higher after injection with 0.15 ml/kg of Nerestin 5A (49.4 x 10^9 spermatozoa/kg), to injection with 4 mg/kg of CPP (12.3 x 10^9 spermatozoa/kg) and compared to the control group (9.3 x 10^9 spermatozoa/kg).

The average daily production of spermatozoa per kilogram of body weight was about 3.5 x 10^9 spermatozoa/day during the 4 after injection with 0.15 ml/kg Nerestin 5A, less (about 3.15 x 10^9 spermatozoa/day) after injection with 0.05 ml/kg Nerestin 5A, but much less (about 1.05 x 10^9 spermatozoa/kg) in males injected with CPP.

The CPP stimulation results were insignificant compared with those obtain in the control group.

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 general condition of the fish used in the experiments was not adversely affected in the 4 days of sperm collection.

Hormonal induction of paddlefish increased the number of spermatozoa by about 4 times that of paddlefish controls. After hormonal injection, the quantity of spermatozoa per male weighing about 10 kg can be increased up to 200 x 10^9 spermatozoa.

Injection of 0.05 to 0.15 ml/kg Nerestin 5A 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 24 hours intervals over a period of 4 days, and storage of the undiluted seminal fluid 3 – 4 days at 4^0C.

The results confirm the data from the literature Mims [4] Brown and Mims [1], on the effects of hormonal stimulation on spermiation and sperm quality in paddlefish males.

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