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Induction of meiotic gynogenesis in sterlet (*Acipenser ruthenus*) using UV-irradiated bester sperm

Dorota Fopp-Bayat [a,*], Ryszard Kolman [b], Pawel Woznicki [a]

[a] *Department of Ichthyology, Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury in Olsztyn, Ul. Oczapowskiego 5, 10-718 Olsztyn-Kortowo, Poland*
[b] *Department of Icthyology, Inland Fisheries Institute in Olsztyn, Ul. Oczapowskiego 10, 10-718 Olsztyn-Kortowo, Poland*

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Abstract

Diploid gynogenesis was induced in sterlet (*Acipenser ruthenus*) using UV-irradiated bester (*Huso huso*×*A. ruthenus*) sperm. The optimal condition for the retention of the second polar body in sterlet was investigated by altering the timing, intensity and duration of heat shock application. A total of 90 gynogens of known parentage from three different experimental treatments were screened using microsatellite DNA analysis, and uniparental transmission in meiogens was confirmed.

Keywords: *Acipenser ruthenus*; Bester; Gynogenesis; Microsatellite DNA; Sterlet; UV-irradiation

1. Introduction

Sturgeons are a very ancient fish group, existing since the Late Cretaceous with a wide distribution in the Northern Hemisphere (Grande and Bemis, 1991). Sturgeon biology is interesting because of important conservation and economic issues involving these fishes. Sturgeons are the source of two high-value products: boneless and very tasteful meat and black caviar. Fish farms producing sturgeon meat and caviar as well as reproducing sturgeons under controlled conditions have emerged (Wade and Fadel, 1997; Chebanov and Billard, 2001). Sturgeons reared under controlled conditions show rapid growth and reach sexual maturity faster than in nature (Chebanov and Billard, 2001). Sterlet (*Acipenser ruthenus*) is the species with relatively small size and rapid sexual maturation within the family Acipenseridae (Nikolskij, 1971; Sokolov and Vasiliev, 1989). These life history traits make the sterlet a useful model for the genome manipulations in Acipenseridae.

Gynogenesis is a developmental manipulation facilitating the inheritance of maternal genetic material alone, which has been accomplished in several fish species (Ihssen et al., 1990; Pandian and Koteeswaran, 1998; Arai, 2001; Paschos et al., 2001). This genome manipulation technique involves the activation of egg development by genetically inactive spermatozoa and diploidy restoration by retention of the second polar body. The first experiments on induced gynogenesis in sturgeons were reported by Romashov et al. (1963). The next studies on techniques for production of meiotic gynogenetic sturgeons were conducted on the American

* Corresponding author. Tel.: +48 89 5234772; fax: +48 89 5233754.
E-mail address: foppik@uwm.edu.pl (D. Fopp-Bayat).

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acipenserids and polyodontid: *Acipenser transmontanus* (Van Eenennaam et al., 1996), *Polyodon spathula* (Mims et al., 1997) and *Scaphirhynchus platorynchus* (Mims and Shelton, 1998). Recoubratsky et al. (2003) successfully induced meiotic gynogenesis in *Acipenser gueldenstaedti* and *Acipenser stellatus*, but in *A. ruthenus* they failed.

The objective of the present study was to induce meiotic gynogenesis in sterlet and to confirm the success of this gynogenesis using microsatellite DNA markers.

2. Material and methods

2.1. Experimental design and gamete collection

The experiment was conducted at the Wasosze fish farm near Konin (Poland) in the winter of 2006 using sterlet oocytes and bester spermatozoa. The experimental design is presented in Table 1. The experiment included a diploid control group, a haploid control group to determine the efficiency of UV inactivation of the sperm (untreated eggs and UV-irradiated sperm), a triploid control group to determine the efficacy of second polar body retention (temperature-shocked eggs and untreated sperm), and a treatment designed to induce gynogenesis (temperature-shocked eggs and UV-irradiated sperm).

Maturation in females was assessed by measuring the diameter of oocytes and observing germinal vesicle migration in ovarian biopsies. One female was induced to spawn by two injections of 6 mg kg^{-1} body weight of *Abramis brama* pituitary extracts and was transferred to a recirculating water system at 15 °C (isolated from the male). One male bester was induced to spermiate by a single injection of 1 g kg^{-1} body weight of Ovopel (mammalian GnRH analogue + metoclopramide). Ovulated oocytes were obtained by stripping the female 20–24 h following hormonal injection. Sperm was drawn from the male into a syringe and kept at 4 °C before use. Sperm motility was checked under light microscopy after activation with freshwater.

Table 1
Experimental design and survival rates at different developmental stages of larvae for meiotic gynogenesis in sterlet

Design	Egg treatment	Sperm treatment[a]/ species	Gast. (%)	Neurul. (%)	Hatch (%)
Diploid	Untreated	Untreated/ sterlet	88	65	53
Diploid	Untreated	Untreated/ bester	74	54	42
Haploid	Untreated	45 s/bester	36	30	20
Haploid	Untreated	60 s/bester	33	21	13
Haploid	Untreated	70 s/bester	30	24	10
Triploid	34 °C–2 min	Untreated/ bester	73	53	41
Gynogen	34 °C–2 min	45 s/bester	35	28	24
Gynogen	34 °C–2 min	60 s/bester	35	29	25
Gynogen	34 °C–2 min	70 s/bester	28	22	19

[a] Sperm treatments involved UV-irradiation for the number of seconds indicated.

2.2. Sperm and ova treatment

Irradiation was carried out using different exposure times (45 s, 60 s and 70 s). For UV-irradiation, 2 ml of sperm was diluted with 18 ml of seminal fluid (supernatant from surplus semen centrifuged at 8000 rpm for 15 min) and put into Petri dishes (diameter: 100 mm) to a depth of approximately 1 mm. These dishes were placed on a gently rotating platform (90 rpm) 50 cm below the UV lamp (Philips 15 W). Sperm was treated with UV-irradiation for 45, 60 or 70 s. After irradiation, 20 ml of 15 °C water from the incubation system was added to the irradiated sperm suspension, and this mixture was immediately added to ova. Eggs were divided into nine approximately equal groups (~1000 eggs), held in individual 2 l beakers and fertilized with 3.25 ml of diluted irradiated or normal sperm at 15 °C. Egg batches were fertilized with control sperm prediluted in seminal fluid or with irradiated spermatozoa, as described above. Three batches of eggs used as a haploid control, activated with UV-irradiated sperm, were not heat-shocked. Eggs to be heat-shocked were transferred into boxes with perforated mesh and kept in water at 34 °C for 2 min. Heat shocks were applied at 18 min after fertilization in experiments by soaking the boxes in a polystyrene incubator containing heated water at 34 °C. The temperature in the incubator was constantly monitored. Immediately after treatment, eggs were transferred into 1000 ml Weiss incubators with their controls in a thermoregulated incubation system at 15 °C. Additionally, ova quality was checked by insemination with sterlet milt (Table 1). Survival of developing eggs and viable fry was recorded at different developmental stages: fertilization (2 h after fertilization—a.f.), neurulation (50–60 h a.f.) and hatching (6 days a.f.). Percent of neurulation was determined by observation of the neural tube closure at 50–60 h after fertilization (Dettlaff, 1993). At 6–8 days post-fertilization, the number of normal, free-swimming larvae was counted for each treatment and percent survival