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Citation	Fisheries Science, 68(Suppl. II), 1271-1272 https://doi.org/10.2331/fishsci.68.sup2_1271
Issue Date	2002
Doc URL	https://hdl.handle.net/2115/35203
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Type	journal article
File Information	arai-70.pdf



Histological observations of gonadal development in gynogenetic diploids and triploids of a hybrid sturgeon, bester

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KEY WORDS: sturgeon, gynogenetic diploid, triploid, gonadal development, sex ratio

INTRODUCTION

In aquaculture, the production of polyploid and gynogenetic fish plays an important role for several species. In recent years, sturgeon has been cultured for the production of both meat and roe for caviar in several countries. Therefore, the ability to produce all-female populations of sturgeon species by chromosome manipulation is of interest to commercial aquaculturists. In sturgeon species, several studies on artificial induction of gynogenesis^{1, 2, 3)} and polyploidy^{2, 3, 4)} have been carried out. However, no detailed information is available on the gonadal development and sex ratio of these fish. Here, we provide histological details of the early gonadal development in gynogenetic diploids and triploids of the hybrid sturgeon, bester.

MATERIALS AND METHODS

Both ova and sperm were collected from the F₁ bester individuals at the Department of Research and Development, Hokkaido Electric Power Co. in 1997. Ovulation and spermiation were induced by injections of 100 µg/kg body weight of the gonadotrophin-releasing hormone analogue des-gly¹⁰, [D-Ala⁶]-LHRH (Sigma).

Gynogenetic diploid and triploid fish were induced with heat shock following egg activation with both UV-treated and normal sperm. For the UV-irradiation treatment, the sperm was diluted five times using the seminal fluid of the bester. The resulting sperm solution (5ml) was spread out on a petri dish (100mm in diameter) and exposed to UV light at an intensity of 21000 erg/mm². Fertilized eggs were kept in water at 15°C for 15-20 min after insemination and were then transferred directly to heated water (34°C, 3-6 min duration). After this heat

shock treatment, eggs were incubated at 15°C, following which neurulation and hatching rates were recorded. Two experiments included both a diploid control group (untreated eggs and sperm) and a haploid group to determine the efficacy of UV exposure in the inactivation of the sperm (untreated eggs and UV irradiated sperm). Treatments within each experiment used ova taken from the same female.

Gonad samples taken from 3-year-old fish were biopsied and fixed in bouin's fluid. Fixed tissues were processed for routine paraffin embedding, and 6µm serial transverse sections of the gonads were stained with Delafield's hematoxylin and eosin. The ploidy status of the resultant progeny was assayed by measuring the relative DNA contents of erythrocytes using a flow cytometer (PA, Partec).

RESULTS

The hatching rate of normal larvae of control diploid and triploid groups were 60-82% in experiment 1 and 38-48% in experiment 2. The hatching rate of normal larvae of haploid and gynogenetic diploid groups were 4 and 36-49% in experiment 1, and 0% and 1-2% in experiment 2, respectively. The neurulation rate of both the haploid and gynogenetic diploid groups was 59-76% in experiment 1, but only 2-4% in experiment 2.

Fig. 1 shows flow cytometric histograms for the DNA content of the progeny of the gynogenetic diploid and triploid groups. Both control (data not shown) and gynogenetic diploid bester (Fig. 1A) showed prominent peaks at the same channel numbers.

In the control diploid and triploid groups, both females and males were present. In contrast, the sex ratio of female, male, undifferentiated fish was 13:1:2

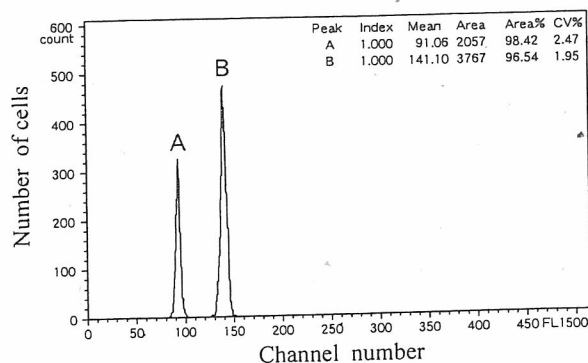


Fig.1 Flow cytometric histograms for DNA content of erythrocytes of gynogenetic diploid (A) and triploid (B).

in the gynogenetic group. In 3-year-old control and gynogenetic diploid females oocytes at the peri-nucleolus stage were observed in the ovaries (Fig. 2A,C); however, in triploid females most oocytes remained at the chromatin-nucleolus stage (Fig. 2B). Spermatozoa were observed in the testes of some 3-year-old control group males but not in the triploid or gynogenetic groups.

DISCUSSION

In the present study, both experiments used sperm from the same male, however, the neurulation and hatching rates of the gynogenetic and haploid groups in experiment 2 were rather lower than those in experiment 1. These results suggest that the efficacy of UV irradiated sperm is due not only to the quality of sperm but also to that of the ova.

Histological differences between control diploid and triploid ovaries would suggest that the triploid female bester was sterile. As one triploid testis sample showed signs of spermatogenesis (data not shown) it is not clear whether the triploid males were sterile. In the gynogenetic group, most progeny were female and no histological differences were detected in ovary between control diploid and gynogenetic female. The reason why a male was observed in the gynogenetic group should be the focus of future studies. In conclusion, our histological observations of gonad samples in the bester suggest that the induction of gynogenesis is a useful technique for the production of all-female progeny and also that triploid females are sterile in sturgeon.

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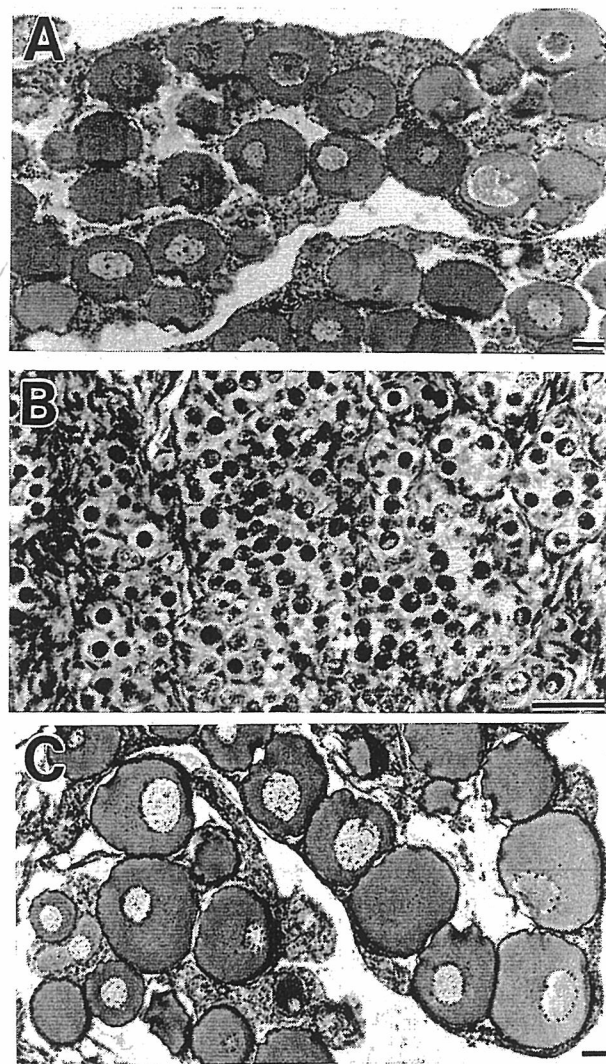


Fig.2 Ovaries of control diploid (A), triploid (B) and gynogenetic diploid (C) three-year-old bester females. Scale bars indicate 50µm.

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