

Diploid Gynogenesis Induced by Heat Shock after Activation with UV-Irradiated Sperm in Rainbow Trout (*Oncorhynchus Mykiss*)

Ioan Bencsik¹, Nicolae Pacala¹, Rodica Caprita¹, Dorel Dronca¹, Gabi Dumitrescu¹, Alena A. Bencsik-Theilen², Valeriu Caraba¹, Liliana Petculescu-Ciochina¹

¹Faculty of Animal Sciences and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine, 300645, Timișoara, Calea Aradului 119, Romania

²Institute of Medical Radiation Biology, University of Duisburg-Essen Medical School, 45122, Essen, Hufelandstr

Abstract

Gynogenesis is a technique used to generate diploid individuals with genetic material exclusively of maternal origin. In this study we test the hypothesis that heat shock treatment of rainbow trout eggs, activated with UV irradiated sperms, leads to the generation of a uniform populations of diploid gynogenetic females. Activation of fertilized eggs with UV irradiated semen, by heat shock at 27.5°C for 10 min. directly after fertilization induces diploid gynogenesis. Diploid individuals are obtained at a rate of 73.33%. Triploid individuals are obtained at a rate of 26.67%. The enhanced number of triploid individuals may be due to insufficient irradiation of heritable material of sperm.

Keywords: gynogenesis, dipoidy, triploidy, rainbow trout, heat shock

1. Introduction

Gynogenesis is a technique to generate diploid fishes with genetical material exclusively of maternal origin. Gynogenesis is an important tool in the study of sex determination in fish [1]. It involves in the first step the generation of gynogenetic haploids by fertilization of eggs with UV-irradiated sperm, followed in the second step by the restoration of diploidy by blocking the extrusion of the second polar body (meiotic gynogenesis) or preventing the first division of the zygote (mitotic gynogenesis) [2,3,4]. Restoration of the diploidy can be achieved by chemical, thermal or pressure treatment [5,6]. An early heat shock right after fertilisation prevents polar body extrusion while a late shock applied to the fertilized eggs blocks the first cleavage of the zygote [7]. The meiotic gynogens may be heterozygous or homozygous and survive better than mitotic gynogens that are homozygous [6].

Gynogenesis typically results in eggs mortality of 30 to 90% [8]. Yields of gynogenetic organisms are generally very low and the optimization of the shock parameters is cumbersome [5].

In this study we use the gynogenesis technique to produce uniform populations of female rainbow trout by heat shock.

2. Materials and methods

The experiment was performed on semen and eggs from rainbow trout (*Oncorhynchus mykiss*) in natural spawning period. Semen (milt) was collected by manual milking of four males at the age of two years. The eggs were collected by manual milking of two females at the age of three to four years. Approximately 4000 eggs were harvested. From these 600 eggs were used to induce gynogenesis and 800 eggs in the control experiment.

To inactivate the hereditary capacity of the paternal origin, seminal material was exposed to ultraviolet (UV). Two ml of semen was diluted with 18 ml of PBS enriched with glucose (54g/l). The biological material was then transferred into a

* Corresponding author: Ioan Bencsik ,
Tel: +40 256-277-102, Fax:+40 256-277-110
Email: bencsik54@animalsci-tm.ro;

dish and homogenized continuously by magnetic stirring to enable uniform irradiation. Irradiation was performed using a UV lamp (5 W/ 254 nm) positioned 12.5 cm above the sample for 2.5 minutes (min.). For fertilization, eggs were added to the irradiated sample and covered with water under controlled slow movement. Two ml of semen were used to fertilize 600 eggs. After two minutes, the excess of sperm was washed out and the eggs were incubated in water for further 8 minutes. Subsequently activation (fertilization), the heat shock of the eggs was induced to restore diploidy. The fertilized eggs were exposed to 27,5°C for 14 min. and incubated at 8°C Celsius for 45 days, representing a total of about 340 degrees Celsius. Through this procedure, we induced ginogenesis to 582 eggs. About 18 eggs were not fertilized (3%) and were not considered for the subsequent development of the experiment. The same applied for the control group were 14 of 800 eggs (1.75%) were not fertilized. The eggs of the experimental and control group were incubated under identical conditions.

Karyotyping was performed to examine the cellular content of DNA. For each time point 30 embryonated eggs were incubated for 6 hours in a

0.005% colchicine solution to block division and obtain metaphase chromosomes. Afterwards these were transferred into a 0.7% NaCl solution and fixed with methanol and acetic acid fixing solution (1:3). After 30 minutes the solution was exchanged by adding 1 ml of fresh fixing solution to each embryo. The embryonated eggs were stored at 4°C until evaluation. The evaluation was performed by microscopy. To detect the methaphase chromosomes, the fixed embryos were stained with 4% Giemsa solution for 10 min. Metaphase chromosomes were counted in at least 10 fields, in order to determine the number of diploid (2n) or triploid (3n) chromosomes sets.

3. Results and discussion

Ginogenesis was induced in 582 eggs by the method described above. The development of the fertilized eggs with UV inactivated sperms was monitored during the incubation period and compared to the 768 control eggs fertilized with unirradiated sperm. The dynamic of developmental changes is summarized in the Table1.

Table 1. Dynamics of embryonic losses in rainbow trout as a result of gynogenesis.

Specification	Experimetal group		Control group	
	Total	%	Total	%
Initial number of unfertilized eggs	600	100	800	100
Number of unfertilized eggs	18	3	14	1.75
Initial number of eggs	582	100	786	100
First decade	31	5.32	30	3.82
Second decade	46	7.90	46	5.85
Third decade	11	1.89	13	1.65
Fourth decade	15	2.58	14	1.78
Last 5 days	1	0.17	3	0.38

It is obvious that the highest loss of eggs was registered in the first two decades of the experiment span. While in the experimental group 104 eggs (17.86%) were lost during the

experimental time, in the control group 106 eggs (13.48%) were lost. This represents an average of $2,31 \pm 0,44$ losses per incubation day for the experimental group and 2.35 ± 0.38 for the control

group. The difference between the two groups is not covered statistically using *t* test at the 5% ($p < 0.0500$).

In figure 1 is presented the evolution of developmental changes in the two groups. It is

obvious that the highest number of losses was recorded during the first and second decade while the lowest number of losses during the final period of incubation.

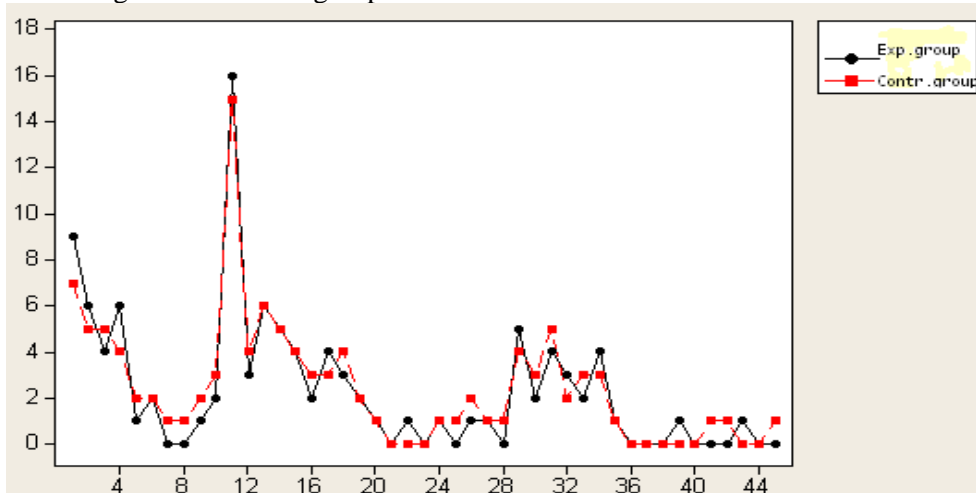


Figure 1. Evolution of egg losses during incubation time in the experimental group (black) and control group (red).

To determine the rate of diploid gynogenesis induction in rainbow trout by the heat shock, the heat treatment of the eggs was applied directly after fertilization to test the hypothesis of retaining the extrusion of the polar body and generate a uniform generation of diploid female individuals.

In Table 2 are shown the results obtained from karyotyping analysis of the embryonated eggs in the two groups. In the experimental sample 73.33% were diploid individuals whereas 26.7% were triploid. In the control group all embryos were diploid.

Table 2. Diploid ginogenesys rate

Specification	Samples	Diploid embryos (2n)	Triploid embryos (3n)
Experimental group	30	22 73.33%	8 26.67%
Control group	30	30 100%	- -

4. Conclusions

Activation of fertilized eggs with UV irradiated semen, by heat shock at 27.5°C for 10 min. directly after fertilization induces diploid gynogenesis.

Diploid individuals are obtained at a rate of 73.33%. Triploid individuals are obtained at a rate of 26.67%.

The enhanced number of triploid individuals may be due to insufficient irradiation of heritable material of sperm.

The highest number of losses during incubation in the experimental group and control were registered during the first two decades.

The enrichment of PBS with glucose in semen used for the dilution of semen has a positive effect on the fertilization ability of the sperm.

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