Acta Hydrobiol., 37 (1995), 2, 87–92. Kraków, November 1995

Comparison of effectiveness of thermal heat and cold shocks applied in the gynogenetic reproduction of common carp (*Cyprinus carpio* L.)

Henryk BIAŁOWĄS

Institute of Ichthyobiology and Aquaculture, Polish Academy of Sciences, Gołysz, 43-520 Chybie, Poland

(Received 9 February 1994, Accepted 11 October 1995)

Abstract – The effects of heat (40 °C, 1.5 min) and cold (2–4 °C, 60 min) shocks applied within 2nd, 8th, 15, and 30th minute after fertilization on the results of gynogenetic reproduction of common carp were compared. The sperm was inactivated using ultraviolet radiation. The temperature of fertilization and incubation of eggs was 22 °C. Greater numbers of larvae were obtained in the case of the heat shocks (0.3–3.4% of the incubated eggs) than of the cold ones (0–0.4%).

Key words: gynogenesis, sperm inactivation, cold shock, heat shock, common carp

Porównanie efektywności szoków termicznych ciepła i zimna stosowanych w gynogenetycznym rozrodzie karpia (*Cyprinus carpio* L.) — Porównano wpływ szoków ciepła (40 °C, 1.5 min) i zimna (2–4 °C, 60 min) zastosowanych w 2, 8, 15 i 30 min po zapłodnieniu na wyniki gynogenetycznego rozrodu karpia. Plemniki inaktywowano przy pomocy promieniowania ultrafioletowego. Temperatura przy zapłodnieniu oraz inkubacji ikry wynosiła 22 °C. Większe ilości larw otrzymano przy zastosowaniu szoku ciepła (0.3–3.4% ilości inkubowanej ikry) niż zimna (0.0–0.4).

1. Introduction

Artificial gynogenesis is composed of 2 basic treatments: the destruction of genetic information in the chromosomes of spermatozoa and the duplication of the haploid number of chromosomes in gynogenetic embryos. Haploid individuals generated from egg cells fertilized with inactivated sperm die during embryonal development or shortly after hatching. To obtain vigorous diploid gynogenetic individuals the number of chromosomes has to be doubled in haploid embryos. One of the methods of duplication of the haploid number of chromosomes consists in thermal shocks of heat or cold.

The aim of the present work was to compare the effectiveness of thermal heat and cold shocks used at different times after fertilization, to duplicate the haploid number of chromosomes in gynogenetic embryos.

2. Material and methods

In two experiments two common carp (*Cyprinus carpio* L.) females of mirror carp scaling (ssnn) and one full scale male of the known, previously checked homozygous genotype of scaling (SSnn) were used for spawning. The method of obtaining eggs and sperm was the same in the two experiments. Before each spawning carp spawners were hypophysed with two injections 12 and 24 h before spawning, using a total dose of 0.9–1.0 mg of pituitary gland homogenate of carp per 1 kg of fish weight.

Sperm portions 2 ml in volume, diluted in 6 ml of 0.85% NaCl solution, were placed on four watch glasses and irradiated with UV-rays emitted by a Philips filament of 30 W and 257.3 nm wavelength. The distance between the surface of sperm solution and the lamp was about 2 cm. During irradiation the sperm was stirred using a magnetic mixer, the temperature of 0–1 °C being maintained. From the start of inactivation to fertilization the sperm was isolated from visible light. The marker of scaling was used in checking the efficiency of DNA inactivation in spermatozoa.

Eggs obtained from the first female was divided into 10 portions of 100 g (about 80 thousand eggs) each. The number of eggs in a portion was determined on the basis of the average weight of the eggs, calculated from 3 random samples of 300 eggs each. One portion was fertilized with the non-inactivated sperm, the remaining nine being fertilized with inactivated spermatic fluid. Eight of them were subjected to thermal shocks of heat or cold, beginning in the 2nd, 8th, 15th, and 30th minute after fertilization. During fertilization and incubation the temperature of the eggs was maintained at 22 °C. The cold shock lasted for 60 min at a temperature of 2–4 °C and the heat one 1.5 min at 40 °C. After termination of the shock the temperature was gradually raised or lowered to 22 °C. The ninth portion of eggs fertilized with inactivated sperm was not treated with either shock. The second experiment was carried out analogically to the first, the only difference lying in the size of the portions of eggs obtained from the second female. They were 50 g in weight and contained about 40 thousand eggs each.

The fertilization, treatment of fertilized eggs after the thermal shocks, and incubation were carried out according to the Woynarovich method (1962). The obtained hatch was reared in flow-through glass aquaria 30 litres in volume. The water temperature was in the range 19–22 °C, the larvae being fed with zooplankton caught in ponds.

The calculations concerned the survival of embryos in the 12th and 24th hour after fertilization (on the basis of 3 random samples of 300 eggs each, sampled from hatch apparatuses), the number of the obtained hatch, and the number and survival (in relation to the hatch) of fish on the 40th day of rearing. The scaling of fish was determined. The survival of embryos, number of hatch, and survival of gynogenetic fish were evaluated using the ANOVA two-factor analysis of variance.

3. Results

In the first experiment good results of fertilization and incubation of eggs were obtained in groups treated with the heat shock in the 8th, 15th, and 30th minute of fertilization (Table I) No hatch was obtained from eggs treated with the cold shock even with the very good results of fertilization of eggs which were found in the group of eggs subjected to the shock in the 30th minute after fertilization.

Table I. Survival of embryos, number of obtained larvae and their survival in conditions of heat or cold shocks carried out at different times after fertilization. Control groups: A — eggs fertilized with inactivated spermatozoa and not treated with shocks, B — eggs fertilized with normal spermatozoa (for further rearing 1000(*) or 500(**) individuals of the hatch were used).

Kind of shock (°C)	Time of beginning the shock (min)	Survival of embryos (%)		Number of individuals			
				hatched		on 40th day	
		12 h	24 h	ind.	%	ind.	%
Experiment 1							
40	2	26	11	243	0.3	54	22.2
40	8	61	52	1914	2.4	420	21 9
40	15	55	47	1703	2.1	236	13.9
40	30	52	46	2692	3 4	386	14.3
2–4	2	18	0	0	0 0	0	0.0
2–4	8	9	0	0	0.0	0	0.0
2–4	15	11	2	0	0.0	0	0.0
2–4	30	49	38	0	0 0	0	0 0
A		8	6	11	0.0	0	0.0
B		79	72	31000*	38.8	883	88.3
Experiment 2							
40	2	30	15	112	0.3	49	43 8
40	8	42	19	238	0.6	83	34.9
40	15	39	20	301	0.8	65	21.6
40	30	44	28	687	1.7	203	29.5
2–4	2	26	14	82	0.2	28	34.1
2–4	8	27	7	59	0.2	23	39.0
2–4	15	8	5	63	0.2	25	39.7
2–4	30	32	21	159	0.4	73	45.9
A		3	1	0	0.0	0	0.0
B		47	45	12400**	31.0	372	74.4

In the second experiment in the case of the heat shock the best results of fertilization and the greatest hatch number were obtained when the shock was begun in the 30th minute, and the poorest for the shock in the 2nd minute after fertilization. In respect of the cold shock the best results were recorded for the treatment in the 30th minute after fertilization (Table I).

In the survival of embryos 12 h after fertilization and in the hatch number statistically significant differences ($p < 0.01$) were evidenced between the group subjected to the heat and cold shocks. No statistically significant differences depending on shocks started at different times after fertilization were observed.

The fertilization was poor in the groups of eggs fertilized with inactivated sperm and not treated with the shocks. Eleven larvae obtained in the first experiment died within three days after hatching. In both experiments the fertilization and the hatch number from eggs fertilized with normal sperm were high, this confirming its good quality.

All the individuals obtained from the gynogenetic reproduction had the mirror carp scaling and the individuals from control groups the full-scale scaling.

4. Discussion

UV-radiation efficiency destroyed the DNA of spermatozoa, as shown by the scaling of individuals form the control and gynogenetic groups. The dominating genes of full-scale scaling (SSnn) of the male were manifested solely in the control group composed of full-scale individuals, this confirming its homozygous genotype of scaling. All the gynogenetic individuals had the recessive mirror carp scaling of the female (ssnn), this demonstrating the destruction of the genetic material of spermatozoa.

When comparing the effectivity of heat and cold shocks applied at different times after fertilization on the same inactivated sperm and the same eggs, better results were obtained with the heat shock. The rate of divisions of fertilized fish eggs depends above all upon the temperature of incubation (Puczkow 1962). According to Ojima and Makino (1978) about 10 min after activation by the spermatozoon the carp egg is between her metaphase and anaphase of the second meiotic division. After the following 20 min the second polar body is formed. Unfortunately, the latter authors did not report the temperature of eggs incubations. According to Komen et al. (1991), during the incubation of carp eggs at 24 °C the first mitotic division occurs between the 20th and 35th minute after fertilization. In the 20th minute the fusion of pronuclei occurs. The prophase of the first mitotic division begins in the 25th minute by the condensation of chromosomes and formation of the division spindle. In the 28th minute the nuclear membrane is dissolved and the chromosomes attach to the threads of the division spindle. During metaphase, between the 30th and 32nd minute after fertilization, chromosomes are arranged in the equatorial plate of the division spindle. Sister chromatids are separated during anaphase (35th minute) and move towards the poles of the spindle. In telophase, 40 min after fertilization, nuclear membrane is developed. Chromosomes are despiralized and form two daughter nuclei. It may be assumed that with the shocks begun in the 2nd, 8th, and 15th minute after fertilization heterozygous gynogenesis was carried out while with the shock in the 30th minute the gynogenesis was homozygous. Differences in the effectiveness of heat and cold shocks probably result from the different mechanisms of their effect on the dividing cells (Bieniarz and Epler 1984, Komen et al 1988).

Analysis of results of the gynogenetic carp reproduction reported by various authors (Table II) and in the present work does not permit the author to determine definitely the most effective time of the shock. The obtained results of the gynogenetic reproduction are probably affected by factors which have not been unobserved by investigators. Little attention is paid to the ways of ending the shock Its beginning has to consist in a rapid change of temperature, while its end and recovery of the normal temperature of incubation should occur gradually. In this case the rapid change affects the eggs as another shock and its effect on the development of gynogenetic embryos cannot be excluded. If the UV-radiation is used for DNA inactivation, photoreactivation may occur. The inactivation chiefly occurs through the formation of pyrimidine photodimers and pyrimidine photoproducts developing between the neighbouring T-C bases. Visible light activates photolyase, a specific enzyme which splits photodimers to monomers and restores DNA to its primary condition (Harm 1980, Pietrzykowska 1987). Only a few authors state in their method of investigation whether the inactivated sperm was isolated from visible light.

The greatest technical problems arise in conducting shocks within a short time of fertilization. A sticky substance on the surface of carp eggs may only be removed

Table II. Results of gynogenetic reproduction of common carp, *Cyprinus carpio* L., depending on the kind and time of the shock.

Temperature of incubation °C	Parameters of the shock °C/min	Time range of starting shocks min	The most efficient time of starting shocks min	Percent of hatched individuals	Author
Cold shocks					
20	4/60	0–100	15, 5	0.9–56.2, 1.1–31.0	Nagy et al. 1978
20	0–4/60	5, 15	15	0.13–2.86	Linhart et al. 1986
24	0/45	0–15	1–2, 7–9	25–53, 23–54	Komen et al. 1988
20	0–2/45–60	1–3.5	1–2	1–31	Cherfas et al. 1990
Heat shocks					
20	39/2	1–15	3–5	< 30	Hollebecq et al. 1986
22	40/2	25–75	40	10.5	Nagy 1987
24	40/2	24–40	28–30	1.4–12.2	Komen et al. 1991

by washing in different solutions, this being a long-lasting process. Eggs subjected to the shock within a few minutes of fertilization are still stuck in lumps. This may play an important role in the case of the heat shock when, owing to its short duration, only the eggs on the surface of the lumps are properly affected. The result of diploidization and hence of fertilization and the number of larvae are significantly changed thereby. This may explain the poor result of fertilization when the heat shock is applied in the 2nd minute after fertilization. Most authors used small numbers of eggs, usually less than 1000, for gynogenesis. These small samples allowed a more precise execution of thermal shocks, which might have significantly affected the number of gynogenetic larvae obtained. In the present work the number of each lot of eggs was 80,000 or 40,000 on account on the planned rearing of gynogenetic carps in ponds and the possible losses in its course. These conditions might have led to poorer results of fertilization and hatch.

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