THE INFLUENCE OF ANTIOXIDANTS ON SPAWN FERTILIZATION AND EARLY EMBRYOGENESIS IN CARP

Vera Granaci, Alisa Morari- Pirlog

State Agrarian University of Moldova
e-mail: granaci@yahoo.com

Abstract

The influence of antioxidants FH_2 and FH_3 on the biological integrity of the conserved by refrigeration (0 - 4°C) Cyprinus carpio semen material was studied. The addition of antioxidants in the milieu contributed to the increase of the frozen spermatozoids survival longevity with 33.33%, fecundity ability with 13.42% (in case of FH_2), respectively with 10.39% (in situation of FH_3), while early embryogenesis with 3.56% and 2.34%, respectively given with control variant.

Key words: antioxidants, fish semen material, biological integrity, conservation, refrigeration

INTRODUCTION

Carp and other cyprinids, artificial fertilization at industrial scale is used in farm production. It presents interest when obtaining fertilized spawn from known producers in cases of hybridizations, blood refreshment, half-breeding, in the case of testing the acclimatization capacity, etc. Anyway, the enterprises that are involved in fish reproduction by means of *in vitro* fertilization, may meet some difficulties caused by non synchronization of maturity and collection of sexual products (spawns and spermatozoids). In some cases the semen material collected from male reproducers is of low quality that could put fertilization at risk. Very often supplementary high quantities of sperm are collected but the spawn is missing.Concerning the above mentioned facts we should pay more attention to the perfection of conservation procedures and we should use the possibilities to increase biological features of semen material in the conditions *in vitro.* These measures would assure normal functioning of technological flow of young fish production and use rationally genetic superior reproducers. This fact proved the study of antioxidants FH2 and FH3 influence on the survival and fertilization abilities of refrigerated semen material (0, +4˚C) and on the early embryogenesis at carp in the conditions of fertilization *in vitro*.

MATERIAL AND METHOD

The stimulation of gametogenesis in the male reproducers has been carried out through the intramuscular injections of pituitary solution in the dorsal part between the beginning of the dorsal fin and lateral line at a depth of about 2 cm. In order to evaluate the influence of antioxidants there has been prepared the basic solution in relation 0.5 mg antioxidant + 10 ml of basic medium variants decreasing the concentration. In order to dilute and preserve the semen material there has been used synthetic medium (Nauc V. et al., 1994) with the following composition: tris-oxiometil- aminomethane-3 g, 1,3 – butilenglicol - 15 ml, the yolk of hen eggs-12,0 ml, doubly distilled water-100ml, the pH has been conditioned at level 8,0 with the solution 0,1N tartric acid. The dilution degree has been 1:2=semen material: synthetic medium. The semen material has been preserved in a refrigerator at +4˚C. In order to maintain constant temperature the bottles with diluted semen material have been put into glasses with ice and covered by cotton-wool.

The semen material from the male species has been collected by massaging the abdomen region towards genital opening. The same method is applied to female species in order to collect the spawn. The testing of the collected semen material has been carried out visually under the microscope after initiating sperms mobility

as the result of sperm dilution with activating solutions according to generally accepted methods. In order to study the influence of antioxidants on spawn fertilization and early embryo development sperm samples from 3 females have been fertilized by the sperm obtained from a carp reproducer, the sperm has been diluted with the best variant established earlier according to survival indices and the parallel samples with the sperm diluted with basic medium. The evaluation of fertilization results has been carried out in 9 hours after fertilization.

RESULTS AND DISCUSSIONS

One of the harmful factors that appears in the conditions of conservation *in vitro* of the semen material and diminishes sperms survival is the process oh lipids per oxidation (Veronica Nauc, 1996; Tulcan, 2005). Normally lipids peroxidation in living systems is in equilibrium and is maintained at an established level owing to proper antioxidant features. Thus, in the obtained conditions the proper antioxidant system of biological objects is sufficient to maintain the processes of lipid per oxidation in the limits inoffensive for cell structures. Anyway, this equilibrium is disturbed when the temperature is lowered to critical levels. In order to mitigate this fact we have tested the features of two compounds with antioxidant characteristics from the class of fenozanel FH_2 and FH_3 in the medium of cryoprotection. As the sperms mobility and viability are important factors in spawn's fertilization, the awareness of the dynamics of these indices has practical importance in spawn's fertilization *in vitro.* The obtained results are shown in table 1.

Table 1. The influience of antioxidants FH_2 and FH_3 in the cryoprotection medium on spermatozoides mobility in the diluted semen material

Spetification	The concentration antioxidants in the cryoprotection medium mg%	The mobility, points	
		FH_{-2}	FH_{-3}
1 (control)	-	6,83±1,14	6,83±0,35
2	1,0	6,17±0,74	6,05±0,20
3	0,5	6,67±0,20	6,69±0,20
4	0,25	7,17±0,74	7,00±0,00
5	0,125	6,87±0,41	6,33±0,20
6	0,0625	6,57±0,41	5,50±0,35

The analysis of the obtained data states that the introduction of the tested compounds in the diluents for the carp semen material shows a tendency to differentiate the mobility according to the medium concentration in the medium exactly after the dilution. Thus, in the basic medium (variant 1) immediately after the dilution there has been stated a tendency of sperms mobility diminution from 7 points to 6.83 points in diluted sperm. The antioxidant concentration of 1.0 mg. %, on average, produces a sudden decrease in comparison to the control group. When reducing antioxidant concentration in the diluents medium, sperms mobility increases. The best results are obtained when using FH2 (variant 4) at the concentration level 0.25mg%. When using FH_3 at the same concentration level, the mobility is lower in comparison with FH_2. If we keep diminishing antioxidant concentration to 0.0625mg %, sperms mobility starts to diminish.

The knowledge of the duration of sperms survival has practical importance in spawn fertilization *in vitro*, it conditions the efficiency of practical usage of this technology in carp growing (Veronica Nauc şi colab., 1993; 1996; Nauc V. şi colab., 1994; Păcală şi colab., 2006). According to the data from the mentioned literature we continued our study that refers to the duration of diluted sperm survival in different variants of medium that contained different concentrations FH_2 and FH_3 in comparison to the control group (table 2 and table 3).

Table 2 The influence of FH_2 on the survival of refrigerated senen material

Concentation of FH_2 in the medium, mg%	**The mobility of spermatozoids after:**					
	Dilution	**2,0 h**	**24 h**	**48 h**	**72 h**	**96 h**
1 (control - basic medium)	6,83±1,14	5,01±0,14	3,11±0,23	1,50±0,09	0,5±0,032	-
BM + 1,0	6,17±0,74	5,11±0,08	2,09±0,12	0,55±0,18	-	-
BM + 0,5	6,67±0,20	5,13±0,17	3,33±0,17	1,50±0,11	0,53±0,013	-
BM + 0,25	7,17±0,74	6,50±0,11	4,25±0,18	2,50±0,11	1,50±0,09	0,53±0,11
BM + 0,125	6,87±0,41	5,11±0,12	3,25±0,20	2,25±0,18	1,0±0,00	0,5±0,00
BM + 0,0625	6,57±0,41	4,08±0,08	223±0,11	1,0±0,00	0,55±0,15	-

The presented results (table 2) show that maximum duration of the survival occurred in variant 4 and experimental variant 5 with concentration of 0.25-0.125 mg % and constituted 96 hours. The obtained data exceed the control group by 33.33%. Both in cases of high concentrations and lower concentrations of the medicine the survival duration is either similar to the results of the control group or it is lower.

According to the data obtained earlier that refer to sperms mobility after dilution and to seminal cells survival the optional concentration of FH_2 in the dilution of carp semen material constitutes 0.25-0.125mg %.

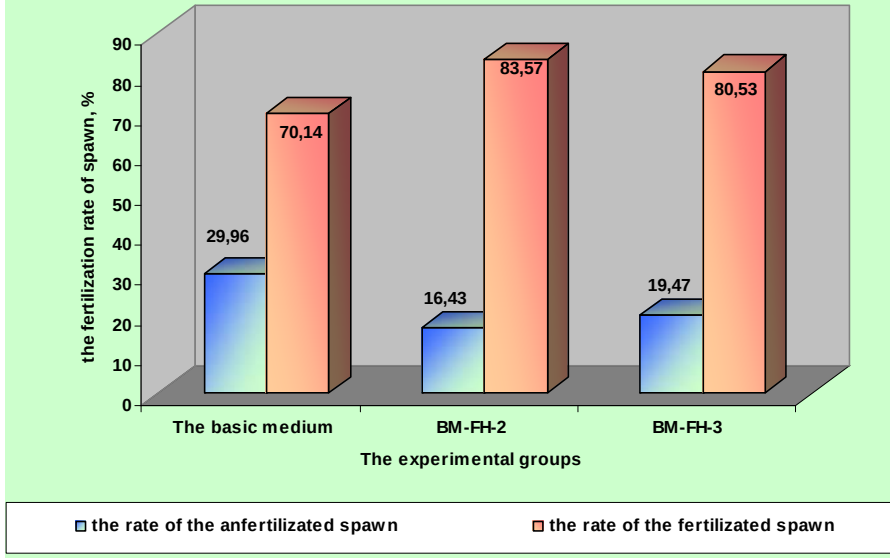
Table 3 The influience of antioxidant FH_3 on the survival of refrigerated semen material

Concentation of FH_3 in the medium, mg%	**The mobility of spermatozoids after:**					
	diluare	2,0 h	24 h	48 h	72 h	96 h
1 (control - basic medium)	6,83±0,35	5,01±0,14	3,11±o,23	1,50±0,09	0,5± 0,00	-
BM + 1,0	6,05±0,20	5,01±0,18	2,49±0,20	0,35±0,11	-	-
BM + 0,5	6,69±0,20	5,48±0,27	2,33±0,07	1,57±0,21	0,33±0,19	-
BM + 0,25	7,00±0,00	6,00±0,00	4,15±0,13	2,53±0,21	1,33±0,19	-
BM + 0,125	6,33±0,20	5,00±0,00	3,33±0,20	2,05±0,14	1,53±0,12	0,5±0,00
BM + 0,0625	5,50±0,35	3,90±0,18	2,00±0,00	1,33±0,05	0,55±0,15	-

The data from table 3 show that the sperm survived the best in variant 5, in which the medicine concentration constituted 0.125mg %. Thus, optimum concentration of FH_3 constituted 0.125mg %.

Considering the data from specialty literature that refer to the factors that compete in fertilization in order to confirm the decisive role of seminal cells' quality in spawn fertilization, we have evaluated these indices, we used for fertilization the diluted and refrigerated sperm in optimal variants 4 and 5 in which the mobility and survival prevailed the control group. The data are presented in figure 1.

Fig. 1.The influence of antioxidants on the fertilization ability of refrigerated semen material

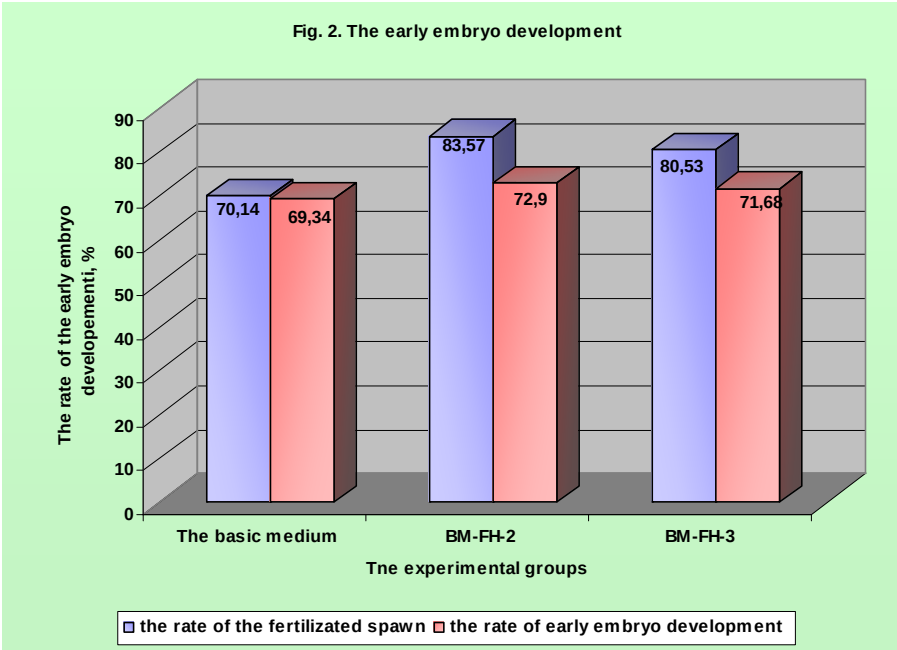


The analysis of the obtained experimental results shows that, if we introduce antioxidant FH_2 in the medium of dilution, it influences essentially the fertilization ability of the diluted and refrigerated sperms. Thus, the fertilization rate of the spawn in the control group constituted 70,14%, 13,43% lower than the results of the experimental group, in which the fertilization was 83,57%. As to the second antioxidant FH_3, the obtained results concerning fertilization ability of the diluted and refrigerated sperms are better than with the data from the control group by 10,39%. In comparison with group 2 (FH_2) fertilization ability of the sperms is lower by 3,04%.

The experimental data concerning the rate of fertilization of spawn in the control group and the experimental groups show that the introduction of FH_2 and FH_3 in the medium of dilution and conservation through refrigeration of carp semen material have contributed to the increase of mobility and survival maintenance of the sperms in refrigeration conditions. Thus, we can state that fertilization process at fish, being very short (to 1 minute), is very much influenced by the quality of seminal cells.

The testing of early embryo development has been carried out at the segmentation stages (early morula compact blastocyst, and hatching stages of early embryos. The obtained data are presented in figure 2.

The experimental results (fig.2) concerning early embryo development in the control variant and in the experimental variants, in which the semen material has been diluted by the medium enriched with the antioxidants, show that during fertilization-hatching (the rupture of pellucid zones, age-2-3 days). There is a tendency of embryos survival to be higher in experimental groups.



CONCLUSIONS

1. The introduction of the antioxidants (FH2 and FH3) in the medium in order to dilute carp semen material has contributed to the increase of sperms mobility and survival duration in the conditions of refrigeration (0-4˚C).

2. During artificial fertilization of the spawn with diluted and refrigerated semen material the rate of spawn fecundity was higher in experimental variants that contained antioxidants (FH2 and FH3) of 13.42% and 10.39% accordingly, in comparison with basic medium.

3. The introduction of antioxidants in the medium in order to dilute carp semen material has contributed to the moderate increase of the maintenance of early embryos viability.

REFERENCES

[1] Nauc, Veronica. Crioconservarea spermei de crap *(Cyprinus carpio L.)*. Autoref. tezei de doctor în biologie. – Chişinău: Secţia poligrafie operativă a USM, 1996. – 20 p.

[2] Nauc Veronica, Toderaş I., Boronciuc Gh. Contribuţii asupra cercetării criobiologiei gameţilor de crap.– Ann. Conf. Şt. USM. – Chişinău, 1993.– p. 273.

[3] Păcală, Nicolae, Bogdan Corbuli, Marian Dumitrescu. Biologia reproducerii peştilor. Timişoara:Edit. Parton, 2006. – 225 p.

[4] Tulcan Camelia Impactul crioconservării asupra sistemelor antioxidante ale celulei spermatice la specia canină. – Revista Politica Ştiinţei şi Scientometrie. – Revista Politica Ştiinţei şi Scientometrie- Număr special, 2005.– ISSN. – p- 1283 – 1293.

[5] Наук, В.А., Борончук, Г.В., Гранач, В.Г., Наук В.В., Крепис О.И. Среда для криоконсервирования спермы рыб. Патент №2010513, Российская Федерация, 1994.