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EVALUATION OF ETHINYLESTRADIOL (EE_2) EFFECT ON EMBRYO DEVELOPMENT IN COMMON CARP (*CYPRINUS CARPIO*)

EVALUAREA EFECTULUI ETINILESTRADIOLULUI (EE_2) ASUPRA DEZVOLTĂRII EMBRIONARE LA CRAPUL COMUN (*CYPRINUS CARPIO*)

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Worldwide, the scientific researches performed during the last years are focused on the determination of the negative effects caused by natural and antropogeneous chemical compounds on aquatic species; these species are more exposed to most pollutants than the land species, for the simple reason that the aquatic environment is the last destination for most residues. Our research team proposed to test the toxic effect caused by ethinylestradiol on embryo development in common carp (Cyprinus carpio). Common carp embryos were purchased from the fish farm S.C. Acva Prod S.R.L. Cefa, Bihor County these were obtained by artificial reproduction. After taking and selection, the fecundated spawns were introduced in 10 Nunk culture plates of 45 ml, where we introduced 40 ml water, too. We created 3 batches, with two replications, namely: batch 1 – control, batch 2 – in water, we added ethinylestradiol (EE_2) in concentration of 1.5 ng L^{-1} and batch 3 – we added in water a concentration of 7 ng L^{-1} EE_2. During the incubation, the Nunk plates were kept in breeding aquariums, at a temperature of 24°C. Successive to the supervision of embryos in batch 3, 48 hours post-fecundation, we could observe evolution stagnations, 70% of them being in the stage of 40 somites of the segmentation period. At the same age, 100% of the control batch- embryos entered the stage of advanced faringula, and in batch 2 all embryos were in the stage of incipient faringula. 60-72 hours post-fecundation, all embryos in the batch 3 died, 90% in the 40 somite stage of the segmentation period and 10% in the stage of incipient faringula. 85 hours post-fecundation, all embryos belonging to the control batch were in the larva stage, while in batch 2, 90% were in the larva stage and 10% died in the stage of advanced faringula.

Keywords: embryo, ethinylestradiol, *Cyprinus carpio*, development

Introduction

The notion of endocrine disruptor appeared at the end of the 20th century, in order to describe all compounds, xenobiotics chemical agents, with hormonal-mimetic properties.

Although, these molecules act in very small doses, they interfere with the function of the endocrine glands on target organs by imitating the natural hormonal action and blocking this action. The endocrine disruptors (ECDs) may interact in this way with estrogenic receptors (ER) modifying the synthesis, release, transport, binding, action or elimination of estrogenic hormones and, consequently, affect body homeostasis, development, reproduction and behaviour. These disorders are more severe as they occur in the early stages of development (foetus, embryo, early stage), because some irreversible effects, like genital malformations, may appear. Also a consequence of the endocrine disruptors' action in fish is represented the process of male womanizing, determined by the induction and increase of vitelogenin secretion (Arukwe et al., 2000, Denslow et al., 1999, Maitre et al., 1985, Le Guellec et al., 1988).

A great part of the about 70,000 artificial chemical substances resulted from different industries get into the aquatic environment.

The residues of the treated waters include mean 17 α-ethinylestradiol (EE2) concentrations of 1-17 ng L^{-1} (Stumpf et al., 1996, Ternes et al., 1999). In the surface waters, the reported EE2 concentration is between 1 and 15 ng L^{-1} (Stumpf et al., 1996, Belfroid et al., 1999, Ternes et al., 1999).

The synthetic oestrogen EE2 is used in oral contraceptives and it is excreted through urine in a conjugated inactive form, but it may be easily activated with the help of the bacterial activity from domestic water and plant treatment water (Guengerich, 1990, Tyler et al., 1998).

EE2 may be considered as one of the strongest estrogenic compounds, its toxic effect being proved *in vivo* and *in vitro* as well.

In the context of the researches performed in the whole world, the problem approached is a present-day one because it is related to the testing of the toxic effect exerted by the main chemical substances causing endocrine disorders. Starting from the idea that estrogens are present in natural water, it was proved that fish are extremely sensible during eclosion to 17 β estradiol and ethinylestradiol even in much reduced concentrations (only a few nanograms/L). Among the reasons of this enhanced sensibility to the substances mentioned, we may specify the long-term continuous exposure and their easiness in entering the fish sanguine circuit through gills.

Materials and Methods

In the context of the researches performed in the whole world, the problems approached by our team aims at the testing of the toxic effect exerted by ethinylestradiol on the embryo development in common carp (*Cyprinus carpio*), an economically-important species in our country.

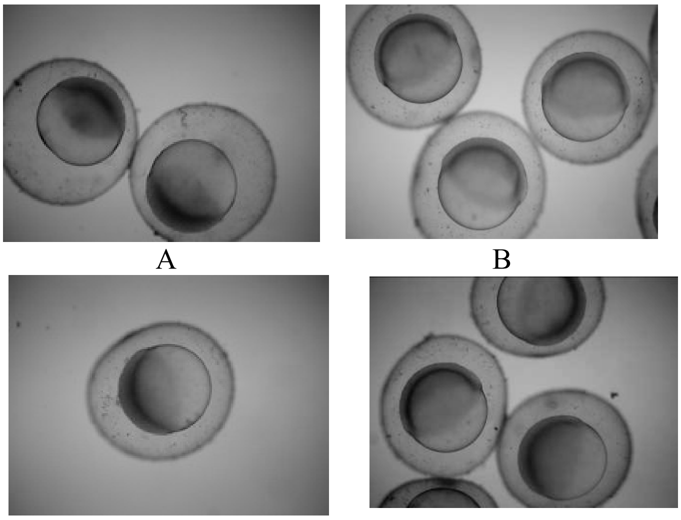
The common carp embryos (*Cyprinus carpio*) were purchased from the Fish farm S.C. Acva Prod S.R.L. Cefa, Bihor County, in June 2008; these embryos were obtained through artificial reproduction. We distributed the embryos in 3 batches, with 2 replications, namely: batch 1 – control, batch 2 – we added ethinylestradiol (EE_2) in water in a concentration of 1.5 ng L^{-1} and batch 3 – we added in water a concentration of 7 ng L^{-1} EE_2. The stock solutions of 1.5g L^{-1} EE_2 (Sigma), respectively of 7g L^{-1} EE_2 were obtained by diluting ethinylestradiol in ethylic alcohol 96^0 (Merck), and after that they were stored at the temperature of 4^0C. The solutions obtained from these stock solutions were processed through dilution in aerated water and stored at 4^0C. We should mention that, in small concentrations, the ethylic alcohol does not have adverse effects on larva development and growth (Van den Belt, K. Et al., 2002, 2003).

During the entire incubation period, the Nunk plates were kept in breeding aquariums at a temperature of 24^0C. Embryo supervision was performed with the research microscope Olympus CX41 endowed with digital camera and software for image analysis. During the process of embryo supervision, we used the main characteristics of embryo development stages in cyprinidae described by Kimmel et al., (1995).

Results and Discussion

The analysis of the data obtained successive to the supervision of common carp embryos leads to the conclusion that the first embryo development stages, respectively zygote, cleavage and blastula, do not differ significantly in the experimental batches compared with the control batch.

In carp, after 9 hours post-fecundation, 20% of the embryos belonging to the control batch were in the shield gastrula stage, 50% in the 50% epiboly stage and 30% in the 30% epiboly. In batches 2 and 3, we may observe an emphasized embryo evolution, so that, at the same period of time, 70% of the embryos belonging to batch 1 were in the shield stage, 20% in 30% epiboly and 10% in the elongation blastula stage, while in batch 3 all embryos were in the shield gastrula stage.

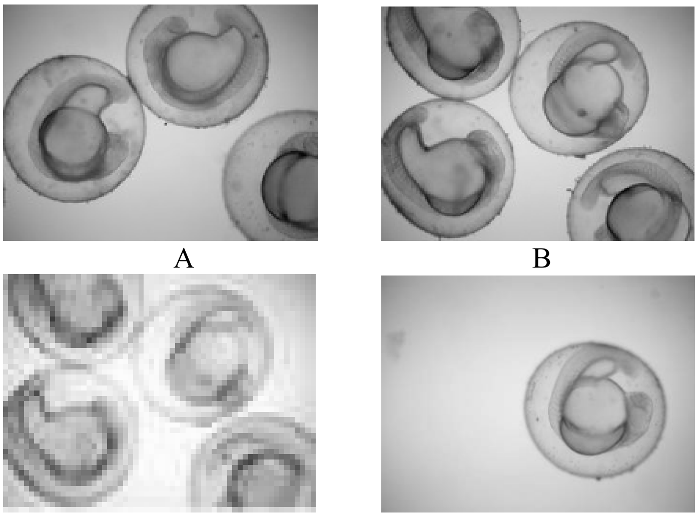


A B

C D

Fig.1. Carp embryos in different gastrulation stages. A – elongated blastula; B – shield; c – 30% epiboly; d. 50% epiboly

After 24 hours post-fecundation, all embryos in the control batch and in the batches 2 and 3 left the gastrula period and entered the segmentation period. So, at this age, in the case of the control batch, 100% of the embryos were in the stage of 20 somites, while in the experimental batches we may notice a slight involution, because 70% of the embryos were in the stage of 18 somites, and 30% in the stage of 16 somites.

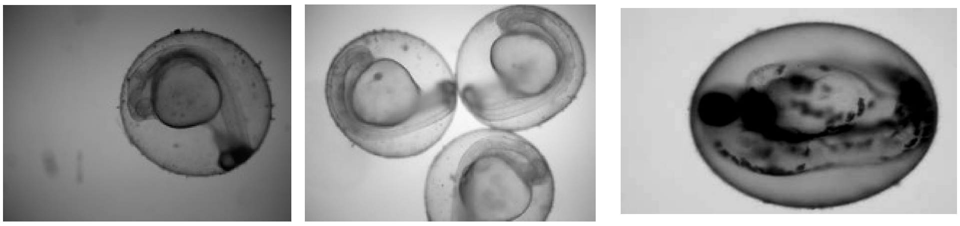


A B

C D

Fig. 2. Carp embryos in different segmentation stages: A - 16 somites; B - 18 somites; C, D - 20 somites

After 35 hours post-fecundation, in the case of the control batch and batch 2 (experimental I), all embryos were in the incipient faringula stage. In batch 2, only 70% of embryos were in this stage, and the difference to 100% was in the stage of 40 somites (10%), respectively 25 somites (20%). 48 hours post-fecundation, we could notice stagnation of evolution in batch 3, too; while in the control batch 100% of embryos were in the advanced faringula stage, all embryos in batch 2 were in the incipient faringula stage. In batch 3, only 30% were in the incipient faringula stage, and the difference of 70% was in the segmentation stage of 40 somites.



A **B** **C**

Fig. 3. Carp embryos: A – segmentation 40 somites; B – incipient faringula; C - advanced faringula

After 60 hours, all embryos in batches 1 and 2 were in the advanced faringula stage. In batch 3, 90% of embryos died, and 10% did not pass the incipient faringula stage, stage where they died at 72 hours. Consequently, at this age, the mortality percentage is 100% in batch 3, in which the ethinylestradiol dose is 7 ng L^{-1}.

Embryo hatching was performed in a proportion of 90% in the control batch after 78 hours post-fecundation, while in batch 2 it was performed only by 10% of embryos, the rest to 100% being in the advanced faringula stage. At 85 hours, the control batch comprised 100% larvae, while batch 2 (experimental I) included 90% larvae and 10% advanced faringula stagnating in evolution and dying in this stage.

In conclusion, the ethinylestradiol, in very small doses, determine a slight stagnation in embryo evolution, while big doses influence a slight stagnation in evolution and also their survival, the mortality in this case being 100% during the advanced faringula stage.

Conclusions

1. In the case of the control batch, the characteristics of the embryo development are similar with the ones described by Kimmel et al., (1995).
2. Embryo eclosion was performed in a proportion of 100% in the control batch 85 hours post-fecundation, while in the batch 2, 90% of individuals reached the larva stage, and 10% died during the advanced faringula stage.
3. In the case of the batch 3, where ethinylestradiol dose was 7 ng L^{-1}, all embryos died between 60 and 72 hours post-fecundation, 90% in the segmentation stage of 40 somites and 10% in the incipient faringula stage.
4. The data obtained show that ethinylestradiol, in small doses, determines a slight stagnation in embryo development, while in big doses it determines stagnation and after that embryo death.

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