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ANALYSIS OF SURVIVAL AND MORPHOMETRIC PARAMETERS OF LOACH EMBRYOS AND PRELARVAE UNDER THE ACTION OF THE POLYETHYLENEGLYCOL DERIVATIVE POLYMERIC NANOCARRIER

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Background. Our previous studies have shown a minor embryotoxic effect of polymeric poly(VEP-*co*-GMA)-graft-mPEG carrier on *Missgurnus fossilis* L. embryos and prelarvae. At the same time, it was found that a 3-day exposure to 10^{-15} M polymer carrier slowed developing, which is characterized by a delayed hatching of larvae. However, in the 10-day experiment on loach larvae exposed to polymeric carrier, anomalies and defects of development have not been identified. The aim of this work was to analyze the embryos and prelarvae survival, as well as determine the morphometric indicators of the loach embryos under the action of a PEG-modified polymer carrier.

Materials and Methods. Ovulation in loach females (*Misgurnus fossilis* L.) was stimulated by intramuscular injection of female chorionic gonadotropin (500 units), eggs were obtained 36 h after stimulation, fertilized in Petri dishes with a suspension of sperm according to A. A. Neifach. The stages of development were observed visually under a binocular microscope MBS-9 with a photo camera in a real-time mode. The experimental embryos were incubated in Goltfreter's solution with the addition of PEG-containing carrier to a final concentration of 10 and 100 μmol/L. The morphological development of the embryo groups was evaluated according to the T. Fujimoto development tables (2004),

and the morphological parameters (diameter and area of roes, blastomers, embryos, embryo sac and blastodisk) were measured using ImageJ and Photoshop (CC 2014v15) programs.

Results. Addition of the 10 μmol/L PEG-containing carrier to the medium initiated significant positive changes in the survival of loach embryos and prelarvae, in contrast to exposure to 100 μmol/L carrier. A significant increase in the area and diameter of blastomers and the embryos themselves under the influence of 100 μmol/L PEG carrier was established, which may indicate swelling of the embryos and a disruption of water-salt exchange that causes the early death of embryos.

Conclusions. (1) Low embryotoxicity of the PEG-polymer was confirmed on the studied model of the loach embryos. Based on the obtained data and the original data of J. Maes *et al.* (2) PEG is an attractive polymeric carrier for the delivery of a variety of compounds to both embryos and prelarvae as well as other model subjects. Despite the fact that we investigated only two concentrations of the PEG-containing carrier, it is quite likely that these concentrations of the carrier are (3) biologically active in themselves and, therefore, a thorough selection of the PEG-carrier concentration for each objective of the model is needed.

Keywords: loach, embryo, prelarva, polyethylene glycol, polymeric carrier, morphometric parameters

INTRODUCTION

The modern technological industry is a major global negative factor that affects human health, especially through environmental pollution and the spread of many chronic diseases such as metabolic, autoimmune ones (Moreira *et al.,* 2023; Sears & Genuis, 2012) and cancer (Alamro *et al.,* 2021; Avramović *et al.,* 2020; Fawzy & Toghan, 2020; Lu *et al.,* 2020). Unfortunately, some of these diseases are still difficult to treat with traditional pharmacotherapy, which depends on many properties of the drugs, such as solubility, bioavailability, biocompatibility, molecular weight, and intermolecular (or hydrogen) bonds, which may hinder the desired positive therapeutic response.

Polymers play an important role in the traditional formulations; they are used as film coating agents in tablets, binding agents in capsules and viscosity enhancers in emulsions (Dardeer *et al.,* 2022). The main function of polymer carriers is to transfer drugs to the active site and protect drugs from interactions with other molecules that cause the loss of their pharmaceutical activity. Modern polymer carriers prevent interaction between the drug and proteins, which leads to the prevention of its arrival at the site of action. In addition, polymer carriers are used as controlled release systems aimed at increasing the effectiveness of the drug therapy (Adepu & Ramakrishna, 2021; Ooya *et al.,* 2000).

The biodegradable polymers (Sung & Kim, 2020) are currently used as drug delivery systems, most commonly in dosage forms, such as poly(N-vinylpyrrolidone) and polyethylene glycol (PEG). PEGs, as polyrotaxanes, have been widely studied due to their biocompatibility and the ease of synthesis (Higashi *et al.,* 2019; Steiner & Saenger, 1996; Yui *et al.,* 1998). However, contrary to the vast clinical experience, not only advantages, but also side effects and complications of the PEG action have been established (Knop *et al.,* 2010). The dangerous and most common effects of the exposure to

PEG include organism hypersensitivity, unexpected changes in pharmacokinetics, toxic byproducts, and antagonism resulting from easy degradation of the polymer, its inability to fully biodegrade, as well as possible accumulation in the body.

Nevertheless, after 30 years of research (Pasut *et al.,* 2008), the advantages of PEGylation (i.e., covalent linking of the PEG strands) have allowed for the introduction of a necessary technology in the field of biopharmaceuticals, which is used to improve the stability, solubility, bioavailability and immunological properties of bioactive proteins and peptides, in particular, in cancer therapy.

The scientists have established a positive effect of PEG on the growth and development of plant species embryos that have low efficiency of somatic maturation of embryos, germination and transformation into seedlings. The increased transcript levels of these genes in immature PEG-treated embryos suggest that PEG may improve the quality of spruce somatic embryos by promoting normal differentiation of the embryonic shoot and root of white spruce (*Picea glauca*) (Jalali *et al.,* 2017). Jalali M. and colleagues (2017) showed the ability of PEG-4000 to stimulate the somatic maturation of the walnut embryo (*Juglans regia* L.). This stimulatory effect was dependent on the carbohydrate source used. Different concentrations of PEG were effective on a number of embryos with a shoot meristem. PEG-4000 (7.5%) and sucrose (3.0%) produced the highest rate (50.0%) of the normal shooting embryos. However, PEG (4000, 6000) and maltose caused an unfavorable effect and increased the frequency of abnormal shaped somatic embryos (Jalali *et al.,* 2017).

Polyethylene glycol is also used for cryoprotective treatment of the immature animal oocytes. For example, da Silva Santos *et al.* (2017) found that PEG did not improve oocyte survival and embryo development, whereas another substance, Supercool X-1000, improved the ability of surviving oocytes to cleave and divide, but not to develop into blastocysts (Santos *et al.*, 2017). T. Somfai *et al.* (2013) attempted to optimize the cryoprotective treatment for vitrification of immature porcine cumulus-oocyte complexes using 35% ethylene glycol (EG) and propylene glycol (PG), or a combination of 17.5% EG and 17.5% PG. The mean survival rate of vitrified oocytes in 35% PG (73.9%) was higher ($P < 0.05$) than that in 35% EG (27.8%) (Somfai *et al.*, 2013). Oocyte maturation rates did not differ among vitrified and non-vitrified control groups. In conclusion, 35% PG enabled a higher oocyte survival rate after vitrification compared with 35% EG. However, PG was greatly toxic to oocytes (Somfai *et al.*, 2013).

However, the studies on PEG and PEG-containing polymers effect on the embryo and larvae of the aquatic organisms, and their development are few and insufficient. Maes and co-authors (2012) determined the maximum tolerated concentration of several commonly used solvents and carriers in zebrafish embryos and larvae at different developmental stages. PEG-400, propylene glycol and methanol as solvents have been found to be relatively well tolerated at various stages of zebrafish development. The study results revealed that acetone was well tolerated by embryos, but not larvae, and 1% cyclodextrin (HPBCD) was well-tolerated by both embryos and larvae, indicating the utility of this carrier for compound screening in zebrafish (Maes *et al.*, 2012).

One of the ways to evaluate the effect of newly synthesized substances on embryonic development is to study their effect on the development of frog (*Misgurnus fossilis* L.) embryos, which, like FETAX (Frog Embryo Teratogenesis Assay – Xenopus (FETAX)) and zebrafish (*Danio rerio*) (Chan *et al.*, 2002), can be used as a screening test for the identification of chemical teratogens (Bantle *et al.*, 1999; Fort *et al.*, 2000).

It has been repeatedly confirmed by previous studies that loach embryos are an adequate test system for studying the influence of chemical (Boiko *et al.*, 2002; Goyda, 1993) and physical (Semochko *et al.*, 2010) factors on the living organisms in our climatic zone, and, due to the short period of embryogenesis, are a convenient object for experimental research. Considering the limited data on the effect of PEG-containing carriers on embryonic development, the aim of the work was to investigate the influence of the polymer carrier on the survival and morphometric parameters of cold-blooded embryos during early embryogenesis.

MATERIALS AND METHODS

Misqurnus husbandry, collection of the gametes, fertilization of eggs, collection of embryos and maintenance was performed as previously described (Fujimoto *et al.*, 2004; Goyda, 1993). Embryotoxicity assays were standardly performed in Petri dishes (wrapped with parafilm to limit solvent evaporation) using 100 embryos per dish in the Holtfreter's medium (110 mM NaCl, 1.4 mM KCl, 1.8 mM $CaCl_2$, and 5 mM Tris-HCl, pH 7,4). In the experiment, 3 females and 3 males were used (to repeat the experiment in a series, 3 cups were placed per pair of the individuals (a total of 900 fertilized eggs)).

Under the experimental conditions, embryos were incubated in Holtfreter's solution with the addition of PEG-containing carriers to final concentrations of 10 and 100 μmol/L. Embryos and larvae of both control and experimental groups were observed for 12 days using a MBS-9 binocular microscope with a photographic attachment in real time. The morphological development of the control and experimental groups of embryos was evaluated according to the development tables of T. Fujimoto (Fujimoto *et al.*, 2004), and the morphological parameters (number of analyzed photoi, **Fig. 1**, n = 7) were calculated using computer programs ImageJ and *Photoshop* (CC 2014v15).

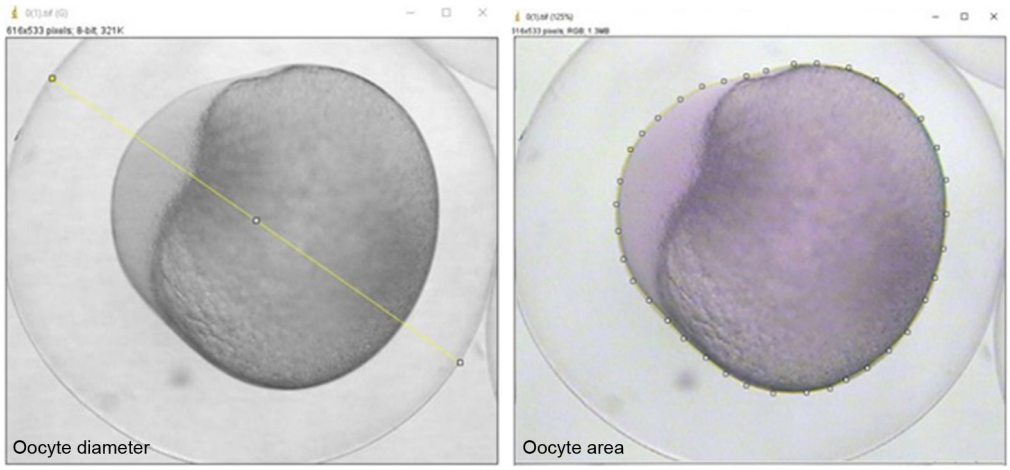

Fig. 1. An example of determining the diameter and area of the oocyte on the microphoto using the ImageJ program

The PEG-containing carrier poly(VEP-co-GMA)-graft-mPEG was synthesized at the Department of Organic Chemistry of Lviv Polytechnic National University. The steps of synthesis were described in detail in the previous publication (Mitina *et al.*, 2020).

Water dispersion of the polymeric carrier on the basis of poly(VEP-co-GMA)-graft-mPEG was prepared by dissolving it in dimethyl sulfoxide (DMSO), and the solution was subsequently transferred in water.

Statistical analysis of the obtained results was carried out using the MS Excel-2016 and Statistica programs. All data are presented as mean (M) ± standard deviation (SD). To determine statistically significant differences between the means of independent investigation groups, one-way analysis of variance (ANOVA) was used. The Shapiro–Wilk's test was used to assess the type of distribution and survival curves were assessed by calculating the Logrank test (Li & Ma, 2013). P values of <0.05 or lower were interpreted as statistically significant.

Manipulations with animals were carried out under the principles of the "General Ethical Principles of Experimentation on Animals" approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) and "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, France, 1985) as well as approved by the Ethics Committee of Ivan Franko National University of Lviv, Ukraine (Protocol No 39-09-2023 of 08.09.2023).

RESULTS AND DISCUSSION

According to our previously conducted studies (Bagday *et al*., 2014), on the first day of the development in control group, the survival of embryos significantly decreased by 19.1±1.9 % compared to the fertilization stage. On the second day of the experiments (48 hours), insignificant decrease in the control embryo survivals was noted – by 2.4±2.0 %. However, at the following stages of the development, changes in survival of the loach prelarvae compared to the first day of the development were significant. Such data are consistent with the critical stages of the development of teleost fish (Fujimoto *et al.*, 2004).

Analysis of the survival of embryos and prelarvae under the influence of PEG-containing polymer. It was found that 87.6 % of the embryos survived under the influence of the 100 μmol/L PEG-containing carrier on the first day after fertilization (significant changes compared to the control, **Fig. 2**).

On days 3–5 of the study, the survival percentage of fish prelarvae (individuals that did not switch to a mixed diet) did not change; on average it was 85.5 %. The prelarvae death gradually decreased up to the 12th day of development after fertilization and the survival percentage of individuals was 73.3 %. In the previous studies of morphological development of the loach, a slowdown in the development was noted on the third day, characterized by a delay in the larvae hatching in the presence of a polymer carrier in the medium (Bagday *et al*., 2014). However, on the 12th day of the experiment, no abnormalities and developmental defects were detected in the larvae loach exposed to the polymer carrier.

The high percentage of mortality on the first day after fertilization can be explained by the natural selection and critical stages of the development. Not all embryos survive this period, the appearance of genetic abnormalities and the development of pathologies is possible (Fujimoto *et al.*, 2004). Also, not all eggs are fertilized by healthy sperm, so this factor must also be taken into account. The lowest mortality rate under the influence of 10 μmol/L PEG-containing carrier was detected on the first day of the development (10.8 %), and the highest level of the mortality – under the 100 μmol/L PEG-carrier influence (25.5 %). On the 12th day of the experiment, the prelarvae survival did not change.

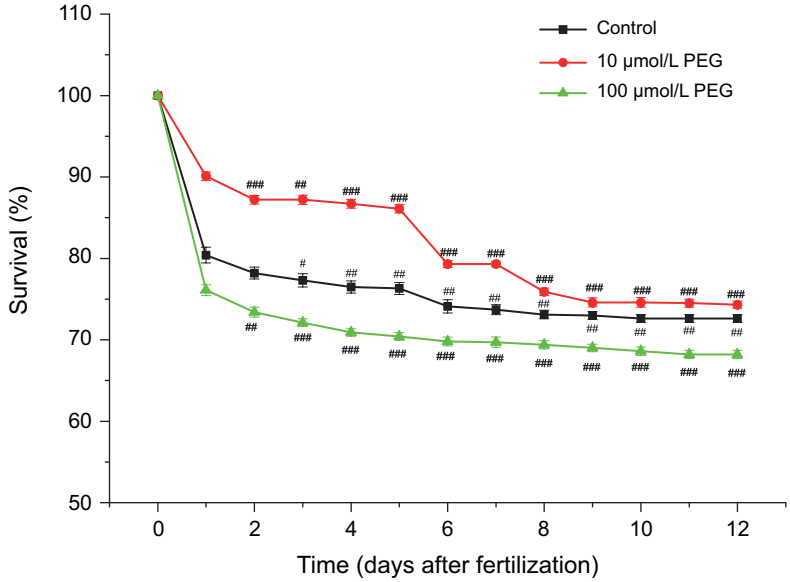

Fig. 2. Survival curves of the loach embryos and prelarvae under the influence of the PEG-containing polymer for 12 days of development (all changes are statistically significant compared to the control under both concentrations of PEG-polymer: *** – P <0.001; statistically significant changes compared to the first day after fertilization; ## – P <0.01, ### – P <0.001)

The Longran criterion (Li & Ma, 2013) was used to analyze the survival data, which allows us to state that the differences between the curves of 10 and 100 μmol/L PEG are statistically significant (see **Table 1**, P <0.001 and P <0.05, respectively).

Table 1. **Survival analysis and survival coefficient of the loach under the action of PEG-containing carrier**

Logrank test	Statistic χ^2	P	Ks (%)	Conclusion
10 PEG	21.43	P <0.001	80.8±1.77	Functions vary
100 PEG	4.08	P = 0.05	70.4±0.68	

J. Maes *et al.* (Maes *et al*., 2012) analyzed the effects of a 24-hour exposure to 11 solvents (acetone, acetonitrile, butanone, dimethyl formamide, DMSO, ethanol, glycerol, isopropanol, methanol, polyethylene glycol (PEG-400), propylene glycol, and solketal) and two carriers on zebrafish embryos and larvae during seven days' post-fertilization. No post-exposure effects until seven days' post-fertilization were detected at the MTCs. Phenotypic effects were recorded at the lowest concentration for each solvent, and at each developmental time point, in those cases where at least 50 % of embryos or larvae were affected (Maes *et al*., 2012).

Under the 10 μmol/L PEG-carrier influence, a better survival of the loach embryos and prelarvae was noted. Thus, an addition of the 10 μmol/L PEG-containing carrier to the incubation medium during the experimental period of the development did not cause significant changes in the morphological parameters, namely the area and diameter of

blastomers and embryos as a whole (data not shown). Examining the tolerance and preservation of biocompatibility is a crucial aspect in the study of properties of newly synthesized carriers. Therefore, the article presents and analyzes the morphometric parameters of the embryo objects under the influence of PEG-carrier in the concentration of 100 μmol/L, addition of which to the incubation medium leads to pronounced and reliable changes in the studied developmental parameters.

Morphometric parameters of loach embryos under the influence of polymer carrier. As experimental animals, representatives of the *Misgurnus* spp. are very interesting from the point of view of chromosome manipulations to induce cyanogenesis, androgenesis and various polyploidies (Fujimoto *et al.*, 2004), biotechnologies to produce of chimeric fish (Nakagawa & Ueno, 2003) and nuclear transgenics (Nam *et al.*, 2001).

Therefore, during the period of blastomer division (**Fig. 3**), no significant differences were found in the development of the embryos that were exposed to the polymer carrier at the concentration of 100 μmol/L compared to the control samples (Bagday *et al.*, 2014).

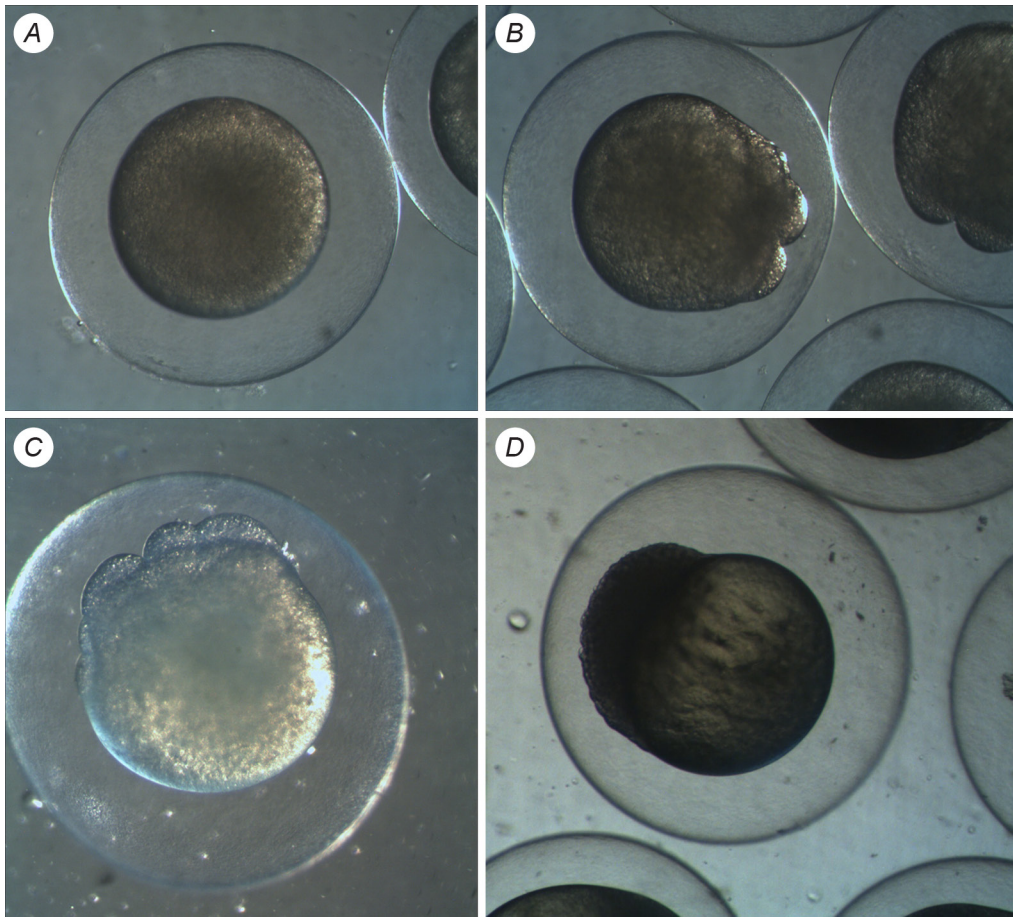

Fig. 3. Morphological development of loach embryos after fertilization (40 min, ***A***), at the stages of the first (***B***), fourth (***C***) and tenth divisions (***D***) under the influence of a PEG-containing polymer carrier at the concentration of 100 μmol/L. Photograph ×7

The cleavage scheme in the loach, as in other teleosts, is typical meroblastic cleavage (cell divisions are synchronous, and each division occurs about every 27–30 min on average (Fujimoto *et al.*, 2004)). The morphometric parameters of fertilized eggs under the action of 100 μmol/L PEG did not change significantly compared to oocytes (data not shown in the **Table 2**). However, there was a significant increase in the diameter and the area of the embryo and blastomer; in addition, a decrease in the area of the yolk sac (see **Fig. 3*A*** and **Table 2**) was noted within an hour after fertilization. Such changes are associated with the intensive use of maternal reserves from the yolk sac.

Table 2. **The morphometric parameters of loach embryos under the effect of 100 μmol/L PEG polymer (number of analyzed photoi, n = 7)**

Stages (min)	Description	Control		PEG-carrier	
		Area (mm^2)	Diameter / height (mm)	Area (mm^2)	Diameter / height (mm)
Fertilization (40±3 min)	Roe	1.68±0.04$^{\#\#\#}$	1.39±0.01	↑**1.99±0.03****	1.42±0.07
	Embryo	0.66±0.03	0.91±0.01$^{\#}$	0.68±0.08	0.90±0.05
	- blastodisk	0.22±0.003	0.19±0.002	0.26±0.02	0.20±0.02
	- yolk sac	0.66±0.03	0.91±0.01$^{\#}$	0.68±0.02	0.90±0.05
1 division 2 blastomers (60±3 min)	Roe	1.92±0.06	1.41±0.04	1.99±0.04	1.43±0.06
	Embryo	0.75±0.008	0.96±0.01	↑**0.78±0.006*****	0.97±0.03
	- blastomer	0.08±0.004	0.19±0.01	↑**0.17±0.01*****	↑**0.29±0.02*****
	- yolk sac	0.63±0.02	0.96±0.02	0.66±0.02	0.98±0.03
4 division 16 blastomers (150 ±3 min)	Roe	2.28±0.03$^{\#\#\#}$	1.53±0.03$^{\#}$	2.27±0.03$^{\#\#\#}$	1.54±0.05
	Embryo	0.89±0.03$^{\#\#\#}$	0.97±0.02	↑**1.18±0.04**$^{***\#\#\#}$	↑**1.07±0.03**$^{**\#}$
	- blastomer	0.12±0.02	0.11±0.01$^{\#\#\#}$	↑**0.19±0.02*****	↑**0.16±0.01**$^{**\#\#\#}$
	- yolk sac	0.77±0.03$^{\#\#\#}$	0.97±0.02	0.77±0.03$^{\#\#}$	0.97±0.02
10 divishion 1024 blastomers (330 ±3 min)	Roe	2.36±0.01$^{\#\#\#}$	1.66±0.01$^{\#\#\#}$	2.38±0.03$^{\#\#\#}$	1.55±0.05
	Embryo	0.95±0.02$^{\#\#\#}$	1.02±0.02$^{\#}$	↑**1.23**±0.04$^{***\#\#\#}$	↑**1.11**±0.03$^{*\#\#}$
	- blastomer	0.22±0.04$^{\#\#\#}$	0.05±0.01$^{\#\#\#}$	↑**0.34**±0.02$^{***\#\#\#}$	↑**0.10**±0.01$^{*\#\#\#}$
	- yolk sac	0.74±0.02$^{\#\#\#}$	0.99±0.02$^{\#}$	0.69±0.02$^{\#}$	0.99±0.02

Comments: changes are valid relative to the control (↑*) and the first division stage of the corresponding sample of dates ($^{\#}$) (* – P <0.05; ** – P <0.01; *** – P < 0.001)

At the 16 blastomers stage, significant changes in the diameter and area of the roe, yolk, embryo and blastomers were found; compared to the control and 2 blastomers stage (see **Fig. 3*C*** and **Table 2**). The diameter and area of blastomers significantly increased compared to the control under the influence of 100 μmol/L PEG, which indicates the swelling of embryo cells and the embryo as a whole. Such changes are probably caused by a change in the membrane permeability for sodium and potassium ions in the embryo plasma membranes, and, accordingly, by a change in the water transport. This is partly confirmed by the general decrease in the size of the roe at this stage of development.

The 10th blastomer division (1024 cells in the blastodisk) is transitional between synchronous and asynchronous divisions of loach. At this stage of the development, under the influence of 100 μmol/L PEG, significant changes in the area of the roes, the area and the diameter of the blastomers compared to the control were detected. Besides, the diameter of the roe significantly decreased compared to the control (see **Fig. 3*D*** and **Table 2**).

Numerous PEGs of varying molecular weights are used as solvents and excipients for the delivery of a broad range of drugs. As is known, polymeric carriers improve the delivery of chemical compounds to the cell. Earlier, it was shown that a 2-amino-5-benzylthiazole derivative in a complex with a PEG-containing carrier (Popovych *et al.*, 2021) did not impair the effect of antineoplastic compounds on lymphoma cells, but caused the lymphoma cells death through apoptotic changes. As C. Yang, H.-Z. Liu and Z.-X. Fu (2012) showed, an antitumor compound oxaliplatin in conjugation with PEG-liposome complex induced a stronger apoptotic response than free oxaliplatin (Yang *et al.*, 2012).

The J. Maes *et al.* (2012) study focused on PEG-400, which was tolerated well by both embryos and larvae. All developmental stages exhibited no adverse effects up to at least 2.5%. Although embryotoxicity studies of PEG-400 and other polyethylene glycols have previously not been reported for zebrafish, mammalian studies have been carried out. For example, rats injected intraperitoneally for a total of three times during the gestation period with polyethylene glycol (PEG-400) at doses equivalent to 50% of the LD_{50} exhibited no increase in pre-implantation mortality. However, mild signs of embryotoxicity and generally retarded development were observed (Smyth *et al.*, 1950), which is partially consistent with our previously obtained data on the morphological development of loach embryos under the influence of PEG-carrier.

Based on our data and the original data by J. Maes and coleagues (2012) (solvents such as PEG-400, propylene glycol, and methanol were studied), PEG is an attractive polymeric carrier for the delivery of various compounds to both embryos and larvae, as well as other model objects. Although we investigated only two concentrations of the PEG-containing carrier, it is likely that these concentrations are biologically active in themselves and therefore could potentially influence the pharmacological or toxicological characteristics of the transported substances. In general, given the relatively small differences between concentrations that are apparently safe, further studies should be conducted to determine which cellular processes and signaling pathways are affected by newly synthesized carriers.

CONCLUSIONS

Based on the obtained data, PEG appears to be an attractive carrier to deliver compounds to both embryos and prelarvae. Low embryotoxicity of the PEG-containing carrier was confirmed on the studied model of loach embryos and prelarvae. Despite the fact that we investigated only two concentrations of the PEG-containing carrier, it is quite likely that these concentrations of the carrier are biologically active in themselves and, therefore, a thorough selection of the PEG-carrier concentration for each model-objective is needed.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: This article does not contain any studies with human subjects performed by any of the authors.

Animal Studies: All international, national, and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [M.B.]; methodology, [M.B.; S.M.; Ya.Sh.]; validation, [M.B.]; formal analysis, [L.B.; M.B].; investigation, [M.B.; O.H.; S.M.]; resources, [Ya.Sh.; M.B.; O.Z.]; data curation, [L.B.]; writing – original draft preparation, [M.B.; O.H.]; writing – review and editing, [M.B.; Ya.Sh.; L.B.]; visualization, [M.B.]; supervision, [M.B.]; project administration, [M.B.]; funding acquisition, [-].

All authors have read and agreed to the published version of the manuscript.

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АНАЛІЗ ВИЖИВАННЯ ТА МОРФОМЕТРИЧНІ ПАРАМЕТРИ ЕМБРІОНІВ І ПЕРЕДЛИЧИНОК В'ЮНА ЗА ДІЇ ПОЛІМЕРНОГО НАНОНОСІЯ ПОХІДНОГО ПОЛІЕТИЛЕНГЛІКОЛЮ

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Актуальність. Попередні наші дослідження продемонстрували незначну ембріотоксичну дію poly(VEP-co-GMA)-graft-mPEG носія на ембріони та личинки *Missgurnus fossilis* L. Водночас з'ясовано, що 3-денна експозиція 10^{-15} М полімерного носія сповільнює розвиток, і це супроводжується уповільненням вилуплення личинок. Проте в 10-денному досліді у личинок в'юна, які зазнали дії полімерного носія, аномалій і вад розвитку не виявлено. Мета роботи – зробити аналіз виживання зародків і передличинок та визначити морфометричні показники ембріонів в'юна за впливу ПЕГ-модифікованого полімерного носія.

Матеріали і методи. Овуляцію у самок в'юна (*Misgurnus fossilis* L.) стимулювали внутрішньом'язовим введенням самкам хоріогонічного гонадотропіну (500 од.), ікру одержували через 36 год після стимуляції, запліднювали в чашках Петрі суспензією спермієв за А. А. Нейфахом. Стадії розвитку контролювали візуально під бінокулярним мікроскопом МБС-9 з фотографічною приставкою в режимі реального часу. Експериментальні ембріони інкубували в розчині Гольтфретера з додаванням ПЕГ-вмісного носія до кінцевої концентрації 10 і 100 мкмоль/л.

Морфологічний розвиток груп ембріонів оцінювали за таблицями розвитку T. Fujimoto (2004), а морфологічні параметри (діаметр і площу ікри, бластомерів, зародків, зародкового мішка та зародкового горбика) вимірювали за допомогою комп'ютерних програм ImageJ та Photoshop (CC 2014v15).

Результати. Додавання до середовища 10 мкмоль/л ПЕГ-вмісного носія ініціювало позитивні достовірні зміни виживання зародків і передличинок в'юна, на відміну від впливу 100 мкмоль/л носія. Встановлено достовірне збільшення площі та діаметра бластомерів і самих ембріонів за впливу 100 мкмоль/л ПЕГ-носія. Це може свідчити про набряк зародків і порушення водно-сольового обміну, що стає причиною ранньої загибелі зародків.

Висновки. На досліджуваній моделі зародків в'юна (1) підтверджено низьку ембріотоксичність досліджуваного ПЕГ-полімеру. Виходячи з отриманих даних і вихідних даних J. Maes та співавт. (2), ПЕГ є привабливим полімерним носієм для доставки різноманітих сполук як до ембріонів і личинок, так і до інших модельних об'єктів. Незважаючи на те, що ми досліджували тільки дві концентрації ПЕГ-вмісного носія, цілком імовірно, що такі концентрації цієї речовини є біологічно активними самі по собі (3), тому виникає потреба в детальному підборі концентрації ПЕГ-носія для кожного модельного об'єкта.

Ключові слова: в'юн, зародки, передличинка, поліетиленгліколь, полімерний носій, морфометричні параметри

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