研究简报

EFFECTS OF SIMULATED MICROGRAVITY ON FERTILIZATION AND EMBRYONIC DEVELOPMENT OF *MISGURNUS ANGUILLICAUDATUS*

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模拟微重力对泥鳅体外受精和胚胎发育的影响

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Researches with aquatic organisms under space conditions became more and more important. The development towards the controlled ecological life supported systems(CELSS) requires understanding of embryogenesis, ecophysiology and ecology in general. Loach is a small animal, adaptable to environment, and its development is rapid, providing the possibility of having all the developmental process during the relative short times of space flight. Therefore, it has been regarded as a biological system by which we could better understand the developmental principles. Its embryonic development had been described detailedly[1,2].

In this study, we examined the effects of simulated microgravity on fertilization in vitro and different stages of embryonic development in a horizontally rotating clinostat.

1 Materials and methods

Experiments were divided into three groups. In the first group, the just fertilized eggs were immediately placed into clinostat. Blastodisc stage was considered fertilized to observe the effects of microgravity on fertilization *in vitro*. The second group fertilized and developed into gastrula in microgravity, then removed into 1g condition to continue the further development, examining the effects of microgravity on early loach embryogenesis. The third group was maintained in clinostat until larvae hatching, which aimed at the effects of microgravity on whole embryonic development.

Experiments were carried out on a horizontally rotating clinostat at 28±1℃, with a light cycle of 12h light / 12h dark. Microgravity (μg) was or less than 10^{-3}g. Each group had a control of 1g.

In order to determine whether there is a difference between the means of two groups, Chi-square analysis ($P < 0.05$) was used.

2 Results

2.1 Fertilization *in vitro* in microgravity

The results were summarized in Table 1. Fertilization was achieved at high frequencies in both 1g condition (83%) and microgravity (82%). We detected no effects of microgravity on normal fertilization *in vitro*.

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Tab.1 effects of microgravity on fertilization of loach

Treatment	No. of eggs	No.(%) of fertilized eggs
1g	121	100(83)
μg	110	90(82)

Note: $P > 0.05$, not statistically significant for both comparisons.

2.2 Development of early embryos fertilized in microgravity

Under 1g control, hatchability was 88%, and normality of larvae was 89%, and 90%, 90% in clinostat-treated embryos, respectively. After Chi-square analysis, there were no statistically significant differences in hatchability and normality in each condition (Table 2).

Tab. 2 Developmental potential of early embryo fertilized in microgravity

Treatment	No. of gastrulas	Hatchability	Normality of larvae
1g	100	88(88%)	78(89%)
μg	95	86(90%)	77(90%)

Note: $P > 0.05$, not statistically significant for both comparisons.

2.3 Embryonic development in microgravity

The third group results showed the hatchability and normality in microgravity were higher compared with those in 1g condition (Table 3). The results consist with that of other animal experiments[3]. Comparing some characteristics during embryonic development indicated that, no differences between in microgravity and under 1g condition.

However, we noticed that embryonic development in microgravity was retarded. Hatching time of the majority (HMT) delayed 5 hours (Table 3).

Tab. 3 Embryonic developmental potential of loach in microgravity

Treatment	No. of eggs	Hatchability	Normality of larvae	HMT(h)
1g	100	90%	89%	26
μg	100	85%	86%	31

Note: $P > 0.05$, not statistically significant for both comparisons.

3 Discussion

Loach, characterized by rapid development, lower requirement of development conditions and easy availability, is a suitable material for embryo experiment. It is unlikely that space aquaculture will actually be used to provide food aboard a spaceflight in the near future, but the accumulating of the need theoretic information is worth pursuing now, to prepare for later space endeavors.

In this study, we examined the effect of microgravity (provided by clinostat) on loach fertilization and early embryogenesis *in vitro*. The first group results indicated that no effects of microgravity on loach fertilization *in vitro*. Fertilization is the critical stage of embryonic development. Souza *et al.*[3] examined the effect of microgravity on amphilian development in the space environment and demonstrated that eggs could be fertilized *in vitro*. Japanese Medaka fish could mate and ovulate in space[4] The microgravity environment did not appear to have a significant impact on mammalian fertilization in vitro[5] We had similar results. A large number of experiments, from lower to higher animals and from simulated microgravity to space flight, demonstrated that fertilization is independent on gravity.

Nishiwaki *et al.*[6] and Ijiri[4] reported that Medaka's embryo developed normally in 3D clinostat and in space, respectively. In 3D clinostat, lamination of retina occurred almost at equal timing, and opsin genes in 3D-clinostat-treated group also express almost at the same time as control. For amphibian embryos, the early embryonic stages showed some abnormalities, but the embryos were able to regulate and produce nearly normal larvae. We examined the effects of exposure to microgravity on early loach embryonic development. Gastrulas fertilized and developed in microgravity could develop in 1g condition with high developmental potential (e.g. hatchability and normality see Table 2). Embryos fertilized in microgravity could develop normally into living-free larvae too. Some characteristic during embryogenesis also appeared at the same stages as control. The larvae developed in microgravity showed no difference in behavior and morphology.

Few researches reported the delay of embryonic development in microgravity. Marco *et al*[7]. reported drosophila's embryos in space developed more slowly than in 1g condition, and this influence was related to flight time. In our experiments, embryogenesis of loach delayed two stages compared with the control. Lymphocytes, cultivated under simulated microgravity in clinostat and in space flight, expressed reduction of proliferation[8]. One reason for the changes in proliferation may be that microgravity seems to affect the expression of cytokines and intracellular signaling mechanisms at the single-cell level[8,9]. To a whole organism, the same may apply to embryonic development.

Our experiments indicate loach fertilization and embryonic development *in vitro could* complete under micro-

gravity. Therefore, we believe that gravity is not necessary for fertilization and development. It means that aquatic animals which are already adapted to low gravity conditions, may be used to enhance the functionality of the controlled ecological life supported system (CELSS) as a whole.

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