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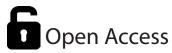
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How the Minuscule Zebrafish Influence on Biomedical Research

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Sangeetha S.^{1*}, Jayasheela J.¹ and Leonal Rabins²

¹Dept. of Pharmacology, Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry (605 502), India ²ICAR-KVK, Tenkasi, Tamil Nadu (627 852), India



Corresponding Author

Sangeetha S. e-mail: sangee1029@gmail.com

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E-mail: bioticapublications@gmail.com



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Abstract

he past few decades, the Zebrafish (Danio rerio) has become an exponentially used animal model for biomedical research. Animal experimentation studies using animal models play a vital role in upcoming research. Though, some animal models have been restored by alternative method of approach, still studies need animal models for development of science that yields better results to apprehend the particular research progress without any risk to the human beings. On the contrary, the research costs carried out in the mammals are high, compared to small animal models. Nevertheless, finding of cost-effective new animal models with the consideration of animal welfare is important. This led to required and management of invertebrates and fishes becomes necessary recently. As a result, the uses of Zebrafish increased extensively as an alternative experimental animal model in biomedical research studies.

Introduction

ebrafish (Danio rerio) is a small tropical freshwater fish inhabitant of river Ganges of Himalayan region of South Asia particularly in India. It's coming under a bony fish (teleost) belongs to the family Cyprinidae under the class Actinopterygii (ray finned fishes). The Zebrafish was first used as a biological animal model by George streisinger (university of orgeon) in the early 70's. Zebrafish has the unique physiological and genetic similarities with mammals especially humans of the brain, digestive tract, musculature, vasculature and innate immune system. Also 70% of human disease genes have physiological and functional similarities with Zebrafish (Figure 1).

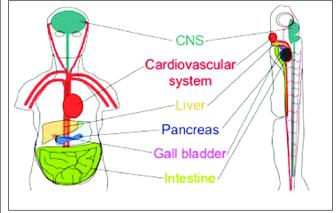


Figure 1: Some of the conserved organ systems between Zebrafish and humans

Physical Characteristics

ebrafish are less than 5 cm in length with the stripes on the side of the body, which extend to the anal fin and onto the caudal fin rays of the tail. Five alternating blueblack stripes of two types of pigment cells, melanophores and iridiophores, and silvery-yellow stripes contain xanthophores and iridophores. Zebrafish exhibit a circadian pattern of day time activity and night time sleep a state which is said to have important similarities with sleep pattern in mammals. They have a maximal recorded life span of 5½ years though an average of 3½ years has been recorded. Many pain related studies have been used Zebrafish as an animal model. Handling and management of Zebrafish requires minimal activity involving less effort than other animal models.

Feeding

Zebrafish can be fed using commercially available dry feed (size range from 100 microns for larvae to 300/400 microns for adult fish) or live feed (brine shrimps). Feed packets available should autoclave or may be undergoing heat treatment to safeguard from outside contaminations. The moisture level needs to be monitored for the fungal contaminations. The amount of feed dispensed at the ratio of 4% of body weight per day not to be overfed as this may increase the nitrate level in the water, which may affect their breeding and viability.

Breeding

reeding play's significant role in order to study the various characteristics of the organism as it develops from embryo to adulthood. Zebrafish scatter non adhesive eggs on the plants and bare substrates but when provided with an artificial spawning site such as the aquarium Zebrafish can be bred easily. Few precautions are needed to protect the eggs for success breeding since the breeders may revert to eating their own eggs. A very common method of breeding Zebrafish is to take a bare fish aquarium of 5 or 10 gallons and put a 2-inch-deep layer of marbles on the bottom. Fill the tank with dechlorinated water only to 1 inch over the marbles. This gives the parents a shallow layer of water for swimming, and the eggs easily fall between the marbles, which prohibit by eating of hungry adults. Another alternative method of breeding zebra fish is to hang a breeder net in a small empty aquarium. Get one with a coarse mesh which allows eggs can fall down at the bottom of the tank.

Preparation of Aquarium Tank Environment

Creation of artificial spawning site with suitable environment parameters is essential for successful Zebrafish breeding.

Parameters to be Maintained for the Breeding

- PH maintained between 6.2 and 7.5.
- Water between 23 and 28 degrees Celsius.
- 14-hour lighting and 10-hour darkness cycle allows the fish to breed at dawn.

• Ultraviolet sterilizing unit.

• Activated carbon filters - remove contaminants and small elements.

- Biological filters remove organic waste.
- Mechanical filters that remove large debris.

Fill the aquarium with fresh dechlorinated water and check the water temperature, pH levels and now set to introduce the breeding stock. Two males for each female (2:1) considered best for successful breeding. Males are slimmer than females, and their colors are often slightly deeper. Set down the Zebrafish into the breeder net. When the fish spawn, the eggs will fall through the net and rest on the aquarium bottom. Generally, the fish spawn the following early morning. Remove the adults and the breeder net addendum. The eggs hatching will takes place in approximately one and a half to two days, but the fry won't become free-swimming for another two days. Avoid feeding the fry until they are all up and start swimming. When kept under optimal conditions the Zebrafish can lay about 200 eggs per week. Under laboratory conditions the Zebrafish spawn throughout the year with surplus availability of off springs.

Salient Features of Zebrafish as an Animal Model

Zebrafish is preferred by many research scientists because of their variety of features that makes it useful as an animal model. Acclimatization of Zebrafish is very compatible than mammals, which smoothen the scientific research. One of the significant, features of Zebrafish embryo is developed outside the mother body and is optically transparent and thus, it is easily accessible for experimentation and observation.

The embryo develops very fast and the life cycle stages completes within 3 days. The blastula stage lasts for 3 hr, while the gastrulation gets completed in 5 hr and the 18 hr old embryo stage has very well developed ears, eyes, segmenting muscles and brain can be viewed as the embryo is transparent. By 72 hr, the embryo hatches out from the egg shell and within next 2 days the Zebrafish fry starts taking feed. In just, couple of 4 days the embryo converts rapidly into a small version of adult and this allows the researchers to study the developmental stages from embryo to adult life. In addition to this features, the adult fish attains sexual maturity very quickly having a generation time of about 10 weeks with high fecundity rate. This features considered the zebra fish has an excellent animal model for developmental and toxicological studies on drug screening. Thus, using the Zebrafish as an animal model, it is possible to replace and reduce the use of mammals in research as well as rising mitigate problems related to the welfare of those animals.

Research and Documentation of Zebrafish as an Animal Model

• There are several good examples where Zebrafish used as a good animal model of human diseases such as Duchenne muscular dystrophy, human melanoma, acute lymphoblastic leukemia, polycystic kidney disease, nephronophthisis, acute kidney injury, Parkinson's disease, Huntington's disease, Alzheimer disease and myocardial infarction.

• Investigated the effect of different toxins, alcohol and different levels of carbohydrate or fat diets on Zebrafish embryos, larvae and adult developmental stages. Application of these substances to the fish tank is a simple technique to study the drug screening.

• Zebrafish was first used as an animal model in cancer research study in the early 60's. They have a very low rate of spontaneous neoplasia, with only 10% of developing a tumor over their life time unless they are exposed to carcinogenic agents.

• Furthermore, Zebrafish has proven to be an ideal animal model to study the malignancy of several tumors by means of tumor transplantation assays. In this regard, the Zebrafish tumor model can also mimic tumor development in the human body.

• Critical pathways that control development in vertebrates are highly conserved between human and Zebrafish. The Zebrafish genome shares lot of similarities with human genome and about 70% of genes associated with disease in humans has functional homology with Zebrafish.

• Important role in the study of hematopoiesis because these fishes have the same sequential multilineage hematopoiesis process as human beings (Paik and Zon, 2010).

• Zebrafish has been used extensively to study the cardiovascular disorders since they have a similar embryonic heart structure as that of human embryos.

• Another, important research area is to understand the neurological disorders. The use of Zebrafish as an animal model in this study because they have the same signaling proteins and neurological disorders equivalent to the brains of human beings (Kalueff *et al.*, 2014).

• Zebrafish has an excellent animal model to study the metabolic disorders. The main cause for development of diabetes mellitus is to failure of pancreatic β -cells to produce insulin, which leads to insulin deficiency (Zhang *et al.*, 2017). These functions and processes are conserved between Zebrafish and humans.

Conclusion

t has been concluded that, the use of Zebrafish has an excellent animal model in biomedical research studies since, they share about 70% of genes associated with disease and have functional & genetic homology with human beings. On the other hand, it is very easy to maintain under laboratory conditions because of its low cost, transparent embryo, easy manipulation, high fecundity and rapid embryonic development makes researchers to study from embryo stage to adult life stage. The Zebrafish features are very similar with human beings which make more comfortable for the researchers to carry out their research work. With the objective of usage of mammals shall be minimized, when the researchers majorly prefer Zebrafish as an animal model. Finally, welfare of the animals also considered as per the CPCSEA guidelines.

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