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Introductory Chapter: Importance of Zebrafish (*Danio rerio*) as Model Organism in Biomedical Research

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1. Introduction

Animal experimentation has an important role in scientific research. Although some models have been replaced by alternative methods, scientific research still needs animal models for development, reliability, and legitimacy of science [1].

From this point of view, biomedical research relies on using of animal models in order to understand a particular disease without causing risk to the human being [2]. On the other hand, as it is known, research costs are high especially carried out with mammals. Therefore, a new model of animal experiments, including invertebrates and fish species, becomes necessary recently. Thus, as a result of searching new experimental models, in order to reduce cost and save time, the zebrafish was discovered.

As a result, using of zebrafish (*Danio rerio*), which is one of the tropical freshwater fish species, has increased its importance as an experimental animal model in the field of biomedical research in recent years.

2. The zebrafish (*Danio rerio*)

The zebrafish belongs to the *Cyprinidae* family, which consists of more than 2000 species [3]. Its former scientific name was *Brachydanio rerio*, but it was changed to *Danio rerio* in 1981 [4], because both genera were very similar by having short dorsal fins and absent or incomplete lateral lines [5].

Zebrafish is a small tropical freshwater fish which lives in river basins of India, Northern Pakistan, Nepal, Bhutan, and South Asia. Their maturation period takes only 2–3 months, which is relatively less laborious and time-saving for generating of transgenic lines. Zebrafish can produce 200–300 fertilized eggs weekly and complete the embryogenesis in 72 h. Pigmentation in the embryos starts about 30–72 h postfertilization [6].

Its adults are about 2.5–4 cm long, and its larval stage is transparent. On the other hand, when it has reached the adult stage, it develops a stripe along with the length of the body, and it looks blue in color. Males are slender and torpedo-shaped, usually with a pink or yellow color.

3. Advantages of zebrafish usage in biomedical research

Although zebrafish (*Danio rerio*) is a primitive vertebrate, it has several advantages over other model organisms. The main reasons for it becoming an excellent model organism in different research fields are easy access to all stages of its body development, transparency of its embryos and larvae, high genetic similarity, and homologous physiology with human beings especially in terms of the central nervous system.

Besides these, adults and embryos of this species are small in size and have a lower cost, and the generation interval is short. Furthermore, they show rapid development and hatches in less than 3 days and become mature in 90 days which the search more rapid [7].

Additionally, microinjection of fertilized eggs is easily accessible and relatively cheap. The embryos of zebrafish develop outside the mother's body and are transparent. The transparency of the zebrafish embryo facilitates the studies in genetic development programs, because it is possible to monitor and manipulate its development without difficulties. Interestingly, the morphology of the brain of mammals and zebrafish is similar, including macro-organization of the brain [7].

Furthermore, their genome sizes are approximately 20–40% of the mammalian genome, and it makes them the only vertebrates available for large-scale mutagenesis. In addition, many routine techniques of molecular biology and genetics, including knock-in, knockdown, and knockout, are well developed in the zebrafish [8].

4. Studies carried out with zebrafish in biomedical research

4.1 Hematopoetical research

Zebrafish play an important role in the study of hematopoiesis. Because these fishes have the same sequential multilineage hematopoiesis process as human beings, these model organisms provide many insights, both in blood lineage development pathways and blood disorders, to the scientists who are studying in the field of medicine [9].

4.2 Cardiovascular research

These fishes are also largely used to explore cardiovascular disorders since they have a similar embryonic heart structure as that of human embryos. Furthermore, zebrafish have the advantage of being able to survive without adequate cardiac circulation. The excellent feature helps the embryo which develops from the initial phases despite cardiovascular defects. For instance, one of the patterns of their use within this field is exploration of the link between inflammation and myocardial infarction [9].

4.3 Cryobiological research

The small size and good fecundity of the zebrafish make it suitable for the cryobiological and genetic studies. However, limited animal facility space and the need to maintain broodstock lines are important restraining factors for the researches carried out with zebrafish [10, 11].

Cryopreservation is the process of freezing the biological material at a temperature of liquid nitrogen (LN₂) (−196°C). This means biological activities discontinue including the biochemical reactions creating cell death and DNA damage at these

low temperatures [12]. In this way, it is possible to store the biological materials unchanged for centuries with the capability of recovering the cell functionality following the thawing process [13].

From this point of view, efficient sperm cryopreservation procedure can help overcome these problems by reducing the number of live fish in a system while maintaining their reproductive capacity [12, 14]. Sperm cryopreservation also provides “genetic insurance” for the recovery of strains in case the living stocks are lost [15, 16] and extends the functional reproductive lifetime of males as long as samples maintain viable in storage [17]. Finally, the technique has been utilized in reverse-genetic mutagenesis approaches, in which a cryopreserved sperm library is used to recover heterozygote mutant fish of interest [18]. Thus, an optimized zebrafish sperm cryopreservation protocol will not only increase the efficacy of this genetic screening method but also benefit the zebrafish community as a whole.

4.4 Neurological research

Another important research field is the curing of neurological disorders with the zebrafish because of the abundance of the same signaling proteins in the brains of human beings. It is also known that several human neurological disorders have also an equivalent in the zebrafish [7].

4.5 Aquaculture research

Zebrafish is also one of the genetically more malleable aquatic species among different fish species in aquaculture. The zebrafish model is used commercially in many areas of aquaculture such as in the identification of genes involved in the development of the muscles, bones, and fats, metabolism of the nutrients, disease and stress pathways, and also behavioral traits.

The drugs affecting the physiology of the fishes can easily be tested in zebrafish especially in terms of their effect on a range of alleles in order to determine their genetic property [19]. Additionally, many researches have been done regarding improvement of diet and their husbandry to improve growth rate and reduce stress and disease in many fish species.

5. Conclusion

It can be concluded that zebrafish is a successful and versatile animal, offering a tool to model regarding gene function, development of various organ systems, cancer studies, toxicology, drug discovery, human disease and disorders, and also aquaculture.

The usefulness of zebrafish has excelled in biomedical research because of its low cost and easy maintenance, transparent embryo, easy manipulation, high fecundity, and rapid embryonic development.

From this point of view, the future of zebrafish as a model organism is very bright. In the coming years, an increased number of reports are expected on the application of zebrafish as an effective bioindicator. On the other hand, there is still much to discover about this species, and also it is necessary to put more efforts so that new information can flow to the understanding of biomedical research combined with the use of zebrafish.

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