

Establishment of an Experimental Aquatic Vertebrate Animal Laboratory within the Framework of Current Legislation: The Case of the Ankara University Faculty of Veterinary Medicine, Türkiye

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ABSTRACT

The necessity to develop alternative methods to reduce experiments on mammals in line with the 4R principle has brought aquatic animals to the fore due to their advantages. Vertebrate and invertebrate aquatic animals are now widely used in experimental studies covering many disciplines, including drug screening and development, preclinical trials, and research into toxicology, oncology, metabolism, the cardiovascular system, the kidneys, the immune system, dermatology, endocrinology, ophthalmology, genetics, infectious diseases and aging. As their use has increased, aquatic experimental animal units have been established around the world, including in Türkiye, and their number is growing daily. In this context, international guidelines containing basic information, recommendations and policies on environmental control for aquatic animals used in experimental studies for scientific purposes have been published. This review aims to provide information on the establishment steps, objectives and basic processes of the zebrafish unit at Ankara University Faculty of Veterinary Medicine's Aquatic Experimental Animal Centre (ADHAM), in line with current national and international guidelines, recommendations, policies and legislation.

INTRODUCTION

Driven by advancing ethical imperatives and aligned with the principles of the 4Rs (Replacement, Reduction, Refinement, and Responsibility), aquatic species have increasingly been recognized as valuable alternatives to traditional mammalian models in biomedical research. Their numerous advantages, including external fertilization, transparent embryonic development, and high genetic and physiological relevance, have positioned both vertebrate and invertebrate aquatic organisms, particularly fish, as powerful tools across a wide range of experimental domains. These include drug discovery and high-throughput screening, preclinical safety and efficacy testing, as well as research in toxicology, oncology, metabolism, cardiovascular and renal function, immunology, dermatology, endocrinology, ophthalmology, genetics, infectious disease, and aging biology (Kiani et al., 2022).

In Türkiye, the use of aquatic vertebrates in scientific research is governed by a comprehensive legislative

framework established by the Ministry of Agriculture and Forestry. This framework includes the 2019 Regulation on the Welfare and Protection of Aquatic Vertebrates Used for Scientific Purposes and its 2022 amendment. These regulations articulate detailed standards concerning the infrastructure and operation of housing and care facilities, the authorization process for research institutions, procedural and ethical use of aquatic vertebrates, qualifications and responsibilities of personnel, systematic record-keeping, and overarching animal welfare and bioethical considerations. Within the scope of this legislation, aquatic vertebrates are defined as vertebrate species such as fish (*Pisces*), amphibians (*Amphibia*), and aquatic or semi-aquatic reptiles that must inhabit aquatic environments during all or part of their life cycle, excluding cetaceans (*Cetacea*). In addition to national regulations, internationally recognized guidelines provide essential directives on environmental parameters including water source and quality, temperature regulation, photoperiod control, noise and vibration levels, and the

operation of life-support and alarm systems. They also offer comprehensive recommendations on animal husbandry practices such as health monitoring, housing design, nutrition, sanitation, transportation, humane euthanasia, individual identification, and detailed documentation. The growing recognition of aquatic models as ethically and scientifically advantageous has led to a marked increase in the establishment of specialized research facilities, particularly zebrafish units, across universities, governmental institutions, and private research centers both in Türkiye and worldwide (OECD, 2002; CCAC, 2005; EC, 2007; FELASA, 2007; OECD, 2009; EC, 2010; NRC, 2011; OECD, 2015; The Ministry of Agriculture and Forestry of the Republic of Türkiye, 2019; CCAC, 2020; CCAC, 2021; The Ministry of Agriculture and Forestry of the Republic of Türkiye, 2022; OECD, 2023; Yipel and Altinok-Yipel, 2025).

Aquatic experimental animal facilities are specialized research infrastructures established and managed in strict accordance with national legislation and internationally recognized guidelines. These units operate within comprehensive regulatory frameworks that encompass animal welfare, scientific rigor, research ethics, and occupational health and safety standards. All procedures involving aquatic vertebrates, including reproductive management, controlled husbandry, environmental monitoring, and their utilization in ecological, physiological, and translational biomedical studies, are conducted by highly trained professionals with advanced expertise in veterinary science and laboratory animal care. The implementation of evidence-based practices ensures both the ethical treatment of research animals and the generation of scientifically valid and reproducible data (Yipel and Altinok-Yipel, 2025).

Zebrafish (*Danio rerio*) have emerged as a widely adopted vertebrate model in numerous stages of the drug discovery and development pipeline, including disease modeling, target identification and validation, pharmacological safety assessment, toxicological profiling, and high-throughput compound screening. Their increasing utilization in biomedical research is largely attributed to their significant genetic, molecular, morphological, and pathological homology with humans. From a veterinary and laboratory animal science perspective, zebrafish offer multiple logistical and scientific advantages. These include their small body size, ease of husbandry, low maintenance costs, expansive and well-characterized genomic resources, absence of ethical constraints in early embryonic experimentation, optical transparency during embryonic and larval stages, and prolific reproductive capacity. Moreover, the growing integration of zebrafish into research protocols aligns with the principles of replacement, reduction, and refinement, which are increasingly mandated by both national and international regulatory frameworks. Compared to traditional rodent models, zebrafish provide a more time-efficient and cost-effective alternative, without compromising translational relevance in preclinical investigations (Yipel, 2020; Yipel and İlhan, 2021; Habjan et al., 2024; Hughes and Hessel, 2024).

This review outlines the conceptualization, establishment, and strategic development of an Aquatic Experimental Animal Facility, using the Aquatic Experimental Animal Center (ADHAM) of Ankara University Faculty of Veterinary Medicine as a case model. The discussion is grounded in up-to-date guidelines, policy documents, and regulatory frameworks issued by both national and international competent

authorities and reference institutions. The critical phases of facility establishment are examined in detail, encompassing physical infrastructure design, comprehensive budget planning, formation of a multidisciplinary research team, acquisition and installation of specialized equipment, navigation of formal application procedures and timelines, procurement of institutional and legal authorizations, transfer and quarantine protocols for initial aquatic specimens, implementation of breeding programs, standardization of husbandry and nutritional regimens, and the development and validation of relevant experimental models. The review further delineates clearly defined institutional goals across temporal categories, including short-term (6 months to 1 year), medium-term (1 to 3 years), and long-term (more than 3 years) objectives. Core procedural workflows specific to zebrafish research units are also described, highlighting key operational standards that align with contemporary veterinary research practices and ethical governance.

Establishment and Operational Licensing Application and Institutional Recognition of the Laboratory

In alignment with applicable regulatory frameworks, the laboratory's foundational infrastructure and aquatic rearing systems were established as illustrated in Figures 1A through 1D. Following these preparatory steps, an official application for operational authorization was submitted to the Directorate of Fisheries and Aquatic Resources under the Ministry of Agriculture and Forestry of the Republic of Türkiye. This submission was made through the "Preliminary Permit Application Form for the Use of Aquatic Vertebrate Animals for Scientific Purposes," as mandated by national legislation. As part of the procedural sequence, a Preliminary Permit was first secured. Subsequently, all requisite documentation for the issuance of a full operational license was compiled in accordance with Article 14, titled "Work Permit," of the relevant regulation.



Figure 1. Infrastructure and compartments of the laboratory. A: Quarantine room, B: Care room, C: Breeding room, D: Examination and operation room.

The documentation included certified copies of academic diplomas for public officials and technical personnel designated to work within the facility, approved by the relevant institutional authority. In addition, an Emergency Action Plan was submitted, detailing the precautionary measures implemented against potential fire

and explosion risks. A comprehensive, authorized inventory was also provided, listing the technical specifications and capacities of all equipment intended for use in ensuring the health and welfare of aquatic vertebrate species, along with all chemical and biological agents to be employed within the facility.

IN THE COMPILED APPLICATION FILE FOR THE OPERATIONAL LICENSE, THE FOLLOWING COMPONENTS WERE INCLUDED IN ACCORDANCE WITH ARTICLE 14 OF THE RELEVANT REGULATION

Under Section 1, Clause (a)

The institutional director officially appointed the personnel designated to work within the facility. Additionally, in compliance with the regulation, a Welfare Unit was formally established within the laboratory. Written notifications of these assignments were issued to the respective personnel, and an official notification addressed to the Ankara Provincial Directorate of Animal Health was appended to the application dossier.

Under Clause (e)

In order to obtain official recognition as an authorized institution to conduct research involving aquatic vertebrates or genetically modified aquatic vertebrates, a formal application was submitted to the General Directorate of Agricultural Research and Policies (TAGEM). Both the official petition and the written response from TAGEM were included in the application file.

Upon completion of the required Standard Operating Procedures (SOPs), laboratory forms, and the laboratory layout plan (Figure 2), the full application for authorization was submitted to the Ankara Provincial Directorate of Agriculture and Forestry, under the authority of the Ankara Governorate.



Figure 2. Schematic overview of the Laboratory infrastructure. A: Quarantine room, B: Care room, C: Breeding room, D: Examination and operation room, E: Feed storage room, F: Disinfection area, G: Corridor, *: Doors.

Following the comprehensive evaluation of the submitted application dossier by the designated commission within the Directorate of Fisheries and Aquatic Products under the Ministry of Agriculture and Forestry of the Republic of Türkiye, an official on-site inspection of the facility was conducted in February 2023. As a result of this evaluation, the Aquatic Experimental Animal Research Center, established within Ankara University Faculty of Veterinary Medicine, was deemed compliant with regulatory standards and was formally approved for the issuance of an operational license.

Subsequently, the application fee mandated by the Directorate was transferred to the Ministry's revolving

fund account, and the finalized species list for which authorization was requested was submitted to the relevant administrative body. The signing process for the official Work Permit was completed in April 2023, and the permit was formally issued to the institution on April 17, 2023 (Figure 3).

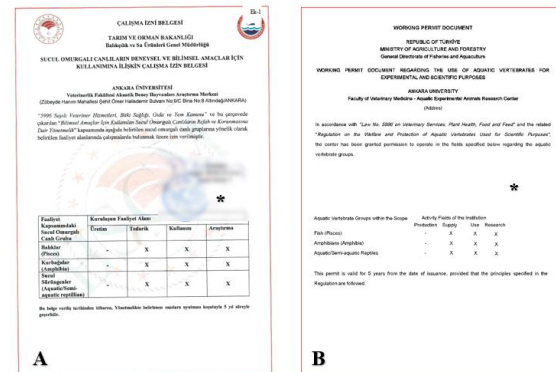


Figure 3. Approval Letter Issued by the General Directorate of Fisheries and Aquatic Products, Ministry of Agriculture and Forestry.

A: original version, B: translated version, * Stamp, date, and signature.

In March 2024, the Aquatic Experimental Animal Center underwent its first annual regulatory inspection, conducted by the Ankara Provincial Directorate of Animal Health. During the audit, a comprehensive overview of the Center's first-year activities was presented. All documentation required under the applicable legislation—including breeding records, health monitoring data, and routine quality control reports—was subjected to detailed examination. Based on the findings of this evaluation, it was determined that the institution remained in full compliance with regulatory expectations, and the continuation of its operational license was formally confirmed.

Zebrafish Reproductive and Embryological Research Unit Equipment

The unit is comprehensively outfitted with specialized infrastructure and high-precision laboratory instrumentation to support zebrafish husbandry and experimental workflows. Core equipment includes a recirculating aquatic housing and breeding system (ZebTEC, Italy), a reverse osmosis water purification unit (Purelab Chorus 2), and ultrapure and distilled water systems (Millipore, Darmstadt, Germany), which together ensure optimal water quality and biosecurity. Microscopy capabilities are supported by a Euromex laboratory microscope, while incubation and sterilization needs are met by a laboratory-grade incubator (Lab Companion), an autoclave sterilizer (Panasonic MLS-3020U), and a programmable temperature-controlled incubator (Jeio Tech IB-11E, Korea).

Precision weighing and volumetric measurement are facilitated through a high-sensitivity analytical balance (Sartorius) and a complete set of adjustable-volume automatic micropipettes (Dlab, Omnipipette). Environmental control within the unit is maintained via a laboratory refrigerator (Panasonic), programmable lighting arrays (Philips), and an automated photoperiod timer (CATA), enabling regulation of circadian cues essential for zebrafish reproduction and embryogenesis. Additionally, a dedicated Artemia hatching system (CE-

PLAN Artemia Hatching Kit) is utilized for the production of live feed critical to larval rearing.

Chemicals

The unit is routinely supplied with high-purity, analytical-grade chemical reagents essential for zebrafish husbandry and experimental protocols. These include sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, CAS No. 10039-32-4), potassium dihydrogen orthophosphate (KH_2PO_4 , CAS No. 7778-77-0), calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, Product No. 12135), magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, CAS No. 10034-99-8), and sodium bicarbonate (NaHCO_3 , CAS No. 144-55-8), which are primarily used in the preparation and stabilization of aquatic media. To ensure accurate pH regulation and buffer formulation, a 1.0N sodium hydroxide solution (NaOH , Product Code: 145057, Reference: MB/26/616/M-552-05/6/16) is utilized. Additional reagents include sodium chloride (NaCl , CAS No. 7647-14-5) and potassium chloride (KCl , CAS No. 7447), both of which are essential for maintaining ionic balance in the aquatic environment. For toxicological and pharmacological studies, compounds such as 3,4-dichloroaniline (CAS No. 95-76-1) and dimethyl sulfoxide (DMSO, CAS No. 67-68-5) are employed as standard toxicants and solvent controls, respectively.

Procurement, Laboratory Transfer, and Quarantine of Adult Zebrafish Broodstock

Wild-type adult AB strain zebrafish, sourced from the Hacettepe University Laboratory Animal Research and Application Center (HÜDHAM), were transferred to the Aquatic Experimental Animal Research Center (ADHAM) of Ankara University Faculty of Veterinary Medicine, which had been established and officially licensed for scientific use under the project framework. In accordance with regulatory requirements, the animals were maintained under quarantine conditions for a period of 21 days. Upon successful completion of the quarantine, the broodstock were introduced into the automated recirculating zebrafish housing system (ZEBTEC, Italy) for acclimatization and continued use in experimental breeding protocols (Figure 4).



Figure 4. Automated zebrafish breeding system and parts (A: Breeding system, B: Control panel, C: Filtration system).

Zebrafish Facility Design, Tank Specifications, Environmental Conditions, Water Quality Monitoring, and Routine Husbandry Protocols

Adult zebrafish were housed in tanks with capacities ranging from 3.5 to 8 liters, depending on the number of individuals per group. Fish were grouped based on age to ensure uniform developmental stages within each tank. Photoperiod regulation essential for maintaining physiological rhythms was achieved through a lighting

system set to 14 hours of light and 10 hours of darkness within the facility.

The ambient temperature was maintained at $28 \pm 1^\circ\text{C}$, with humidity stabilized at 61%, both controlled through an automated environmental system. The water pH was adjusted and stabilized within the range of 6.9 to 7.2. Water quality was preserved using an integrated filtration system comprising both mechanical and ultraviolet (UV) components.

In accordance with the manufacturer's recommended schedule automatic system sensor checks and tank cleaning were carried out routinely. Key environmental parameters, including pH, water temperature, conductivity, and ambient room temperature, were monitored daily and documented using a standardized recording form (Figure 5).

[illegible]

Figure 5. Water Parameter Monitoring Form.

Feeding Procedures

Feeding practices were implemented in accordance with the developmental stages of the zebrafish. Feed quantities were adjusted to ensure complete consumption within five minutes, thereby reducing waste and maintaining water quality. Adult fish were provided with a daily ration consisting of one feeding of dry feed and one feeding of live *Artemia nauplii* (brine shrimp). Juveniles, defined as individuals aged 15 days post-fertilization or older, were fed twice daily with dry feed and once daily with live food. Larvae received three feedings of dry feed and one feeding of *Artemia nauplii* per day (Figure 6).



Figure 6. Zebrafish nutrition by live and commercial feeds in the facility.

Embryos between 0 and 6 days post-fertilization were not externally fed, as they subsisted on endogenous yolk sac reserves. During this period, nutritional intake and

development were monitored without exogenous feeding (Table 1).

Table 1. Weekly Feeding Schedule for Zebrafish

Day	Larval Feeding Time and Type				Juvenile Feeding Time and Type				Adult Feeding Time and Type			
Monday	10:00	-	Otohime	A1	10:00	-	Otohime	B1	10:00	-	Otohime	B2
	12:00	-	Otohime	A1	12:00	-	Otohime	B1	15:00	-	Artemia	
	14:00	-	Otohime	A1	15:00	-	Artemia					
	15:00	-	Artemia									
Tuesday	10:00	-	Otohime	A1	10:00	-	Otohime	B1	10:00	-	Otohime	B2
	12:00	-	Otohime	A1	12:00	-	Otohime	B1	15:00	-	Artemia	
	14:00	-	Otohime	A1	15:00	-	Artemia					
	15:00	-	Artemia									
Wednesday	10:00	-	Otohime	A1	10:00	-	Otohime	B1	10:00	-	Otohime	B2
	12:00	-	Otohime	A1	12:00	-	Otohime	B1	15:00	-	Artemia	
	14:00	-	Otohime	A1	15:00	-	Artemia					
	15:00	-	Artemia									
Thursday	10:00	-	Otohime	A1	10:00	-	Otohime	B1	10:00	-	Otohime	B2
	12:00	-	Otohime	A1	12:00	-	Otohime	B1	15:00	-	Artemia	
	14:00	-	Otohime	A1	15:00	-	Artemia					
	15:00	-	Artemia									
Friday	10:00	-	Otohime	A1	10:00	-	Otohime	B1	10:00	-	Otohime	B2
	12:00	-	Otohime	A1	12:00	-	Otohime	B1	15:00	-	Artemia	
	14:00	-	Otohime	A1	15:00	-	Artemia					
	15:00	-	Artemia									
Saturday	11:00	-	Otohime	A1	11:00	-	Otohime	B1	11:00	-	Otohime	B2
	14:00	-	Otohime	A1	14:00	-	Otohime	B1	14:00	-	Otohime	B2
Sunday	11:00	-	Otohime	A1	11:00	-	Otohime	B1	11:00	-	Otohime	B2
	14:00	-	Otohime	A1	14:00	-	Otohime	B1	14:00	-	Otohime	B2

Artemia Culture and Live Feed Preparation

Live feed in the form of *Artemia nauplii* was cultured daily by the research team to ensure optimal nutritional quality and bioavailability for zebrafish. Commercially sourced dormant *Artemia* cysts (Salt Lake Aquafeed Premium *Artemia* Cysts) were introduced into a conical hatching vessel containing saline water adjusted to a salinity of 14‰ (Figure 7A). The cysts were incubated under continuous aeration and constant illumination for 24 to 36 hours, in accordance with established hatching protocols (Figure 7B).

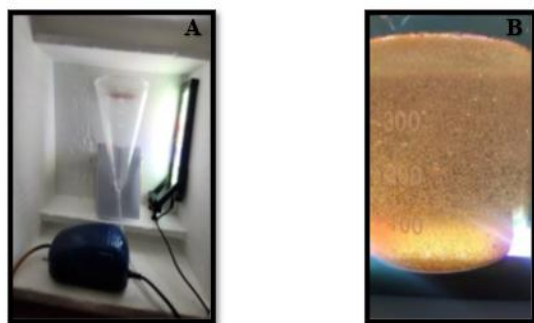


Figure 7. Cultivation of *Artemia nauplii*.
(A: *Artemia* separation funnel, B: *Artemia* larvae under illumination).

Upon completion of the incubation period, both unhatched cysts and newly hatched nauplii were collected through the bottom outlet of the separation funnel. The harvested material was passed through a fine mesh to remove excess salinity and debris. The nauplii were then resuspended in clean, oxygenated tank water to maintain viability and prevent osmotic stress. Hatched nauplii, which accumulated at the water surface, were gently siphoned and administered directly to the zebrafish as live feed.

Unhatched cysts were rinsed and returned to a freshly prepared hatching vessel for continued incubation. This procedure ensured a continuous supply of high-quality live feed. Feeding was performed daily in alignment with the stage-specific nutritional regimen outlined in the zebrafish feeding schedule.

Reproductive Classification Based on Morphological Traits

Phenotypic differentiation between male and female zebrafish (*Danio rerio*) was carried out by experienced personnel using established external morphological markers. Individuals with a slender, streamlined body shape and a yellow to golden hue were classified as males. In contrast, individuals exhibiting an enlarged abdominal profile-indicative of oocyte presence-and a prominent silvery-blue lateral stripe were classified as females. These visually distinguishable traits, representing clear sexual dimorphism, allowed for accurate non-invasive reproductive classification (Figure 8).

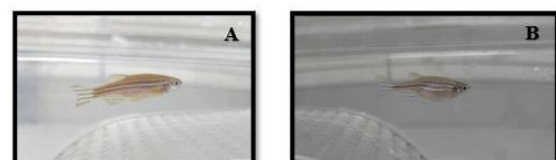


Figure 8. Adult Zebrafish.
A: Male, B: Female.

Breeding and Embryo Collection

Breeding was carried out using specially designed spawning tanks sourced from the manufacturer, optimized for egg collection and mating behavior. Adult males and females (6–12 months old) were placed in breeding tanks at a ratio of 1:2 or 5:8, depending on broodstock availability. The sexes were initially separated within the

tanks using dividers. Fish designated for breeding were fasted the day prior to spawning. In alignment with the circadian cycle, the broodstock were transferred into fresh, clean spawning tanks in the morning. The divider was removed to allow mating interactions, which were observed until sufficient egg deposition was achieved. Once adequate spawning had occurred, the broodstock were returned to their respective holding tanks. Fertilized eggs, which accumulated at the bottom of the spawning tank (Figure 9A), were carefully collected using a fine mesh strainer and transferred into sterile glass Petri dishes for further observation and incubation (Figure 9B).

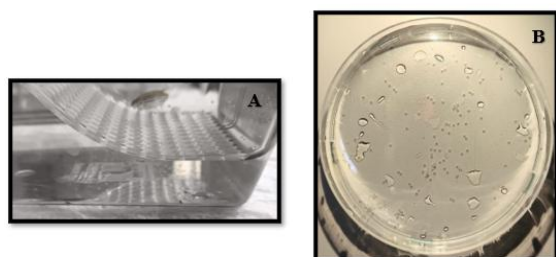


Figure 9. A: Embryos collected at the bottom of the spawning tank, B: Embryos transferred into petri dishes.

Embryo Collection and Handling

Eggs collected from the spawning tanks were rinsed with distilled water to remove debris and residual waste. Clean and intact embryos were transferred into embryo medium that had been freshly prepared the previous evening. Each embryo was individually assessed under a stereomicroscope to verify fertilization status and determine the developmental stage.

During microscopic examination, embryos exhibiting transparency were classified as viable (Figure 10A), while those with an opaque crescent-shaped appearance were considered non-viable or dead (Figure 10B). Non-viable embryos were separated and placed in a different Petri dish to avoid contamination.

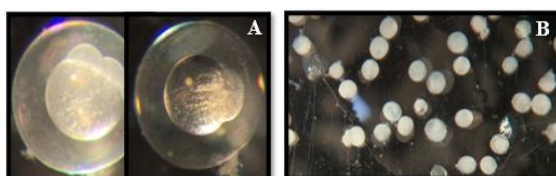


Figure 10. A: Fertilized zebrafish embryos, B: Coagulated (non-viable) zebrafish embryos.

Viable embryos were then transferred to an incubator pre-set to 28°C. Embryos and resulting larvae were maintained in the incubator for no more than seven days. Developmental progression was monitored daily under the microscope, and embryo media were refreshed three to five times per day to ensure optimal water quality and embryo viability.

CONCLUSION

As Türkiye's first faculty of veterinary medicine, the Faculty of Veterinary Medicine at Ankara University established the Aquatic Experimental Animal Research Center (ADHAM) with the dual aim of advancing academic training for both undergraduate and postgraduate students and providing a regulatory and procedural blueprint for the development of similar facilities across

other institutions. Beyond serving as a zebrafish model laboratory, ADHAM was strategically conceptualized to address environmental toxicology within the scope of the One Health paradigm, offering a multidisciplinary research platform distinct from existing aquatic laboratories in the country.

In pursuit of this vision, a collaborative research team was formed, comprising experts from Physiology, Pharmacology-Toxicology, and Histology-Embryology. Together, they initiated work on embryotoxicity modeling, positioning the center at the intersection of veterinary biomedical science, environmental health, and regulatory toxicology. The establishment process, along with the outcomes achieved thus far, has laid a robust foundation that serves as both a national reference model and a scientific benchmark for faculties of veterinary medicine and related institutions across Türkiye.

The successful operationalization of the center is expected to contribute substantially to veterinary education, scholarly output, and interdisciplinary collaboration, while simultaneously providing a roadmap for future research in aquatic toxicology and animal welfare sciences. As veterinary surgeons and researchers, we recognize the critical importance of such infrastructure in generating translational knowledge and shaping evidence-based approaches to aquatic animal health.

In alignment with the short-term objectives (6 months to 1 year), the center has successfully developed zebrafish-based embryotoxicity models, submitted multiple institutional and inter-institutional research project applications, and initiated preparations for international scientific partnerships.

In the medium-term phase (1 to 3 years), the center has expanded its research capacity by incorporating alternative aquatic vertebrate models beyond zebrafish, launched scientific research services, and begun engaging with the private sector, particularly through educational offerings and initiatives aimed at establishing sustainable revenue streams via the university's revolving fund system.

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Ethical Declaration

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authorship contributions

Concept: E.Y., M.Y., F.G.A., S.S., S.N.A., A.G.B., H.Ö., Methodology: E.Y., M.Y., F.G.A., S.N.A., Writing of Original Draft: M.Y., F.G.A., Final Review and Editing: E.Y., M.Y., S.S., A.G.B., H.Ö.

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