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Rejeneratif tıpta model organizma; Aksolotl (*Ambystoma Mexicanum*)

Model organism in regenerative medicine; Axolotl (Ambystoma Mexicanum)

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ABSTRACT

The axolotl has an extraordinary capacity to regenerate damaged and lost structures, especially the nervous system, limbs, organs such as the eye and heart, without causing scarring. It has become a very important model organism by attracting the attention of scientists working in both developmental biology and regenerative medicine and stem cell biology. The axolotl, which is amphibian and tetrapod, is a more preferred model due to its ease of maintenance and reproduction compared to other organisms such as African clawed frog (*Xenopus laevis*) or zebrafish (*Danio rerio*), which are relatively difficult to study. The main purposes of this review are the definition and origin of the axolotl, its taxonomy, anatomy, reproduction, nutrition, habitat, to give a perspective to scientists who want to work on this model organism by giving examples to the scientific data and study fields of the last 20 years by addressing issues such as how it contributes to scientific studies as a model organism.

Keywords: Axolotl, regeneration, model organism.

ÖZ

Sinir sistemi, ektremiteler, göz ve kalp gibi organlar olmak üzere hasarlanan ve yitirilen yapılarını skar oluşturmaksızın olağanüstü derecede yenileme kapasitesine sahip olan aksolotl; hem gelişimsel biyolojide hem de rejeneratif tıp ve kök hücre biyolojisi alanında çalışan bilim insanlarının dikkatini çekerek oldukça önemli bir model organizma haline gelmiştir. Amfibi ve tetrapod olan aksolotl, nispeten çalışılması zor olan Afrika pençeli kurbağası (Xenopus laevis) veya zebra balığı (Danio rerio) gibi diğer organizmalara göre bakımı ve üremesinin kolaylığı sebebiyle daha çok tercih edilen bir modeldir. Bu derlemenin temel amaçları; aksolotlın tanımı ve kökeninden başlayarak, taksonomisi, anatomisi, üremesi, beslenmesi, yaşam alanını, bilimsel çalışmalara model organizma olarak nasıl katkı sağladığı gibi konulara değinerek son 20 yıldaki bilimsel verileri ve çalışma alanlarına örnekler vererek bu model organizma üzerinde çalışmak isteyen bilim insanlarına bakış açısı kazandırmaktır.

Anahtar Sözcükler: *Aksolotl, rejenerasyon, model organizma.*

1. DEFINITION AND CLASSIFICATION

Axolotls (*Ambystoma mexicanum*), cute appearance world-famous salamanders and a national icon in Mexico live around Lake Xochimilco. This lake is located at an altitude of about 2200 m above sea level and its surroundings are a region Aztecs lived for centuries. The God Xolotl, the twin brother of Quetzalcoatl according to Aztec mythology and is considered sacred by the local people. The name 'Axolotl' refers to the god Xolotl, who was able to transform himself into an axolotl to escape his enemies (1). Seventeen ambystomatid salamander species are living in Mexico (2).

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However, these days wild axolotls are threatened with extinction due to population growth in Mexico City and pollution of the lake. Today, axolotls are protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (3). The CITES regulates the international trade of axolotl (including laboratory-raised animals) (2). Since most of the lakes and lagoons in their habitats have disappeared, water channels and lakes with the few remaining axolotls have been declared cultural heritage sites for humanity by UNESCO and have been under special protection since 2001 (4). *Ambystoma mexicanum* is the neotenic larva of Ambystoma and belongs to the family *Ambystomidae*, class *Urodela* (salamanders), *Amphibia*. It was initially mistakenly included in *the Perennibranchiates group* that did not undergo metamorphosis and was named *Siredon piscijormis* (5). The first living axolotl was brought to Europe in 1864 (6). Subsequent to Axolotls spontaneously metamorphosed in the Paris Botanical Garden, they were renamed *Ambystoma mexicanum* (7). Metamorphosed forms, lives in cold mountain lakes in natural habitat in Mexico year-round, are also called Ambystoma. Karyotype of the axolotl was defined by Signoret, Hauschka, and Brunst and it consists of 28 chromosomes, 7 pairs of metacentric and 7 pairs of acrocentric (1). Axolotl genome has approximately $32x10^9$ base pairs located on 14 haploid chromosomes thus it is the largest of all tetrapods (8).

2. ANATOMY

Bearing in mind the general body plan of the axolotl, it is thought to be among the most primitive members of tetrapods (9). Axolotl's lengths varies between 15-45 cm (Figure-1) (1). Females' abdomens are generally larger than males. Contrasting females, males have very prominent, strongly developed cloacal swellings at the base of the tail (10). Unlike most salamanders, axolotls do not undergo metamorphosis unless artificially stimulated by the addition of thyroid hormone (11). They reach adulthood growing in water and their characteristic hairy external gills are permanent throughout their lives. When axolotls are artificially stimulated, they can metamorphose like adult tiger salamanders (*Ambystoma tigrinum*). However, it has been shown that the regenerative rate and quality of regeneration decrease as a result of experimentally induced metamorphosis (12). Axolotls do not have a middle ear structure but they use their sense of smell greatly in foraging and selection. Although the olfactory systems of urodels are quite complex, this system allows them to distinguish similar odours and chemicals (1). The axolotl eye has poor vision limited to detecting movement. However, the retina is stratified similar to vertebrates (1). It has a skeleton composed of calcified cartilage anatomically similar to real teeth and mammalian joints (13). Axolotls have a different skeleton from most vertebrates as they are not fully ossified. In general, the amphibian skull has a greater number of cartilaginous structures than the bony ones and has canals that connect the nasal cavity with the buccal cavity called choanae or internal nostrils. The number of vertebrae in the tail region can vary between 30 and 35, and the total number of vertebrae is approximately 50 depending on this change.

The spine can be divided into four sections: cervical, thoracic, sacral and caudal. Another interesting aspect is that they have rudimentary ribs that can be seen throughout the body. Axolotls have four limbs that developing strated from the third week. The two anterior extremities have four toes, while the two hind extremities have five toes. When the systematic anatomy of the axolotl is examined, its most characteristic feature is red gills. Axolotls, capable to skin and lung respiration in addition to gill respiration, do not lose the gills gained during the larval period throughout their lives (14). Thanks to its three pairs of external gills, axolotl can exchange oxygen and also exchanges gases with skin respiration. They have primitive lungs and obtain at least 40-60% of their oxygen through surface respiration. The lungs are long and translucent and course parallel to the spine for nearly the entire length of the ventral body cavity (15). It has a dorsal caudal fin that runs along its spine. The axolotl has a three-chambered heart consisting of two atria and one ventricle. The pulmonary arteries and the aorta carry blood to the rest of the body, arise from the ventricle (16). Oxygenated blood leaves the lungs and is pumped back into the heart's single ventricle to mixes with deoxygenated blood circulating throughout the body. This three-chamber system is less effective than the four-chamber system seen in mammals and birds, as it causes deoxygenated blood to enter the general

circulation (17). Amphibian heart doesn't have a ventricular septum unless mammals and whereas most of the contractile force in mammals comes from the myocardium, amphibians rely on the contraction of a complex network of muscle fibers within the ventricle (18). Organs such as the liver and pancreas assist the digestive system functions. The liver stores fat and protein and helps the digestion of fats by producing bile fluids that are poured into the initial part of the small intestine. In aquatic amphibians, the liver has a minimal role in processing nitrogen excreted in the ammonia formula from the skin and kidneys (10). The pancreas, located between the stomach and the foregut, produces pancreatic enzymes that play a role in digestion. Both bile fluids and pancreatic enzymes enter the intestine through ducts that flow into the anterior part of the small intestine where nutrient absorption takes place. Axolotls have a lateral line system that functions both to detect electrical currents (with their cranially located ampullary organs) and to control their movement in water (via mechanical neuromasts that run along the side of their body) (19). The lateral line develops from neurogenic plaques and the study in related to these developmental stages guide scientists about the evolution of vertebrate sensory systems (Figure-2).

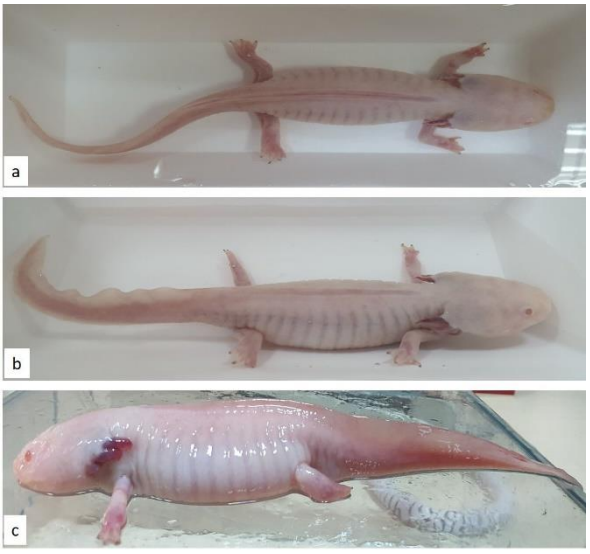

Figure-1. Photographs of *Axolotl mexicanum* in dorsal view (a), right view (b), and left view (c).

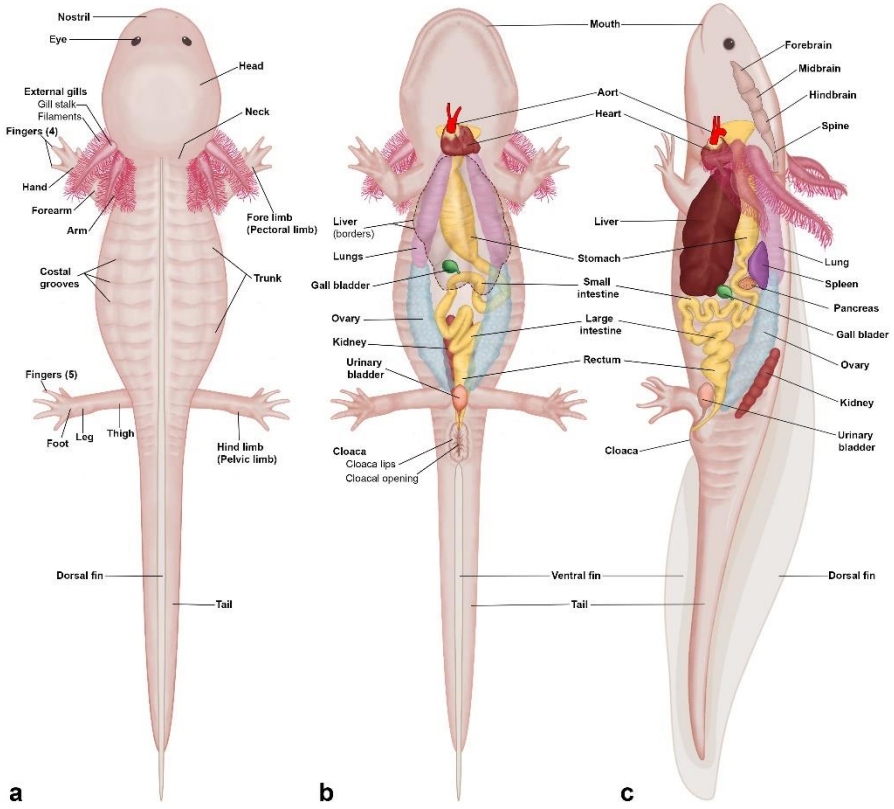

Figure-2. Illustrations present internal organs of *Axolotl mexicanum*. (a) Dorsal view (b) Ventral view and (c) Left lateral view.

3. CARE AND REPRODUCTION

The main reasons for using axolotls as model organisms in laboratories; their reproductive convenience, long breeding seasons, rapid sexual maturity, and relatively larger eggs compared to other model organisms. Axolotls can easily live in the aquarium and can be easily transported to many laboratories around the world. Although the lifespan of wild axolotls is not known exactly, it is usually between 10-15 years in the natural environment and in laboratories they can live up to 8-10 years under optimal conditions (20). The temperature tolerance range varies between 8-24°C (21). Useful information about the summaries and bibliography of the researches on housing and reproduction conditions, diseases and mutant types of the colonies in different laboratories; has been published since 1976 in the Axolotl Newsletter published by Indiana University (10). At Indiana University, adult axolotls are kept singly in small aquariums, clear containers or small ponds in 50% modified Holtfreter's solution (22). The water pH should be 7.5-8.5. If water is dirty within a few hours after feeding, it should be changed and its level should be kept at approximately 35-40 cm (1). Each container should have its evacuation in laboratories and should be illuminated with fluorescent lamps to provide a 12-hour day and 12-hour night cycle. Female axolotls reach sexual maturity between 9 and 12 months, while males can take 12 to 15 months. A female can lay eggs 2-3 times a year (14). The sexual maturity can be observed with the increase of cloacal swelling in males and abdominal swelling in females, and in some cases, mating behaviour can be stimulated by temperature changes (10). Mating begins when the male axolotl releases its spermatophores into the environment, which are taken up by the female axolotl and stored in the cloaca until the time spermatozoa lay eggs. When the eggs leave the oviduct and enter the cloacal space, spermatozoa encounter the egg and more than one sperm enter the egg cytoplasm (23). The eggs are usually arranged in rows and surrounded by a thick layer of sticky jelly that must be removed before experiments. Most female axolotls lay 200-1000 eggs per spawning. In addition, reproductive status is affected by seasonal variability and peaks in the spring, while reproductive abilities gradually decreasing during the summer period (24). Although axolotls are cultivated widely, easily and reliably in the laboratory environment, there are many areas to research about development of their reproductive systems.

4. NUTRITION AND IMMUNE SYSTEMS

Axolotls can be fed finely cut beef, pieces of beef liver, earthworms, fish, frozen bloodworms or standard pellet fish food. It is necessary to feed juvenile axolotls six times a week while feeding adults three times is sufficient (10). It is necessary to patiently feed the small piece of meat held by long forceps or ready-made commercial feed, shaking it in front of the axolotl's head. Another issue to be considered for the axolotls kept together in aquarium is that they can bite each other's legs in case of hunger. Infected axolotls should be quickly isolated from others. The sick axolotl should be fed three times a week with beef cut into strips mixed with multivitamins and various minerals, free of fat and hard fibres (25). When the immune systems are examined; it is seen that they have a very primitive adaptive immune system, which can be called immunodeficiency (26).

Immunodeficiency are commonly seen in urodels, so that axolotls do not reject tissue transplanted from other salamanders (27). Head transplantation trials are being carried out in some laboratories in Europe, there are efforts to create favourable conditions for organ transplantation. Heads transplanted onto the backs of other axolotls grow rapidly and may show the characteristic features of a normal axolotl head (28). In regard of microsurgical procedures for research, Axolotls are at moderate risk of bacterial infection for 2 weeks after operation. In addition, chronic stress is an important factor for fungal, viral and bacterial infections. *Ambystoma tigrinum* virus (ATV) can reduce their population both in the wild and in the laboratory, and mortality can exceed 90% (29). This hypersensitivity is probably due to the lack of lymphocyte proliferation when exposed to the virus (30). Despite the high degree of cell proliferation in their tissues, they are surprisingly highly resistant to cancer (31). A deeper understanding of these processes would be possible due to the study of regenerative medicine, and an increase of scientists focusing on axolotl development or cancer treatments will pave the way for the discovery of new therapeutic approaches in cancer treatment and will shed light on the mechanisms linking regeneration and cancer.

5. USE IN REGENERATIVE MEDICINE AND OTHER FIELDS

Axolotls have become one of the most ideal model organisms for scientists working on developmental biology. Axolotl eggs, including nuclear transplantations, has been used in studies such as primary embryonic induction and ectoderm development, mesoderm formation, inducing effect of endoderm and somitogenesis, localization and structure of primordial germ cells (32). By examining their developmental processes; numerous subjects such as the dynamics of nuclear transformation during fertilization, duration of different mitotic phases, reorganization of the cell cycle during division and the occurrence of RNA synthesis, and their relationship with cytokinesis and nuclear division have been studied (33). Early gastrula stages were revealed for axolotl embryos and the dynamics of morphogenetic changes during gastrulation were investigated in these studies (34). Axolotls' ability to regenerate their limbs and tails makes them important in regeneration studies because of their uniqueness among adult vertebrates in this regard. After tail amputation, epithelial wound healing begins with the formation of the progenitor cell region called the blastema, and this region firstly expands, then undergoes morphogenesis and differentiation and after that becomes a protrusion (35). Regeneration can be observed in the tail, jaw, spine, gills, brain, heart and all extremities. It occurs both without leaving a trace or loss in the functional sense (12). In addition, in recent studies, it has been reported that a rich extracellular matrix (ECM) is synthesized, which controls the behaviour of cells that lead to regeneration of the extremities (36). In another study comparing ECMs in axolotl and mouse extremities, extremity regeneration in the axolotl has been shown to occur through heparan sulphate and fibroblast growth factor (FGF) (37). In another study showed that adult axolotls can regenerate a natural neuronal diversity in response to brain injury. These data show that electrophysiologic functional neurons can regenerate (38). It fact proves that its brain has plasticity and can change throughout life, and axolotls will be a suitable model for neural plasticity studies.

6. AXOLOTL STUDIES FROM PAST TO PRESENT

Lazzaro Spallanzani first described limb regeneration in salamanders in 1768 (39). In 1850, the first axolotl laboratories were established and taxonomic comparison and species identification studies were carried out in these laboratories (40). Today, axolotls are laboratory experimental animals used commonly as model organisms in many research areas, mostly in regeneration and developmental biology studies. Analysis of axolotl studies shows that it is used more and more in researches over increasingly last 20 years.

Today, the newly developed single-cell RNA sequencing method (scRNA-seq) enables observe cellular and molecular dynamics of axolotl regeneration in a single cell (41). It has been found miRNAs play an important role in timing and control of gene expression to regulate and organize processes in blastema formation (42). In addition, studies on transgenic axolotls are increasing. In a study on planar cell polarity (PCP) mediated neural stem cell induction during spinal cord regeneration; change of homeostatic balance towards regenerative stem cells that restore the lost tissue reveals the key role of PCP in coordinating the morphogenesis of spinal cord growth (43). In comparative biology researches, there are studies on the similarities and differences in transcriptomic, ECM modelling and gene expression models between the two species after spinal cord injury in axolotls and rats (44). Researches in comparative embryology, regeneration and evolution continue to be the main research areas of axolotl studies (45).

CONCLUSION

As mentioned throughout the review, the axolotl, which is a very important model organism, due to its potential to clarify different scientific problems than the experimental animals routinely used in studies in areas such as evolutionary studies, regenerative medicine, organ transplantation, and cancer if attracts necessary attention as an experimental model in our country it can be answer of important scientific problems, creates new ones and will pave the way for high-quality international studies with its biological mechanisms waiting to be clarified.

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