Experimental models and tools in animal research: From classical approaches to emerging trends

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Abstract

From invertebrates like fruit flies to vertebrates such as mice and aquatic organisms, animal models have long been central to biological discovery. Despite ethical debates and growing regulatory constraints, animal research remains a cornerstone for understanding pathophysiology and developing new therapies for both humans and animals. Significant advances have refined these models, improving their relevance in scientific research and experimental education. However, the emergence of innovative systems, including computer simulations, organ-on-a-chip technologies, and genetically modified organisms, offers promising alternatives that can complement or, in some cases, replace animal experimentation. These tools accelerate scientific progress, enhance educational training, and improve the translation of results to human biology. Yet, systematic reviews have revealed discrepancies between animal and human responses, calling into question the predictive validity of traditional models. This ongoing debate highlights the need for a balanced integration of classical and emerging approaches to strengthen scientific reliability, educational value, and ethical standards. This review aims to provide an overview of experimental models, contrasting classical and novel systems, and to discuss their applications, limitations, and the evolving regulatory frameworks that guide their use in modern research and education.

Keywords: Animal models, Education, Animal research, Emerging models, Classical models, Animal Ethics

INTRODUCTION

Since ancient times, humans have depended on animals to supply them with their demands in various fields. However, the role of animals is not just restricted to these traditional applications. Animal models have been contributing largely to scientific experiments for centuries and their use in the pursuit of medical knowledge and research has continued for millennia(Ericsson *et al*., 2014). The term model is frequently used to describe an experimental system, the "animal model" derives from the Latin word *anima* (meaning soul or spirit) and the word *model*, which refers to something that imitates or resembles another (Claudia Janeth Juárez-Portilla, 2019).

Animal models are based on the principle of comparative medicine (Jota Baptista *et al*., 2021), serving as tools that can replicate physiological and pathological processes (Swearengen, 2018). Already in ancient Greece, animals were employed by Aristotle to study their living organisms. But it was in the 18th and 19th centuries that usage of animal models grew exponentially. Jean Baptiste Van Helmont, Francesco Redi, John Needham, Lazzaro Spallanzani, Lavoisier, and Pasteur were among the scientists who employed experiments to ascertain the origin of life (Oparin, 1957; Andersen, 2017). It is important to recognize that traditional models are responsible for nearly 90% of Nobel Prizes in physiology and medicine, which involved animal models in their research (Andersen, 2017).

Currently, the use of laboratory animals extends across various fields, particularly in experimental teaching and scientific research. In addition, animals play a crucial role in teaching as a medium for studying students of life sciences, medicine, and veterinary medicine, by providing their fist contact with laboratory animals (Carnovale *et al*., 2021). This helps students learn, especially anatomy, animal physiology, pathology, pharmacology, and experimental procedures, more comprehensively. It is also important to emphasize that the most of the experimental operations are still inseparable from frogs, mice, rats, rabbits, and other laboratory animals. However, in teaching and scientific research, laboratory animal welfare and ethical education have attracted significant attention from society and should be widely integrated into education (Kang *et al*., 2022).

Animal models have also played a crucial role in advancing biomedical science, contributing to the development of vaccines and antibiotics, as well as enhancing our fundamental understanding of human disease processes. For example, monkeys were used in the development of the polio vaccine, canines contributed to the discovery of insulin, and genetically modified mice and rats have underpinned diabetes research. Moreover, most antibiotics are tested on animals before human use (Domínguez-Oliva *et al*., 2023). Notably, the current pandemics' pathology, such as the 2019 Coronavirus disease, has been studied in primate, rodent, and porcine models to develop treatment strategies (Domínguez-Oliva *et al*., 2023). Compared to the traditional approach, the new approaches still have their place (Xu *et al*., 2018). In recent years, the need to find appropriate alternatives has become increasingly important for advancing experimental teaching and scientific research, particu-

larly with the emergence of virtual simulations and 3D experimental learning. Public awareness and discussion regarding animal experiments and alternative methods have also grown significantly. In this context, the concept of the "Three Rs" - Replacement, Reduction, and Refinement of animal experiments - is central. A primary goal within the Three Rs, scientific community is to develop predictive non-animal models and to effectively integrate all available data from *in vitro*, *in silico*, and omics approaches, thereby improving both the quality of research and educational outcomes (Neuhaus *et al*., 2022). This review aimed to highlight the significance of classical experimental models in educational processes, and scientific investigation, and how these tools and models evolved over time, by giving a short overview on their advantages and limitations.

EXPERIMENTAL MODELS

Conventional models

While researchers most often use rodents and other mammals as animal models for scientific research, a variety of other models also exist, such as zebrafish, the nematode *Caenorhabditis elegans,* and the fruit fly *Drosophila melanogaster*. These models offer advantages in cost, efficiency and ethics (Vila *et al*., 2014). Wild-type or natural models refer to animals that have not undergone genetic manipulation or experimental treatment. Classical experimental models have long been the foundation of physiological investigations. Rodents (rats, mice, guinea pigs, hamsters and rabbits) are extensively used due their genetic closeness to humans, short reproductive cycles, and well-documented physiology (Robert, 1997). Other traditional Models, such as amphibians, zebrafish, *C. elegans*, and *D. melanogaster,*provide complementary advantages depending on the research objectives (Vila *et al*., 2014). For example, *Drosophila melanogaster* is utilized to study neurological diseases like epilepsy (Lasko and Lüthy, 2021). *C. elegans* for mechanisms underlying obesity (Benditha *et al*., 2021), and aquatic animals to model metabolic diseases, such as diabetes (Zang *et al*., 2018). To illustrate the evolution of animal model usage in scientific research, a bibliometric analysis of PubMed data from 1990 to 2024 was conducted. This analysis highlights the changing trends in publication frequency among classical models such as the mouse, rat, rabbit, guinea pig, amphibians (*Xenopus*), and small fish (zebrafish), reflecting shifts in research priorities and ethical considerations over the past decades (Bédard *et al*., 2020).

Rodents

To date, rodents remain the predominant species used teaching and scientific research (Domínguez-Oliva *et al*., 2023; Makowska and Weary, 2019; Robinson *et al*., 2019), they have been employed in teaching, scientific, veterinary, and comparative medical research to investigate physiological mechanisms and gene mutations. Rodents, particularly (*Mus musculus*) and rats (*Rattus norvegicus*), have long been central to each research owing to their genetic tractability, cost-effectiveness and practicality in laboratory settings (Ferreira *et al*., 2025). Over the past 25 years, the laboratory mouse has become the dominant model in biomedical research, while the use of most non-rodent mammals has steadily declined (Ericsson *et al*., 2014). Rodent models play a vital role in evaluating the early-stage therapeutic effects of new drugs and remain essential for advancing translational research (Ericsson *et al*., 2014). Rabbits also hold great value in both education and research due to their biological and physiological characteristics, which are comparable to those of humans. As intermediate models, they combine the ease of handling of smaller species with greater physiological relevance than rodents (Ferreira *et al*., 2025).They are used in various biomedical fields, including cardiovascular, immunological, ophthalmological, and neurological studies, as well as in toxicology testing, vaccine development, and behavioral research. In educational settings, rabbits are frequently utilized in practical laboratory classes, particularly in physiology and pharmacology. Readily available and generally docile, they represent a practical model, when ethical guidelines and research protocols are carefully respected (Thomas *et al*., 2012).

The Guinea pig

The term 'guinea pig' has become synonymous with scientific experimentation. Classified as a non-rodent (Noguchi *et al*., 1994), *Cavia porcellus* has been used in research for approximately 200 years. Historically, guinea pig were pivotal in the study of infectious diseases such as tuberculosis and diphtheria, efforts that contributed to Nobel Prizes winning discoveries (Padilla-Carlin *et al*., 2008). Today, guinea pig remain valuable models for investigating bacterial infections of the lungs, reproductive tract, eyes, ears, as well as viral infections, including influenza (Lowen *et al*., 2014). They also play a key role in the development of vaccines and other preventive measures to combat these infections (Padilla-Carlin *et al*., 2008). Despite their contributions, guinea pigs are less frequently used than other animal models, largely due to higher costs and limited availability of immunological reagents (McMurray, 2001). Notably, they are considered essential in asthma research, especially for pharmacological studies, where their physiological responses are closer to humans than those of mice (Adner *et al*., 2020). Guinea pigs are also used in educational settings to teach immunology and pharmacology, allowing students to observe physiological responses in a live mammalian model.

Amphibians

Amphibians have long been a cornerstone in the study of physiology. Early physiologists favored them due to their small body size, local availability, and tolerance of surgical procedures. This diverse group of tetrapods has been instrumental in advancing our understanding of musculoskeletal, cardiovascular, renal, respiratory, reproductive, and sensory system physiology (Burggren and Warburton, 2007). Amphibians offer several advantages for experimental studies (Bédard *et al*., 2020),

including accessibility, ease of handling, and suitability for detailed physiological investigations, making them highly valuable for generating new insights in fundamental physiological research aimed at generating new insights**,** and strong applicability in physiological research. Amphibians are still widely used in educational laboratories to demonstrate physiological principles, such as nerve-muscle function, heart activity, and respiratory mechanisms, providing students with hands-on learning experiences (Rees *et al*., 2025).

Fish

Over the past decade, small fish such as zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) have increasingly been used as animal models for studying human diseases, due to the high degree of conservation in genome organization and physiology with humans. A major advantage of these models is their suitability for real-time live imaging of various biological processes, including skeletal development and repair, which has contributed to a significant increase in publications involving teleosts in biomedical research (Lleras-Forero *et al*., 2020). Today, zebrafish remain the predominant small fish model species in laboratories worldwide, with medaka being used to a lesser extent (Parichy *et al*., 2009; Wittbrodt *et al*., 2002). Their short life cycles allow researchers to generate large numbers of subjects in a short period, facilitating high-throughput experiments (Vila *et al*., 2014). Beyond fundamental research, zebrafish have provided valuable insights for the pharmaceutical industry (Patton *et al*., 2021). They serve as comparative models in a wide range of fields, including cardiovascular research, bone biology, immunology, and cancer studies (Van Rooijen, 2017; Lleras-Forero *et al*., 2020; Tessadori *et al*., 2018). Several characteristics make these small fish especially suitable as laboratory models: external fertilization, rapid embryonic development, small body size, ease of handling, low maintenance costs, and optical transparency of embryos and adult fish (Bauer *et al*., 2021). Despite their aquatic nature and differences from terrestrial mammals, teleosts can faithfully replicate many important aspects of human physiology and disease phenotypes (Lleras-Forero *et al*., 2020). Small fish models are increasingly integrated into educational laboratories, allowing students to observe embryogenesis, organ development, and disease phenotypes in real time, enhancing hands-on learning experiences (Gladys *et al*., 2015; Wilk *et al*., 2018).

Non-Human Primates (NHPs)

Non-human primates (NHPs) are considered the most suitable experimental models for biomedical research due to their close phylogenetic relationship with humans (Zhang *et al*., 2014). Historically, four NHP species, chimpanzees, cynomolgus monkeys, rhesus monkeys, and marmosets, have been widely used in biomedical studies with notable success (Johnsen *et al*., 2012) . However, chimpanzees and other great apes are now banned from invasive biomedical research in many countries (Johnsen *et al*., 2012). NHPs are particularly valuable because their anatomy, physiology, immunology, and neurology closely resemble those of humans, facilitating the study of disease mechanisms and the evaluation of new therapeutic interventions (Cauvin *et al*., 2015; Zhang *et al*., 2014). Several primate models have been established to investigate human diseases, including infectious, cardiovascular, endocrine, reproductive, neurological, and ophthalmic disorders. Despite their scientific value, the use of NHPs is severely limited by ethical concerns, high costs, and restricted availability (Nakamura *et al*., 2021; Zhang *et al*., 2014). NHPs are occasionally used in advanced educational settings, such as comparative anatomy or neuroscience courses, to demonstrate physiological and behavioral principles that are highly translatable to humans (Takemura *et al*., 2019).

ALTERNATIVES TO ANIMAL MODELS

By the early twentieth century, the use of animal models had expanded considerably. Despite ongoing ethical concerns, animal experimentation became the standard approach for demonstrating biological significance. Over more than 150 years of research, science has progressed from uncovering fundamental biological principles to reproducing complex physiological and pathological events in the laboratory (Ericsson *et al*., 2014). Non-animal alternatives, including cell cultures, 3D tissue cultures, organs-on-chips, mathematical models, stem cells, bioprinting, *in silico* testing, and high-performance computer simulations, have gained increasing popularity in recent years and show great promise for the future (NIH, 2023). With improvements in alternative methods, the concept of reducing, refining, and replacing the use of animal models in research, the 3Rs increases more and more feasible. In major research countries and territories, such as the United States, United Kingdom, China, Germany, Japan, Canada, and across the European Union, the use of animals in scientific research is strictly regulated (Conroy, 2022). These regulations aim to ensure proof of ethical practices, promote animal welfare, and encourage the development and adoption of alternative techniques wherever feasible, due to their potential to significantly reduce the number of animals used in experiments and to offer high reproducibility of results (Wysoczański *et al*., 2024).

Organ-on-a-chip models

Recent advances in the microfluidics-based Organ-on-a-Chip (OOC) technology, also known as microphysiological systems, have provided a promising alternative to animal testing (Ma *et al*., 2021). These microfluidic devices culture living cells in continuously perfused, micrometer-scale chambers, replicating the physiology and functionality of tissues and organs on a chip. The origin of OOC technology dates back over three decades, beginning with the application of microfluidic devices for cell culture and biological analysis (Bhatia and Ingber, 2014). Progress in OOC has been largely driven by advancements in microfluidics since the late twentieth century (Ma *et al*., 2021). OOC systems offer unique advantages over conventional *in vitro* and *in*

vivo models, enabling high-resolution, real-time imaging and detailed investigation of biochemical, genetic, and metabolic processes in functional tissue and organ contexts. They have significant potential to advance understanding of tissue development, organ function, and disease pathogenesis. In drug discovery and development, OOC platforms facilitate the study of molecular mechanisms, lead compound selection, toxicity testing, and biomarker identification (Srivastava *et al*., 2024). Furthermore, OOC technology provides a powerful alternative in teaching laboratories, allowing students to explore organ-level physiology in a controlled, human-relevant system. For instance, a liver-on-a-chip can be used to measure hepatocyte responses to varying drug concentrations, assess cell viability, and analyze metabolite production. Such hands-on experiments enhance learning outcomes by improving understanding of organ-specific drug metabolism, experimental data interpretation, and appreciation of the ethical benefits of reducing animal use (Koyilot *et al*., 2022).

Genetically modified models

Rodent models, particularly mice, have long been central to biomedical research, with several hundred mouse stocks containing spontaneous or induced mutations used as models of human disease and for the study of metabolic processes. Genetically modified mouse models in which a specific gene is removed or replaced, particularly by a human gene, have proven to be invaluable tools for investigating gene function and its relationship to disease. Moreover, these models serve as powerful systems for the identification and validation of target genes, and for advancing our understanding of the molecular mechanisms underlying drug-induced toxicity through mechanistic studies. Traditionally, mutations either occurred spontaneously or were induced chemically or through radiation; however, recent advances now allow the generation of mutant strains through precise genomic engineering, with increasing specificity and efficiency. The development of knockout and humanized mouse models has therefore provided unprecedented opportunities for researchers in the field of drug metabolism and transport. From an educational perspective, genetically modified organisms such as transgenic mice, zebrafish, or *Drosophila* are also being introduced in teaching laboratories to illustrate gene expression, inheritance, and physiological regulation *in vivo*, helping students connect genotype to phenotype while promoting ethical awareness through refinement and reduction of animal use (Auer and Del Bene, 2014).

Computer simulation models

When scientists consider alternatives to traditional animal testing, they usually speak of tissue cultures, isolated organ preparations, biochemical baths, or other *in vitro* biosystems. Only within the last few decades have computer models reached a realistic potential as alternatives to the use of animals in biomedical research. Computer-based simulations now represent an important step in the scientific method, allowing hypotheses to be tested *in silico* before being validated experimentally, thereby reducing the number of animals used in biomedical experimentation. In educational contexts, platforms such as PhysioEx, SimBio Virtual Labs, and Virtual Physiology™ provide interactive modules where students can explore cardiovascular regulation, endocrine control, or pharmacological responses in virtual organisms. These tools offer immediate feedback, customizable experimental parameters, and reproducible results that help students grasp complex physiological mechanisms without ethical or safety concerns. However, while virtual simulations promote conceptual understanding and analytical reasoning, they cannot fully replicate the tactile, procedural, and sensory experiences of hands-on laboratory work. Therefore, integrating computer simulations as pre-laboratory or complementary learning tools, rather than complete substitutes, can significantly enhance students' preparedness for real experimental settings (De Jong *et al*., 2013; Rutten *et al*., 2012). Ongoing advances in artificial intelligence, haptic interfaces, and physically based rendering techniques are expected to increase the realism and educational value of simulation systems, paving the way for more ethical and efficient biomedical training (Malone *et al*., 2010).

COMPARATIVE ANALYSIS: CLASSICAL VS. EMERGING MODELS

Most research is conducted using classical model species, such as laboratory mice (*Mus musculus*), rats (*Rattus norvegicus domestica*), the fruit fly (*Drosophila melanogaster*), small fish and Non-Human Primates. These models offer clear advantages, including access to well-characterized strains and a wealth of high-quality preexisting data (Holtze *et al*., 2021) i.e., laboratory mouse (*Mus musculus)*. Selecting an appropriate animal model is essential for advancing scientific research, evaluating therapeutic strategies, and translating preclinical findings into clinical applications. Each model has unique strengths and limitations that influence its applicability (Ferreira *et al*., 2025). However, classical models can introduce interpretation biases due to species-specific characteristics, which may lead to flawed or misleading generalizations. As noted by Greek and Menache (2013), traditional animal models often fail to accurately predict human responses, underscoring the need for more human-relevant and ethically responsible alternatives (Greek and Menache, 2013).While animal models remain foundational and rich in investigative potential, ethical considerations increasingly shape their use. The global adoption of the Three Rs principle (Reduction, Replacement, and Refinement) has encouraged the development of alternative methods to animal use (Kiani *et al*., 2022); rats, mice and purpose-bred birds comprise almost 90% of the animals that are used for research purpose. However, growing awareness of the sentience of animals and their experience of pain and suffering has led to strong opposition to animal research among many scientists and the general public. In addition, the usefulness of extrapolating animal data to humans has been questioned. This has led to

Ethical Committees' adoption of the 'four Rs' principles. Emerging strategies, including integrative experimental models, *in silico* simulations, and artificial intelligence-driven approaches, aim to refine our understanding of biological mechanisms and accelerate the development of personalized therapies (Singh, 2022). In educational settings, simulator training in laboratory animal science has been shown to effectively support the 3Rs, particularly for inexperienced students, by reducing the need for live animal exercises (Humpenöder *et al*., 2021). However, current simulators cannot fully replace live animals, highlighting the need for more realistic designs and further research for broader implementation. Similarly, Andrew Knight (2007) demonstrated that non-harmful teaching alternatives - such as simulations, ethically sourced cadavers, and surgical models - can achieve equivalent or superior learning outcomes in veterinary education, while enhancing student confidence, satisfaction, and compliance with animal welfare standards.

Despite these advances, suitable alternatives are not yet available for all areas of scientific and educational research, due to prior validation requirements and substantial financial and technical investments. Consequently, classical animal models remain essential in many contexts to provide reliable and reproducible data. Table 1 summarizes the main characteristics, advantages, and limitations of both classical and emerging experimental models, emphasizing their relevance and applications in both research and teaching contexts.

CONCLUSION

This review has described and contextualized classical experimental models and their evolution. Although alternative approaches are continually developing, animal research remains essential, and the relevance of animals for advancing both veterinary and human health is undeniable. Over time, the continued use of various animal models has been crucial in academic and research activities. Researchers employing animals must justify their use and ensure that their work adheres to ethical principles and high standards of laboratory practice.

Given increasing concerns over animal welfare, the development of innovative technologies offers promising alternatives, including genetically engineered organisms, organs-on-chips, and *in silico* simulations. Animal ethics is as important as human welfare, and the introduction of alternatives requires reexamining the use of sentient animals in both research and teaching, with a focus on reduction, refinement, and, ultimately, replacement. Nevertheless, these alternatives cannot yet fully replace classical models.

In the meantime, animal models continue to provide a solid foundation and remain a key component of vital research. Innovation in tools and models is crucial for advancing experimental teaching and scientific research. While these technologies cannot completely eliminate the need for animal use, they aim to complement or partially replace classical approaches. Moreover, despite their advantages, these technologies are often limited by high costs and the sophisticated equipment required, which partly explains why their widespread adoption remains constrained. Future research should focus on enhancing the realism and applicability of alternatives, such as organ-on-a-chip systems, 3D tissue cultures, and *in silico* models. While promising for research and teaching, classical animal models remain essential where alternatives are not fully validated. Broader adoption will require training, funding, and supportive policies to ensure ethical and effective implementation.

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Table 1: Comparative overview of classical and emerging experimental models: main characteristics, advantages, limitations, and applications in research and teaching

Model Type	Species / Technology	Advantages	Limitations	Applications
Classical models	Rodents (mice, rats), rabbits, NHPs, zebrafish, *C. elegans, Drosophila*	Well-characterized physiology; high reproducibility; cost-effective (small mammals); strong historical dataset	Species differences limit human translation; ethical concerns; invasive procedures	Fundamental research, translational research, teaching in anatomy, physiology, pharmacology
Emerging models	Organ-on-a-chip, 3D tissue culture, stem cells, *in silico* models, microfluidics, simulations	Human-relevant data; reduced animal use; real-time observation; ethical advantages; customizable	High cost; technical expertise required; limited validation in all areas; cannot fully replace animals yet	Drug testing, toxicology, mechanistic studies, teaching (e.g., organ-level physiology)

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