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Animal Welfare of Rodents in Laboratory Research: Applications and Challenges of the 3Rs

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Authors' contributions

This work was carried out in collaboration among all authors. All authors contributed equally to the writing and revision of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Animal models, particularly rodents, are indispensable in biomedical research due to their physiological and genetic similarities to humans, enabling advancements in understanding diseases, developing therapies, and ensuring safety in drug testing. Rats and mice have been

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extensively utilized in diverse fields such as neuroscience, endocrinology, cardiovascular research, and toxicology, owing to their adaptability, rapid reproductive cycles, and cost-effectiveness. However, the ethical implications of animal research needs a balanced approach that prioritizes animal welfare while advancing scientific knowledge. The Three Rs (3Rs) principle — Replacement, Reduction, and Refinement—provides a framework for minimizing animal use, refining experimental conditions to reduce suffering, and exploring alternative methods. This review examines the advantages and limitations of laboratory rodents in scientific research through the lens of the 3Rs, highlighting their recent contributions to fields such as renal disease, pharmacology, toxicology, and regenerative medicine. Additionally, it explores emerging alternatives, including *in vitro* models, organ-on-a-chip systems, and computational approaches, which aim to reduce reliance on animal models while maintaining scientific rigor. By integrating ethical considerations with innovative methodologies, this review underscores the importance of advancing both animal welfare and biomedical research.

Keywords: Clinical research; animal welfare; research ethics; rat model; mice model; rodents for scientific research.

1. INTRODUCTION

Many scientific studies fundamentally rely on animal models as substitutes for healthy humans in safety and toxicity testing, and for studying human diseases in efficacy testing (Marshall et al., 2023). Animal models provide an experimental foundation that enhances the understanding of biological processes and the evaluation of therapeutic interventions before their application in humans, ensuring greater accuracy and safety in the results obtained (Domínguez-Oliva et al., 2023).

Rats have been used in science for many years due to their physiological similarities to humans and the ease of genetic manipulation. However, mice have recently increasingly dominated rodent models in biomedical research due to their larger genetic toolbox (Ellenbroek et al., 2016). Animal welfare is essential in animal studies, as they are often conducted under less-than-ideal conditions (Soulsbury et al., 2020).

The principle of the Three Rs (3Rs) has been gaining prominence as an important framework for enhancing animal welfare in research. This principle advocates for the replacement of animal models whenever possible; the reduction in the number of animals used in experiments, and the refinement of practices to minimize suffering and improve the conditions of the animals. There is widespread acceptance that the welfare of research animals should be optimized through the rigorous application of the 3Rs, promoting more ethical and sustainable approaches in science (Rácz et al., 2021).

The implementation of the 3Rs principle has prompted a shift in drug development practices, leading to updates in policies, regulations and the introduction of innovative safety assessment methods in different countries (Poh & Stanslas, 2024). Therefore, the aim of this review is to analyze the advantages, limitations and comparisons of rodents in scientific research from the perspective of the 3Rs framework, examining how their physiological and genetic characteristics align with the principles of replacement, reduction and refinement, while exploring their contributions to research and addressing the ethical considerations and potential alternatives to their use.

2. MATERIAL AND METHODS

This study is a narrative and qualitative literature review, conducted through a systematic search for scientific articles in databases such as *ScienceDirect*, *Scientific Electronic Library Online (SciELO)*, *PubMed*, and *Google Scholar*. The manual search was conducted between October 2024 and January 2025.

The inclusion criteria adopted for article selection were as follows: publications available in Portuguese, English and Spanish; studies aligned with the research theme, involving animal models, particularly rodents such as laboratory rats and mice, as well as the 3Rs principles and their applications in scientific research; availability of the full text; and titles and abstracts consistent with the objectives of this investigation. Additionally, only studies that used rats or mice as experimental animal models were included.

The search strategy was based on keywords such as *animal models*, *rodents*, *laboratory rats*, *3Rs principles*, *animal welfare*, *biomedical research*, *ethical considerations*, and *alternative methods*. These terms were combined using Boolean operators (AND, OR) to refine and broaden the search results. The selection criteria were applied independently by three authors, and any discrepancies were resolved by consensus.

The review was conducted through reading, analyzing, interpreting, and critically synthesizing the information extracted from the selected articles, with emphasis on those that provided significant contributions to the topic under investigation. The article screening process occurred in two main stages: reading of titles and abstracts to exclude studies that, although containing relevant keywords, did not directly address the central research focus; and full-text reading to assess the depth of the approach, methodological clarity, coherence of the data presented, and the relevance of the results to the objectives of this review.

The exclusion criteria included: studies with no direct relevance to the topic, even if they addressed animal models in a generic way; articles whose results or objectives did not contribute meaningfully to the discussion on the use of rodents in scientific research and the 3Rs principles; publications not available in the selected databases or restricted by paywalls; duplicate studies across different databases; and opinion-based documents or those with low methodological quality and lacking solid scientific grounding.

3. RESULTS AND DISCUSSION

3.1 Advantages of Clinical Research in Rodents

For over 2,400 years, animal studies have provided insights into human biology, evolving into a foundation of biomedical research across diverse fields like immunology, oncology, and behavior (Ericsson et al., 2013).

The use of rodents in research is rooted in comparative medicine, which relies on information from one species to understand similar processes in another. This approach enables the study of physiological, behavioral, and other characteristics, with the findings applied to understanding these processes in humans. Similarly, insights from human medicine can be utilized to advance veterinary medicine, highlighting the shared traits among species that form the foundation of comparative medicine (Bryda, 2013).

Therefore, rodents are the most commonly employed laboratory animals in medical research due to their biological similarities to humans, ease of use, small size, low cost, and rapid reproductive cycle (Peter et al., 2017). Although significant progress has been made in the search for alternatives to animal testing, the number of animals used remains high and continues to increase (Clarkson et al., 2022). A study published by Carbone et al. (2021) estimated that more than 111 million mice and rats are used annually in biomedical research in the United States, representing approximately 99.3% of all mammals employed in these studies. In the European Union, according to data from The European Animal Research Association (2023), just over 4.5 million mice and rats are used, accounting for 92.29% of the mammals involved in scientific research. Among European countries, France, Germany, and Spain are highlighted as the leading users of these animal models. Mice and rats, in particular, are extensively used in various research fields due to their genetic manipulability and similarity to humans. In neuroscience, they are vital for studying brain disorders and neuropsychiatric conditions, with mice being the primary model due to available genetic tools (Ellenbroek et al., 2016). In endocrinology, they help investigate the effects of hormonal changes during aging and menopause (Koebele & Bimonte-Nelson, 2016). For cardiovascular research, rodents, especially transgenic mice, are ideal for studying diseases like atherosclerosis (Zhao et al., 2020). In pain research, rodents are used to explore nociception and pain management (Deuis et al., 2017). Rodent models also play a key role in malaria research, cancer studies, and ecological investigations, where they help in understanding disease mechanisms, testing therapies, and examining environmental interactions (Čepelka et al., 2021; De Niz & Heussler, 2018; Tian et al., 2020).

These models are also widely used to investigate the underlying mechanisms of stress-related disorders, such as anxiety, depression, cognitive impairments, and post-traumatic stress disorder. Moreover, rodent models have played a critical role in drug screening and the development of treatments for stress-induced conditions (Atrooz et al., 2021). This underscores the need to consider the mental health of the animals, as stressors or unfavorable conditions can skew behavioral data and ultimately affect the reliability of the results. Therefore, understanding and ensuring the mental well-being of rodents is crucial for obtaining accurate outcomes in preclinical research.

Animals such as mice and rats in the wild thrive in complex environments, exhibiting a wide range of behaviors such as foraging, hunting, and capturing prey, while also serving as prey for many other species (Dennis et al., 2021). When handling these animals in the laboratory, it is important to be aware if the animal is actually in distress, pain, or illness, as these conditions cannot be managed if unrecognized (Gaskill et al., 2013). In addition, social needs are essential for rodents well-being, as interaction influences their health and behavior, with isolation leading to stress and aggression. Proper social conditions are crucial for their mental and physical health in research (Kappel et al., 2017). In summary, although rodents are used in research and medicine, there are ethical challenges associated with their use, as the benefits of such research must be balanced against concerns for animal welfare.

3.2 Limits of Clinical Research

The use of animals in research presents significant ethical challenges, including balancing animal welfare with the potential benefits for humans (Yeates. 2024). A major difficulty lies in addressing different laws, divergent ethical values ranging from total permissiveness in the use of animals to complete rejection based on animal rights and the current use of animals in research (Mertz et al., 2024). The inability of animals to consent further highlights the ethical complexity, raising concerns about exploitation and the adequacy of harm-benefit assessments in protecting animal interests. Tackling these challenges requires an interdisciplinary and international effort to create robust ethical standards that integrate diverse perspectives while prioritizing transparency and accountability (Fine & Griffin. 2024).

Biomedical research requires a delicate balance between advancing scientific knowledge and respecting animal welfare. Traditional frameworks, such as the 3Rs (Reduction, Refinement, and Replacement), aim to minimize harm by encouraging the use of fewer animals, reducing suffering, and exploring alternatives to animal models (Louis-Maerten., et al 2024). By implementing strategies such as reducing unnecessary animal use, refining study designs to improve welfare, and exploring alternative methods like *in vitro* and *in silico* approaches, the 3Rs not only advances animal welfare but also strengthens scientific outcomes. Furthermore, the cross-functional membership of groups with diverse expertise, enabling the development of comprehensive and impactful practices that align with regulatory standards and society expectations. This proactive approach underscores the importance of balancing ethical responsibility with scientific progress (Graham., et al 2023).

A way to improve animal welfare is by the concept of Humane Intervention Points (HIPs) that represents a progressive refinement in laboratory animal welfare, shifting the focus from euthanasia-based Humane Endpoints (HEPs) to a broader spectrum of non-lethal intervention options (Williams., et al 2022). By adopting HIPs, researchers can identify specific time points and humane actions such as providing analgesia, supportive care, or modifying husbandry practices to address pain or distress without ending the animal's life. This approach aligns more closely with the principle of the 3Rs by minimizing harm while preserving scientific integrity. HIPs not only enhance animal welfare but also reduce the need for additional animals, as humane interventions allow more animals to remain in study and achieve experimental goals. Incorporating HIPs into research protocols fosters innovation, improves collaboration, and ensures a more ethical and effective use of animals in research (Nunamaker., et al 2021).

The financial costs associated with improving animal welfare are a crucial consideration. Policies that aim to enhance animal welfare, such as banning harmful practices or improving living conditions, typically require significant investments in infrastructure, compliance monitoring, and alternative methods of production or research. Despite these financial burdens, the willingness-to-pay (WTP) studies indicate strong public support for such measures, reflecting a society's recognition of the intrinsic and ethical value of animal well-being. Balancing these costs with the expected benefits both for animals and for human utility requires transparent and comprehensive economic assessments to ensure sustainable and socially supported policy implementation (Lusk., 2011).

3.3 Application of the 3rs in Clinical Research

The reduction of the number of animals used in research is an ethical and scientific priority, supported by innovative strategies that enable more efficient studies with minimal impact on animals. Among these strategies, the use of robust experimental designs, such as randomization and blinding, stands out for ensuring reliable results with fewer experimental subjects. Additionally, approaches like *in silico* models, which simulate biological interactions through computational methods, and the development of *in vitro* techniques, such as three-dimensional cell cultures, have significantly contributed to reducing the reliance on animal models. The integration of databases and the sharing of information among researchers also prevent the duplication of experiments and optimize resources (Poh & Stanslas, 2024).

Refinement methods are essential to ensure maximum welfare for laboratory rodents during scientific research, prioritizing both ethical responsibility and scientific validity. Key strategies include enhancing housing conditions through environmental enrichment, such as providing nesting materials and opportunities for natural behaviors like burrowing and climbing (Rinwa., et al 2024). Pain management is another critical component, with the use of effective anesthesia and analgesia to minimize discomfort during and after procedures. Behavioral assessments, such as monitoring social play and grooming patterns, allow researchers to early detect distress and adjust conditions accordingly. Additionally, innovative tools like facial expression analysis and automated home-cage tracking systems enable the detection of subtle signs of pain or stress, facilitating timely interventions. Promoting positive affective states, rather than merely avoiding negative ones, has become a focal point of modern refinement efforts. This approach not only benefits the animals but also improves the reliability of research outcomes (Jirkof, Rudeck & Lewejohann, 2019).

Advancements in alternative methods had significant development in replacing the use of rats in scientific research, offering ethical and scientific alternatives. New Approach Methodologies like organ-on-a-chip systems, advanced *in vitro* models using human cells and tissues, and computational techniques such as artificial intelligence (AI) are proving to be very predictive. These methods address limitations inherent to animal models, such as interspecies differences, which often hinder the translation of findings to clinical applications. For instance, these methodologies have demonstrated their utility in fields like toxicology and drug development by providing accurate and human-relevant data (Hutchinson., et al 2022). Additionally, advancements in 3D bioprinting and microfluidic technologies are enabling the creation of human tissue analogs for drug testing and disease modeling, further reducing reliance on rats and other animals. These initiatives not only enhance the precision of biomedical research but also pave the way for globally harmonized standards, ensuring the ethical and scientific rigor of studies. As these alternatives gain traction, they highlight the importance of continuous investment and education in non-animal methodologies to accelerate their broader implementation (Kiliccioglu., et al 2024).

The adoption of alternative methods is also being driven by international policy changes and collaborative initiatives aimed at reducing animal use. Regulatory bodies, including the U.S. FDA and the European Medicines Agency, are increasingly endorsing and integrating these alternatives into safety and efficacy assessments. These innovations not only reduce the reliance on animal models but also align with global ethical standards, marking a critical shift towards effective scientific practices (Dulisch., et al 2017).

3.4 Comparison between Clinical Research

The laboratory rat is widely recognized as one of the most emblematic model organisms, extensively used in various fields of scientific research, including neuroscience, physiology, and toxicology. Its popularity as an experimental model stems from factors such as wide availability, low maintenance costs, a rapid reproductive cycle, and remarkable adaptability to controlled environments. These characteristics make rats ideal for experimental studies, significantly contributing to advances in understanding biological processes and developing new therapies (Modlinska, 2020).

For example, renal diseases remain a devastating global health problem, characterized by unacceptably high rates of mortality and morbidity. Animal models play a critical role in deepening our understanding of renal disease mechanisms and developing effective therapeutic strategies. A study conducted by Demirkiran et al. (2019) utilized rats as experimental models to investigate the effects of the anesthetic ketamine, administered in varying doses, on reactive oxygen species and pro-inflammatory components associated with renal ischemia-reperfusion injury. Renal ischemia-reperfusion is a phenomenon characterized by the interruption and subsequent restoration of renal blood flow, one of the leading causes of acute kidney injury (AKI), a potentially fatal condition. The study demonstrated that ketamine exhibits antioxidant properties, with varying intensities depending on the administered dose. The most prominent antioxidant effects were observed with lower doses, highlighting its therapeutic potential in mitigating damage associated with renal ischemia-reperfusion injury (Demirkiran et al., 2019).

Another study by Mhaibes and Abdul-Wahab (2023) evaluated the nephroprotective effects of vitamin D against renal injury. This study utilized 42 rats subjected to renal injury induced by intraperitoneal injections of levofloxacin, a substance that damages kidneys by generating reactive oxygen species, resulting in oxidative stress, cellular damage, and renal injury. Results indicated that vitamin D mitigates renal dysfunction, suggesting its antioxidant properties and potential for preventing renal injury (Mhaibes & Abdul-Wahab, 2023). In both studies, rats proved to be invaluable models for simulating renal damage induced by different agents, enabling the evaluation of therapeutic substances' effects. Their use as experimental models allows for a deeper understanding of renal diseases and contributes to developing treatments that can eventually be applied clinically.

In addition to renal damage studies, rats play a crucial role in pharmacological testing, as demonstrated by Müller et al. (2020). This study investigated the effects of tetrahydrocannabinol (THC), the main pharmacological compound of cannabis, on the minimum alveolar concentration (MAC) of sevoflurane, the most widely used inhaled anesthetic today. The study involved 38 adult Wistar rats and found that THC significantly reduced the MAC of sevoflurane in these animals. MAC, a widely used parameter in anesthesiology, measures the potency of inhaled anesthetics; its reduction indicates that lower anesthetic concentrations are required to achieve the same effect. This is desirable, as lower doses reduce potential adverse effects associated with anesthetic agents (Müller et al., 2020).

Another study by Javorac et al. (2023) utilized rats as models to investigate the toxic effects of lead (Pb) in low-level exposure scenarios, focusing on its reproductive impacts. Among toxic metals, lead is one of the most significant environmental pollutants, historically used for its favorable physicochemical properties. Lead exposure is a key factor in developing male infertility of unknown etiology (MANI et al., 2021). The study involved 42 Wistar rats, administered Pb in increasing doses (0.1, 0.5, 1, 3, 7, and 15 mg Pb/kg body weight) daily through oral gavage for 28 days. Results showed that Pb affects male reproductive organs and testosterone levels at very low doses. A dose-dependent decrease in testosterone levels and a corresponding dose-dependent increase in testicular zinc levels were observed. These findings highlight Pb's role in male reproductive health disruption and serve as a foundation for further investigations into lead exposure's effects (Javorac et al., 2023). These studies underscore the importance of animal models, especially rats, in exploring both pharmacological effects and toxicological impacts of substances on living organisms, contributing significantly to advances in medicine and toxicology.

An emerging and increasingly prominent field is anxiety and depression, which are two highly prevalent and debilitating psychiatric disorders affecting individuals of all ages. Studies indicate that these disorders share several risk factors, such as intensely stressful life events or chronic stress exposure (Tang et al., 2019). Prolonged or extreme stress often results in significant physiological and psychological alterations, leading to a variety of mood and psychiatric disorders, particularly depression and anxiety.

Animal models have proven indispensable for investigating the mechanisms underlying these disorders. In a study by Tang et al. (2019), rats underwent an eight-week Chronic Mild Stress (CMS) protocol, a validated procedure for inducing prolonged stress and generating behaviors akin to depression and anxiety. This research identified several hippocampal protein candidates associated with susceptibility to stress-induced depression and anxiety, as well as stress resilience. These protein candidates were linked to specific molecular pathways mediating vulnerability or resistance to chronic stress, contributing to understanding the biological mechanisms underlying these psychiatric disorders (Tang et al., 2019).

A similar study by Hadipour et al. (2023) employed restraint stress to induce anxiety-like behaviors and emotional abnormalities. The authors tested the effects of hydroalcoholic extract from the spathe of the date palm (HEDPP) on stress-induced electrophysiological alterations. Findings revealed that HEDPP administration improved stress-induced impairments in learning and memory, reduced anxiety-like behaviors, and prevented adverse effects on synaptic plasticity in the hippocampus and amygdala (Hadipour et al., 2023). The use of rats as experimental models was critical for these studies, allowing for controlled experimental variables and replicable stress-induced behaviors, while laying a foundation for developing new therapeutic strategies.

In addition to these areas, regenerative medicine has emerged as an innovative approach focused on the regeneration or replacement of damaged cells, tissues, and organs, aiming to restore normal bodily functions. This therapeutic strategy relies on the unique properties of various types of stem cells, particularly their capacity for self-renewal, cellular differentiation, and immunomodulatory action. The latter enables modulation of the inflammatory process, thereby enhancing tissue regeneration in a more efficient manner (Fugger et al., 2020).

Qiu et al. (2020) investigated the enhancement of periodontal tissue regeneration using conditioned medium from mesenchymal stem cells (MSCs) derived from gingiva or periodontal ligaments. For this purpose, 90 rats were utilized as experimental models. Periodontal defects were surgically induced on the buccal side of the first molar in the left mandible of the animals, and the treatment involved applying MSC-conditioned medium (GMSC-CM). Results demonstrated that GMSC-CM significantly promoted periodontal regeneration in rats, suggesting its potential as a promising treatment for periodontal defects (Qiu et al., 2020).

Additionally, studies on pulmonary diseases have also benefited from using rats as experimental models. Nassar et al. (2022) explored the potential of stem cells in treating pulmonary fibrosis (PF), a terminal condition associated with various lung diseases. This study involved 32 Wistar rats treated with stem cells and sodium hydrosulfide (NaHS). Results indicated that the combination of stem cells and NaHS significantly inhibited pulmonary fibrosis, highlighting its potential to improve the prognosis of patients affected by this condition (Nassar et al., 2022).

Furthermore, the application of stem cells in rat models has explored various fields, including inflammatory modulation, bone tissue regeneration, skin lesion repair, and treatments for renal, hepatic, and ocular diseases (Gad et al., 2020; Barrientos et al., 2020; Cui et al., 2020; Ishiuchi et al., 2020; He et al., 2021; Nieto-Nicolau et al., 2021). These studies emphasize the versatility of rats as experimental models and their critical role in advancing innovative therapeutic strategies in regenerative medicine.

4. CONCLUSION

Although rodents have been essential in biomedical research, the need for more ethical and human-relevant approaches is becoming increasingly clear. Applying the 3Rs is not just about minimizing animal use; it also improves the quality and applicability of research. Advances in *in vitro or in sillico* models like organ-on-a-chip systems and computational methods offer promising alternatives, but their widespread adoption depends on scientific validation, regulatory approval, and investment in new technologies. Therefore, balancing traditional models with innovative approaches will be key to ensuring that biomedical research continues to advance in an ethical and scientifically rigorous way in the next years.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. The generative AI technology used was ChatGPT, which is based on large language models (LLMs) developed by OpenAI. The applied version is the most recent in the series, belonging to the GPT-4 (Generative Pre-trained Transformer 4) family. All of the generative artificial

intelligence behind ChatGPT was developed and trained by OpenAI.

2. The technology was used strictly to assist in the translation of the manuscript from Brazilian Portuguese to English for the purpose of publication in the current journal. After the translation, the text was reviewed again by team members to identify and correct any potential translation errors.
3. The input prompt used was: "Translate the text from Portuguese to English, without altering the meaning of the sentences."

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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