



Review Article

Errors, Contaminations, and Confounding Factors in *In vivo* Studies: Challenges and Practical Solutions

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ABSTRACT

In vivo studies remain a cornerstone of biomedical, pharmacological, and toxicological studies, providing critical insights into the safety and efficacy of novel interventions. However, the reliability and translational significance of these experiments are often compromised by methodological mistakes, hidden contaminations, and uncontrolled confounding factors. By identifying threats to validity and offering practical solutions, the current review aimed to enhance reproducibility, reduce unnecessary expenditure of time and resources, and improve the ethical and scientific integrity of *in vivo* studies. Poor study design, insufficient randomization, and operator-related inconsistencies introduce variability that may obscure true biological effects. Similarly, viral or microbial infections, environmental contaminants in feed or bedding, and cross-contamination between animals can profoundly alter immune, metabolic, or behavioral outcomes, often without being detected until results prove inconsistent. Furthermore, factors such as temperature, light cycles, handling stress, circadian rhythms, and biological characteristics of the animals introduce additional layers of complexity, leading to irreproducible or contradictory findings. The present study consolidated the existing evidence on the primary sources of errors, contamination, and confounding factors in *in vivo* studies, supported by practical case examples. Additionally, the present study emphasized best practices for mitigation, such as standardizing protocols, following animal research guidelines in *in vivo* experiments, utilizing specific-pathogen-free animals, continuously monitoring environmental and health parameters, and providing comprehensive staff training. Thus, emerging solutions such as automation, artificial intelligence, and the increasing incorporation of *in vitro* and *in silico* alternatives were explored as methods to decrease reliance on animal testing models.

1. Introduction

Preclinical *in vivo* experiments form the backbone of translational biomedical studies¹. Animal models remain essential for exploring complex physiological interactions, validating drug targets, characterizing pharmacokinetics and toxicology, and modeling disease processes that cannot yet be fully replicated *in vitro*². Well-designed *in vivo* studies generate mechanistic insights and serve as the main evidence base for determining first-in-human doses, safety margins, and difficult decisions for a drug development³. Beyond drug discovery, *in vivo* studies support vaccine development, biomarker validation, and regulatory toxicology, where

biological complexity, whole-organ system responses, and integrated immune and metabolic interactions are important⁴.

Despite their central role, *in vivo* studies are especially susceptible to sources of error and confounding factors that can significantly weaken internal validity, external generalizability, and translational potential reliability⁵. Methodological shortcomings such as insufficient randomization, absence of blinding, small sample sizes, and poor reporting can create systematic bias and increase false positive results rates^{6,7}. Biological and environmental deviations are equally important and

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often go unnoticed in standard methods sections. These include subclinical infections, batch differences in diet, vendor-dependent microbiomes, subtle shifts in housing conditions such as temperature, light, and noise, and operator-dependent handling techniques. These factors can alter fundamental biology by modulating immune tone, metabolism, behavior, and drug disposition, thereby changing the direction or magnitude of an effect⁸. The result is not just scientific ambiguity but also ethical and economic inefficiency. Non-reproducible findings cause repeated experiments, misuse of animals and resources, and hinder or misdirect the translation of clinical applications⁹.

Compounding the problem, many of these confounders remain underreported or unmeasured¹⁰. Standard reporting practices often omit critical metadata such as cage density, circadian timing of procedures, feed lot or batch numbers, or pathogen screening results that would enable readers and reviewers to assess reproducibility risks or to replicate experiments faithfully¹¹. When such metadata are absent, explanatory post hoc analyses are severely constrained, and potential systematic errors go undetected until costly clinical efforts have begun¹². Moreover, the interplay of multiple small biases can produce large, non-linear effects. A low-grade pathogen combined with a high-phytoestrogen diet and inconsistent handling may together produce an apparent drug effect that is wholly artifactual¹².

Practical, field-oriented solutions exist; however, their adoption remains limited uneven¹³. Guidelines and checklists aimed at improving design and reporting in randomization, blinding, and sample size justification have been published and adopted in many journals. Yet, adherence varies, and guideline checklists alone do not eliminate biological confounders¹⁴. Technical mitigations, such as regular pathogen surveillance, stable sourcing of dietary ingredients, environmental monitoring, personnel training, and routine instrument calibration, help reduce known risks. However, their implementation requires a robust laboratory infrastructure, prepared staff, and clear standard operating procedures (SOPs)¹⁵. Finally, emerging technological tools, including automation, telemetry, continuous environmental logging, and advanced statistical methods, have demonstrated promising results in detecting and correcting hidden variability; however, practical examples and best-practice workflows remain to be consolidated for routine use^{16,17}.

The present study aimed to identify key sources of error, contamination, and confounding in modern *in vivo* studies across pharmacology, toxicology, and disease modeling; demonstrate these issues with real-world examples showing how they distort biological inference; and provide practical, prioritized mitigation strategies and checklists that laboratories can easily implement affordably.

2. Experimental errors

Experimental errors represent one of the most preventable yet most pervasive threats to validity in *in vivo*

studies¹⁸. Unlike biological or environmental confounders, which may be more challenging to detect and control, errors at the level of study design and execution directly reflect human decisions and methodological rigor¹⁹. These errors can be broadly categorized into flaws in experimental design and human or technical errors occurring during implementation.

2.1. Design-related errors

A fundamental design flaw in many preclinical studies is inadequate sample size²⁰. Underpowered experiments not only increase the risk of false negatives but also tend to overestimate effect sizes when they reach statistical significance²¹. This phenomenon, sometimes referred to as the winner's curse, has been documented across fields ranging from behavioral neuroscience to oncology^{22,23}. Small cohorts amplify the impact of random biological variability, making it difficult to distinguish true medicine effects from random fluctuations²⁴. Randomization can distort effect estimates and reduce comparability between groups; the use of suitable controls is equally important²⁵. Many studies rely on only a single control group or fail to match controls for key factors, such as sex, age, or handling conditions. In toxicology and pharmacology, ignoring vehicle controls or overlooking circadian timing can result in incorrectly attributing effects to the experimental compound instead of underlying physiological rhythms or the excipient properties^{17,22}.

2.2. Human and instrumental errors

Even when study designs are theoretically sound, errors during execution can compromise results. Operator skill varies greatly, and in fields such as behavioral testing, surgical procedures, or dosing by gavage, small differences in technique can affect stress responses and medicine absorption²⁶. Lack of proper training or inconsistent adherence to protocols causes variability in outcomes between technicians or even within the same laboratory over time²⁷.

Instrumental issues are equally important. Uncalibrated balances, pipettes, or analytical instruments can cause systematic measurement errors²⁸. In long-term studies, equipment accuracy drift often remains unnoticed until differences between datasets become apparent²⁹. Additionally, inconsistent data recording or dependence on manual input without proper audit trails increases the likelihood of transcription errors and selective reporting³⁰.

2.3. Common methodological errors

The reproducibility crisis in cancer biology indicated how design and operator mistakes lead to unreliable results³¹. Independent efforts to repeat studies have found that many well-known preclinical cancer studies cannot be replicated, mainly due to small sample sizes and incomplete randomization³². Similarly, in neuroscience, differences in handling techniques and a lack of blinded outcome assessment have led to conflicting results regarding the effectiveness of antidepressants in rodent models²². Another example is about toxicology, where the

failure to include properly matched vehicle controls resulted in false warnings about a compound's toxicity²². Re-analysis later showed that the adverse effects were caused by contaminants in the delivery medium, not the active compound³³. These examples demonstrated how simple oversights during the design phase can waste resources and lead to misleading scientific claims.

Collectively, these errors reduce reproducibility, increase false discovery rates, and slow the translation of promising preclinical findings into clinical benefits. Importantly, they are mostly preventable through rigorous design, transparent reporting, proper training, and systematic equipment management calibration³⁴. Being aware of these challenges and implementing concrete procedural safeguards is the first step towards reducing error-driven bias in *in vivo* science.

3. Contaminations

While experimental design errors often stem from methodological choices, contaminations represent hidden biological and environmental intrusions that compromise the integrity of *in vivo* studies³⁵. These issues are harmful because they often go unnoticed until late in a study or even after publication, when inconsistencies across different laboratories emerge. Contaminations can be categorized into microbial or viral infections, environmental exposures, and cross-contamination across experiment groups³⁶.

3.1. Microbial and viral infections in laboratory animals

Laboratory rodents, even when kept under specific-pathogen-free (SPF) conditions, can carry subclinical infections that influence physiology and immune responses³⁷. Murine norovirus (MNV) has been shown to alter baseline immune activation and affect susceptibility to experimental models of colitis and cancer²³. Likewise, infection with *Helicobacter hepaticus* (*H. hepaticus*) can lead to spontaneous intestinal inflammation and hepatocellular carcinoma, which can significantly impact toxicological or immunological assessments³⁸. These infections may remain asymptomatic, meaning that routine observation of animals does not reveal any apparent signs of illness. However, they can subtly deviate results by exaggerating or masking the effects of treatment³⁹. Significantly, because different vendors and facilities have varying standards in microbial monitoring and husbandry, reproducibility between laboratories can be compromised when unrecognized infections differ among study sites⁴⁰.

3.2. Environmental contaminations

Even when animals do not have visible pathogens, environmental factors are a significant source of contamination. Diet often goes unnoticed as a confounding element; differences in feed composition among suppliers or even variations in batches from the same supplier can greatly affect endocrine, metabolic, and behavioral results⁴¹. The phytoestrogen content in soy-based diets has been repeatedly linked to varying results in

reproductive toxicology and cancer studies. Water quality is another important factor⁴². Trace contaminants such as heavy metals, residual disinfectants, or microbial byproducts in animal drinking water can affect physiology and experimental outcomes⁴³. Similarly, volatile compounds or cleaning chemicals present in the housing environment might serve as unintentional exposures, particularly in studies assessing endocrine or neurobehavioral outcomes⁴⁴.

3.3. Cross-contamination

Cross-contamination occurs when animals, cages, or personnel accidentally transfer biological material between experimental groups⁴⁵. This can happen through shared equipment, improper handling of bedding, or inadequate personal protective practices⁴⁶. For instance, pathogens such as pinworms or *Helicobacter* species can spread via contaminated gloves or instruments, jeopardizing the separation between treatment and control groups⁴⁴. Besides pathogens, microbial transfer between cages, such as fecal microbiota exchange through airborne particles or bedding dust, can homogenize microbiomes across groups^{44,46}. Such transfer undermines efforts to compare treatment conditions that are believed to act through microbiome-mediated mechanisms pathways⁴⁷.

3.4. Practical case examples

Several documented cases illustrated the impact of contamination on *in vivo* science⁴⁸. A well-known example involved MNV contamination across multiple vivariums, which led to the retraction and repetition of immunology studies after it was discovered that the viral status fundamentally altered experimental outcomes⁴⁹. In another case, an endocrine toxicology study was postponed because it was discovered that varying levels of phytoestrogens in feed batches caused inconsistent estrogenic activity, obscuring the actual effects of the compound being studied⁵⁰. Similarly, contamination with *H. hepaticus* resulted in false-positive findings of medicine-induced colitis in rodent models, which was later recognized as infection-driven pathology⁵¹. These examples demonstrated how contamination can disrupt studies, lead to resource waste, and threaten the validity of results credibility⁵¹.

Contaminations pose a dual challenge that is hard to detect without proactive monitoring, and their effects can be significant⁵². Unlike random fluctuations, which merely increase variability, contaminations frequently introduce systematic bias, resulting in a consistent shift of the baseline physiology⁵³. Regular microbial monitoring, strict environmental management, verified sourcing of feed and water, and rigorous cage and staff protocols are crucial. Ignoring these safety measures can result in failed experiments and misguided translational efforts⁵⁴.

4. Confounding factors

Confounding factors are nuanced variables that can systematically influence results, yet they have no connection

to the experimental hypothesis, unlike apparent errors or contaminations⁵⁵. The role of confounding factors is to distort the perceived connection between an intervention and its observed effect, often without acknowledgment or reporting⁵⁴. In *in vivo* studies, confounders originate from environmental factors, the inherent biological traits of the animals, handling procedures, and physiological variables rhythms⁵⁶. Managing these factors is challenging because many are inherent to standard animal care or experimental procedures^{17,54}.

4.1. Environmental conditions

Light, temperature, ventilation, and background noise are foundational elements of animal housing that strongly shape physiology⁵⁷. Circadian-regulated processes such as hormone secretion, feeding, and metabolism are entrained by light-dark cycles; altering light intensity, spectrum, or timing can therefore shift experimental outcomes in metabolic or behavioral studies⁵⁸. Similarly, ambient temperature is critical because most laboratory rodents are housed below their thermoneutral zone, which elevates baseline sympathetic activity and energy expenditure⁵⁹. This overlooked stressor affects cardiovascular and metabolic outcomes, making it harder to interpret the effectiveness or toxicity of treatments⁵⁶. Noise and vibration are additional factors that can also act as confounders⁵⁶. Even modest acoustic disturbances from equipment or human activity can elevate stress hormones and alter neurobehavioral readouts⁶⁰. Poor ventilation or fluctuating air exchange rates may further exacerbate stress or immune responsiveness⁶¹. Variability across facilities and difficulties in inter-study comparisons can occur if these environmental conditions are not standardized or reported⁶².

4.2. Biological characteristics of animals

Intrinsic characteristics of the animals themselves often cause unexpected differences⁶³. Age is a particularly strong factor⁶⁴. Young rodents tend to show heightened regenerative or metabolic responses, while older animals display immune senescence, comorbidities, and altered pharmacokinetics⁵⁰. Sex differences introduce another layer; hormonal cycles in females or sex-specific gene expression patterns influence outcomes in cardiovascular, neurological, and immunological models⁶⁵. Traditionally, many studies have used only male animals to avoid perceived variability; however, this approach has decreased external validity and obscured sex-specific effects relevant to humans⁶⁶. Strain and genetic background also affect responses⁶⁷. The C57BL/6 mice differ markedly from BALB/c in immune and metabolic phenotypes, leading to divergent results in infection or diabetes models⁶⁸. Metabolic status, such as diet-induced obesity or latent insulin resistance, introduces yet another layer of variability, particularly in toxicology and pharmacology studies where drug disposition is closely linked to metabolic health⁵⁴.

4.3. Stress and handling

Handling procedures exert strong, often underestimated influences on animal physiology⁶⁸. The method of restraint, frequency of injections, or even the familiarity of the handler can alter corticosterone levels, immune tone, and behavior⁶⁹. Repeated intraperitoneal injections may sensitize animals to procedural stress, confounding the interpretation of treatment-related effects⁶⁹. Studies in rodents have demonstrated that handling techniques such as tail picking versus tunnel handling produce divergent anxiety-like behaviors in standard assays, underscoring how procedural nuances shape outcomes⁷⁰.

4.4. Inter-individual and physiological rhythms

Biological processes differ dynamically throughout the circadian cycle, and ignoring the timing can lead to significant issues⁷¹. The absorption, metabolism, and toxicity of medicine often vary depending on whether administration occurs during the light or dark phase⁵⁷. Likewise, immune responses and hormonal secretion exhibit circadian oscillations that can either mask or exaggerate treatment effects. Inter-individual variation, even within genetically identical inbred strains, further complicates analysis, and minor differences in microbiome composition or early-life experiences can manifest as measurable physiological divergence⁷².

4.5. Practical case examples

Several cases illustrated how confounders derail experimental interpretation³³. In metabolic studies, variations in glucose tolerance test results across laboratories were due to differences in fasting times and the timing of the tests in relation to the circadian cycle⁴⁸. In neurobehavioral studies, differing outcomes for anxiety-reducing medicine were connected not to the pharmacology of the medicine but to handling procedures during testing⁷¹. In toxicology, inconsistent evidence of liver toxicity in candidate compounds was later attributed to strain-specific vulnerabilities rather than the inherent toxicity of the compounds⁷³.

Confounders pose particular challenges because they are not errors, but genuine biological and environmental factors that need to be acknowledged, controlled, or transparently reported³³. Their impact can be reduced by standardizing housing conditions, including both sexes in the study design, stratifying analyses by age or strain using low-stress handling techniques, and carefully timing experiments in relation to circadian cycles^{66,72}. Being transparent about these variables enables others to interpret and replicate the findings more accurately, ultimately enhancing the translational relevance of *in vivo* studies⁷⁴. The prevalent issues compromising *in vivo* studies' integrity, categorizing them as experimental errors, contaminations, or confounding factors, are summarized in [Table 1](#).

Table 1. Summary of common errors, contaminations, and confounding factors in *in vivo* studies and their mitigation strategies

Category	Type of error, contamination, and confounder	Source/cause	Effect on the study	Mitigation/control
Experimental errors	Poor design, including a small sample size, inadequate randomization, and insufficient controls	Study design decisions	Inflated random effects, reduced validity	Adequate sample size calculation, rigorous randomization, and properly matched control groups
Experimental errors	Human/operator error	Inadequate skills, protocol deviations	Increased uncontrolled variability	Staff training and assessment, adherence to SOPs, and instrument calibration
Contaminations	Viral/microbial	MNV, <i>Helicobacter</i> , pinworms	Altered immune response, inflammation, metabolic changes	Use SPF animals, regular pathogen screening, and microbiome management
Contaminations	Environmental	Diet, water, bedding, and chemical exposure	Metabolic, hormonal, or behavioral alterations	Standardized feed and water, environmental monitoring, control of bedding and chemicals
Contaminations	Cross-contamination	Animal transfer, personnel, shared equipment	Group mixing, microbiome homogenization	The SOPs for handling and equipment, personnel training, and preventing cross-contact
Confounding factors	Environmental conditions	Light, temperature, noise, ventilation	Hormonal, behavioral, or drug response changes	Environmental monitoring, strict control of housing parameters
Confounding factors	Animal characteristics	Age, sex, strain, metabolic status	Differential drug responses, phenotype variability	Appropriate animal selection, detailed reporting, and stratified analysis
Confounding factors	Handling/stress	Repeated injections, the restraint method	Elevated stress, altered immune or behavioral responses	Low-stress handling techniques, standardization of procedures
Confounding factors	Circadian rhythm	Timing of dosing or sampling	Altered metabolism, hormone levels, and medication response	Standardize timing of interventions, report schedules precisely

MNV: Murine norovirus, SPF: Specific-pathogen-free, SOPs: Standard operating procedures

5. Strategies to reduce errors and contamination

Mitigating sources of error and contamination in *in vivo* studies requires a systematic approach that integrates methodological rigor, standardized practices, and continuous quality assurance. While some variability is inevitable in biological systems, many sources of bias can be prevented with careful planning and adherence to established best practices⁷⁵.

5.1. Standardization of protocols

Developing and enforcing detailed SOPs ensures consistency across experiments, operators, and laboratories⁷⁶. Protocol standardization should extend beyond dosing schedules and outcome measures to include animal handling, housing conditions, feeding regimens, and data recording practices⁷⁷. Detailed SOPs reduce operator-dependent variability, facilitate training of new personnel, and support inter-laboratory reproducibility⁷⁸. Importantly, standardized documentation allows deviations to be identified and analyzed, rather than remaining hidden sources of variability.

5.2. Implementation of reporting guidelines

Transparent reporting is essential for reproducibility and critical appraisal⁷⁵. The animal studies provided a structured framework for reporting experimental design elements, including randomization, blinding, sample size determination, and animal characteristics⁷⁹. Adoption of

such guidelines enables reviewers and readers to evaluate methodological rigor and identify potential risks of bias⁵⁴. Journals and funding agencies increasingly require adherence to reporting checklists. However, their real influence relies on researchers incorporating them as a core part of study design⁸⁰.

5.3. Use of specific pathogen-free animals

Maintaining colonies under SPF conditions minimizes the risk of hidden microbial or viral infections that can alter physiology and immune status⁸¹. Routine health monitoring, including screening for MNV, *Helicobacter* spp., and pinworm infestations, further reduces the likelihood of undetected infections⁸². Although SPF facilities require significant resources, the investment pays off in improved data reliability and decreases the need for costly rework of contaminated samples experiments⁶⁵. For studies where immune challenges are intrinsic to the design, controlled introduction of pathogens or dirty microbiota can be performed intentionally rather than as inadvertent confounders³³.

5.4. Training and calibration of research teams

Human error remains one of the most persistent sources of variability⁸³. Continuous training programs ensure that staff are proficient in handling techniques, dosing procedures, and data recording⁷³. Inter-operator calibration, where multiple technicians perform the same procedure and compare outcomes, helps identify discrepancies early⁸¹. Regular refresher sessions and competency assessments promote uniformity across

personnel and prevent skill drift over time⁸¹. Cultivating a laboratory culture that values precision and accountability is as important as technical training itself⁸⁴.

5.5. Continuous monitoring of environment and animal health

Environmental monitoring systems, which track temperature, humidity, light cycles, and noise, offer crucial context for understanding experimental data outcomes⁸⁵. Automated sensors and digital logs minimize the need for manual checks, facilitating the detection of deviations⁸³. Likewise, ongoing monitoring of animal health, encompassing body weight, clinical scores, and microbiome analysis, enables prompt intervention when confounding factors arise⁷⁹. Recording these parameters improves reproducibility and supports the ethical refinement of animal studies principles. These strategies create a multi-layered defense against both overt errors and hidden confounders⁸⁶. Standardization ensures procedural consistency, guidelines enforce transparency, SPF animals

minimize biological noise, training reduces human variability, and monitoring provides real-time assurance of environmental and animal health⁸⁷. While no system can eliminate variability entirely, implementing these safeguards transforms *in vivo* studies from a fragile enterprise into a robust, reproducible foundation for translational science⁸⁸.

6. Challenges and future directions

Despite significant advances in experimental design, housing standards, and quality control, achieving full control over all confounding factors in *in vivo* studies remains unattainable⁸⁶. Animal models, by their nature, encompass complex biological variability that cannot be fully standardized⁸⁹. The challenge, therefore, is not the complete elimination of variability, but the transparent recognition, minimization, and reporting of sources of bias, so that results remain interpretable and reproducible (Figure 1).

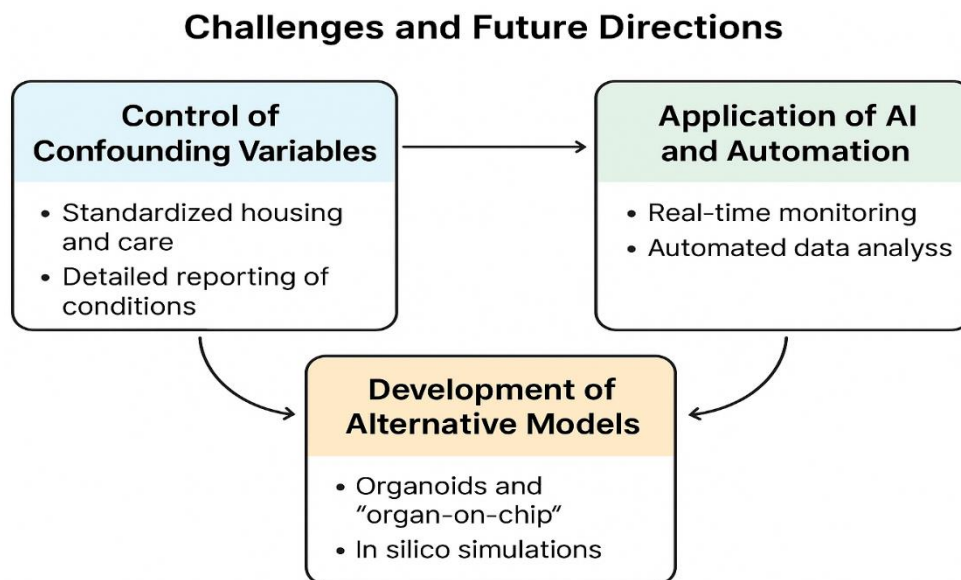


Figure 1. Challenges and future directions in *in vivo* studies

6.1. Limits of controlling confounding variables

Even in highly controlled SPF facilities, hidden variability persists⁸⁶. Subtle differences in microbiome composition, early-life experiences, or genetic drift within inbred strains can influence phenotype⁹⁰. Attempts to tightly regulate one variable, such as diet, may inadvertently amplify another, including stress due to dietary restriction. Moreover, environmental parameters such as noise, vibration, or light spectrum cannot be fully homogenized across facilities, meaning that cross-laboratory reproducibility will always face inherent challenges^{5,54,86}. Recognizing these limitations shifts the goal from absolute control to systematic documentation and mitigation⁹¹.

6.2. The Central role of transparency

Transparency in reporting experimental conditions is

crucial for scientific integrity⁹⁰. Underreporting of seemingly minor details such as light cycle timing, cage density, or feed batch can obscure critical confounders⁸⁹. Additionally, the ARRIVE guidelines have been established to emphasize comprehensive methodological reporting as a prerequisite for publication or funding⁹⁰. Standardized metadata frameworks, shared databases, and digital laboratory notebooks offer tools to make such reporting more practical and consistent across study environments⁹¹.

6.3. Artificial intelligence and automation

Emerging technologies promise new insights for error reduction. Automated cage monitoring systems can continuously log environmental variables, detect behavioral anomalies, and reduce reliance on subjective human observations⁹². Artificial intelligence tools are being developed to analyze complex datasets, flagging anomalies

that may indicate hidden confounders or equipment drift⁹³. Machine learning algorithms can also assist in experimental design, suggesting optimal randomization strategies and sample sizes based on historical datasets⁶⁵. While these technologies are not yet universally implemented, their integration into preclinical workflows could transform quality assurance from a reactive to a proactive process⁶⁸.

6.4. The rise of *in vitro* and *in silico* alternatives

An important frontier is the gradual reduction of reliance on animal models through alternative approaches². Advanced organoid systems, microfluidic organ-on-chip platforms, and computational modeling offered increasingly sophisticated means to simulate human biology *in silico*⁹². Pharmacokinetic and toxicological models are already being applied in regulatory contexts, while *in vitro* systems provided human-specific mechanistic insights that animal models sometimes fail to capture^{2,38}.

The future of *in vivo* science will not be defined by the elimination of variability, but by more intelligent management of it⁸⁶. Combining transparency, technological innovation, and integration of alternative models provides a realistic path forward^{2,90,92}. Animal studies will remain essential in the near term, but their credibility and efficiency will increasingly rely on laboratories adopting automation, thorough reporting, and hybrid experimental systems that combine *in vivo*, *in vitro*, and *in silico* methods.

7. Conclusion

The reliability and translational value of *in vivo* studies depend on how rigorously and precisely experiments are designed and conducted. Experimental errors, hidden microbial or viral contaminations, environmental inconsistencies, and biological confounders collectively threaten reproducibility and can distort the interpretation of results. Addressing these challenges through standardized protocols, rigorous reporting, use of SPF animals, operator training, and environmental monitoring is essential. Future studies should proactively manage these sources of variability to improve scientific validity, reduce unnecessary expenditure of time, resources, and animal lives, and ultimately enhance the ethical and translational impact of preclinical study.

Declarations

Authors' contributions

Hadis Farokhmoradi and Faezeh Salari-Kakhk have done the conceptualization, literature search, data extraction, and manuscript drafting. Both authors read and approved the final edition of the manuscript.

Availability of data and materials

The data of the present study can be obtained upon reasonable request.

Competing interests

The authors declared no conflict of interest related to the

design, execution, interpretation, or publication of this study.

Ethical considerations

As this study was a systematic review of publicly available literature, no ethical approval or informed consent was required. All data used were from peer-reviewed, published sources, and ethical standards regarding citation, attribution, and data integrity were fully observed.

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