ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

VIÉS DE SEXO EM PESQUISAS COM ANIMAIS: IMPLICAÇÕES BIOÉTICAS, CIENTÍFICAS E PARA A EQUIDADE EM SAÚDE

SEX BIAS IN ANIMAL RESEARCH: BIOETHICAL AND SCIENTIFIC IMPLICATIONS FOR HEALTH EQUITY

SESGO DE SEXO EN LA INVESTIGACIÓN CON ANIMALES: IMPLICACIONES BIOÉTICAS, CIENTÍFICAS Y PARA LA EQUIDAD EN SALUD

Bruno Luis Lima Soares
Master, UFMA, Brasil
E-mail: brunoluissoares@gmail.com

Luana Mara Batista Sousa
Specialist, IFFar, Brasil
E-mail: luanamarabsousa@gmail.com

Geraldo Gomes de Oliveira Junior
Doctor, IFSULDEMINAS, Brasil.
E-mail: geraldo.junior@muz.ifsuldeminas.edu.br

Resumo

A experimentação pré-clínica apresenta um viés de sexo sistemático, caracterizado pela predominância de animais machos, omissão do sexo como variável e escassez de análises estratificadas, comprometendo a validade científica, a reprodutibilidade e a translação clínica, além de aprofundar desigualdades em saúde. Esta revisão integrativa objetivou identificar as implicações bioéticas desse viés, sintetizando práticas metodológicas e suas consequências para a aplicabilidade dos resultados e a equidade em saúde. A busca foi realizada nas bases PubMed, ScienceDirect, SciELO e Google Acadêmico, incluindo estudos publicados entre 2010 e 2025, em português e inglês. Os dados foram organizados em três eixos: abordagem metodológica do sexo; implicações éticas à

ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

luz dos 3Rs; e impactos na qualidade e generalização dos achados. Os resultados evidenciam que o viés masculino é endêmico em áreas como neurociência, cardiologia, cirurgia e parasitologia, manifestando-se tanto pelo uso exclusivo de machos quanto pela omissão do sexo. Mesmo com a inclusão de ambos os sexos, persistem falhas analíticas, como o agrupamento de dados sem teste de interação. A exclusão de fêmeas, historicamente justificada pela variabilidade do ciclo estral, foi refutada. Observa-se ainda a má interpretação do princípio da Redução, gerando excedente biológico e prejuízo ao bem-estar animal. Conclui-se que o viés de sexo é um problema bioético que exige mudança cultural, letramento estatístico e atuação das CEUAs para garantir validade científica e equidade em saúde.

Palavras-chave: Bioética; Equidade em saúde; Pesquisa pré-clínica; Princípios dos 3Rs; Viés de sexo.

Abstract

Preclinical research exhibits a systematic sex bias, characterized by the predominance of male animals, omission of sex as a biological variable, and limited use of stratified analyses. This bias compromises scientific validity, reproducibility, and clinical translation, while reinforcing health inequalities. This integrative review aimed to identify the bioethical implications of sex bias in animal research, synthesizing methodological practices and their consequences for the applicability of findings and health equity. A literature search was conducted in PubMed, ScienceDirect, SciELO, and Google Scholar, including studies published between 2010 and 2025 in English and Portuguese. Data were organized into three analytical axes: methodological approaches to sex in experimental design; ethical implications based on the 3Rs principles; and impacts on the quality and generalizability of results. Findings indicate that male bias is endemic across fields such as neuroscience, cardiology, surgery, and parasitology, expressed through both exclusive use of males and failure to report sex. Even when both sexes are included, analytical limitations persist, particularly the pooling of data without testing for interaction effects. The exclusion of females, historically justified by estrous cycle variability, has been refuted. Misinterpretation of the Reduction principle also contributes to biological surplus and compromised animal welfare. Sex bias in preclinical research constitutes a bioethical issue requiring cultural change, statistical literacy, and stronger oversight by Animal Ethics Committees to ensure scientific validity and health equity.

Keywords: 3Rs principles; Bioethics; Health equity; Preclinical research; Sex bias.

Resumen

La investigación preclínica presenta un sesgo de sexo sistemático, caracterizado por la predominancia

ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

de animales machos, la omisión del sexo como variable biológica y la escasa utilización de análisis estratificados. Este sesgo compromete la validez científica, la reproducibilidad y la traslación clínica de los hallazgos, además de profundizar las desigualdades en salud. Esta revisión integradora tuvo como objetivo identificar las implicaciones bioéticas del sesgo de sexo en la investigación con animales, sintetizando las prácticas metodológicas y sus consecuencias para la aplicabilidad de los resultados y la equidad en salud. La búsqueda se realizó en PubMed, ScienceDirect, SciELO y Google Académico, incluyendo estudios publicados entre 2010 y 2025 en inglés y portugués. Los datos se organizaron en tres ejes analíticos: abordaje metodológico del sexo en los diseños experimentales; implicaciones éticas a la luz de los principios de las 3R; e impactos en la calidad y generalización de los resultados. Los hallazgos evidencian que el sesgo masculino es endémico en áreas como neurociencia, cardiología, cirugía y parasitología, manifestándose tanto en el uso exclusivo de machos como en la omisión del sexo en los reportes. Incluso cuando se incluyen ambos sexos, persisten limitaciones analíticas, como la agrupación de datos sin pruebas de interacción. La exclusión de hembras, históricamente justificada por la variabilidad del ciclo estral, ha sido refutada. Se concluye que el sesgo de sexo constituye un problema bioético que exige cambios culturales, alfabetización estadística y una mayor actuación de los comités de ética para garantizar la validez científica y la equidad en salud.

Palabras clave: Bioética; Equidad en salud; Investigación preclínica; Principios de las 3R; Sesgo de sexo.

1. Introduction

Animal-based biomedical research is indispensable to the advancement of scientific knowledge and the development of health interventions. However, there is broad consensus in the literature that such research is characterized by a systematic and persistent sex bias, defined by the predominance of male subjects, the omission of sex as a reportable variable, and the scarcity of sex-stratified analyses (Zucker; Beery, 2010; Beery; Zucker, 2011; Karp *et al*., 2025). The exclusion of females has been historically rationalized on several grounds: the assumption that hormonal variability associated with the estrous cycle would compromise experimental homogeneity; the premise that findings derived from male subjects are generalizable to females; and a broader androcentrism that treats the male as the default biological reference and female-specific variation as anomalous (Beery; Zucker, 2011).

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

For the purposes of this study, the following conceptual distinctions are adopted: "sex" refers to a biological variable determined by chromosomal, gonadal, and hormonal characteristics (NIH, 2015; Plevkova *et al*., 2020), whereas "gender" denotes a sociocultural construct that organizes identities, roles, and social relations (Plevkova *et al*., 2020; Gualtierotti, 2025). In preclinical animal research, the primary focus is necessarily on biological sex; however, the clinical and social consequences discussed throughout this review also involve gender-mediated dynamics, which will be explicitly signaled where relevant. The same distinction applies to "health equity," understood here as the diagnostic and therapeutic adequacy to the biological differences between sexes, ensuring safety, efficacy, and differentiated clinical responsiveness for both men and women (Whitehead, 1992; Warren; Garrett; Frame, 2025).

This pervasive pattern carries substantial scientific and social consequences. From a methodological standpoint, it undermines the validity, reproducibility, and clinical translation of research findings (Zucker; Beery, 2010; Beery; Zucker, 2011; Flórez-Vargas *et al*., 2016; Will *et al*., 2017; Becegato; Silva, 2022; Karp *et al*., 2025). From a social standpoint, it deepens health inequities by generating a body of evidence that inadequately reflects the biological diversity between men and women. Although studies have demonstrated that female animals perform comparably to males across a wide range of experimental models — thereby undermining the traditional rationale for their exclusion — entrenched barriers to reform remain (Becegato; Silva, 2022; Karp *et al*., 2025).

These barriers include culturally ingrained misconceptions, such as the belief that including females increases data variability, reduces statistical power, or necessitates larger sample sizes — all of which contradict the Reduction principle of the 3Rs framework. Logistical challenges, including the need for individual housing of aggressive males, and resistance to methodological change further impede progress (Karp *et al*., 2025). The systematic exclusion of females also contravenes recommendations issued by major funding and regulatory bodies. The National

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

Institutes of Health (NIH) formally recognizes sex as a biological variable essential to experimental design (NIH, 2015), and current practices are in direct conflict with the ethical principles governing animal experimentation — particularly the Refinement principle, proposed by Russell and Burch (1959) within the 3Rs framework, which mandates the continuous improvement of methodologies in the service of scientific quality and ethical integrity.

The 3Rs — Replacement, Reduction, and Refinement — were formulated by Russell and Burch (1959) as an ethical framework to guide animal experimentation in a humane and scientifically rigorous manner. Replacement advocates the adoption of alternative methods that avoid the use of animals whenever feasible; Reduction aims to minimize the number of animals used without compromising the statistical validity of experiments; and Refinement seeks to continuously improve procedures and husbandry conditions to reduce suffering and promote animal welfare (Russell; Burch, 1959; Fenwick; Griffin; Gauthier, 2009). These principles have been progressively incorporated into legislation and research funding policies across numerous countries, becoming the normative cornerstone of bioethics applied to preclinical research (Tannenbaum; Bennett, 2015).

In the context of sex bias, the 3Rs acquire particular salience. The exclusion of females not only violates the Refinement principle by disregarding biological variables that directly influence both animal welfare and experimental responses, but also subverts the Reduction principle by paradoxically generating systematic culling of the excluded sex in animal facilities, resulting in the gratuitous loss of animal lives (Nunamaker; Turner, 2023). Far from constituting an ethically neutral practice, the exclusive use of male subjects represents a structural violation of the very principles that should govern responsible animal research.

1.1 General Objective

This study aims to examine how sex bias has been addressed in animal research

ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

and to characterize its bioethical and methodological implications in light of the 3Rs principles.

2. Materials and Methods

This study employs an integrative literature review (ILR), a qualitative methodology that enables the synthesis of evidence from studies employing diverse research approaches (Sousa; Bezerra; Egypto, 2023). The investigation followed the six-stage framework proposed by those authors: formulation of the guiding question, establishment of eligibility criteria and search strategy, study selection, data extraction and analysis, and interpretation of results. The guiding question was: How has sex bias been addressed in animal research, and what are its bioethical and methodological implications in light of the 3Rs principles?

The literature search was conducted across PubMed, ScienceDirect, SciELO, and Google Scholar, using controlled and uncontrolled descriptors in both Portuguese and English, combined through Boolean operators AND and OR: "viés de sexo," "sex bias," "animal research," "preclinical studies," "bioethics," "3Rs," "female exclusion," "biological sex variable," and "pesquisa pré-clínica." Eligible studies were those published between 2010 and 2025 in English or Portuguese that addressed differential treatment of male and female animals in biomedical research or examined the ethical and methodological implications of such distinctions.

Studies that did not specify animal sex, conference abstracts, dissertations, theses, and texts without full-text access were excluded. Given the integrative nature of the review, however, sources beyond primary studies and structured reviews were also included — specifically critical essays, reply commentaries, narrative reviews, and institutional documents — provided they were directly relevant to the research question and fully accessible. Each document type is identified throughout the analysis to preserve the epistemological distinction between empirical evidence, normative argumentation, and institutional positioning.

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

As this study constitutes a bibliographic review without direct intervention involving animals or human subjects, submission to an Animal Ethics Committee (CEUA) was not required. The study was conducted in accordance with the principles of scientific integrity outlined by Rosaneli and Fischer (2024).

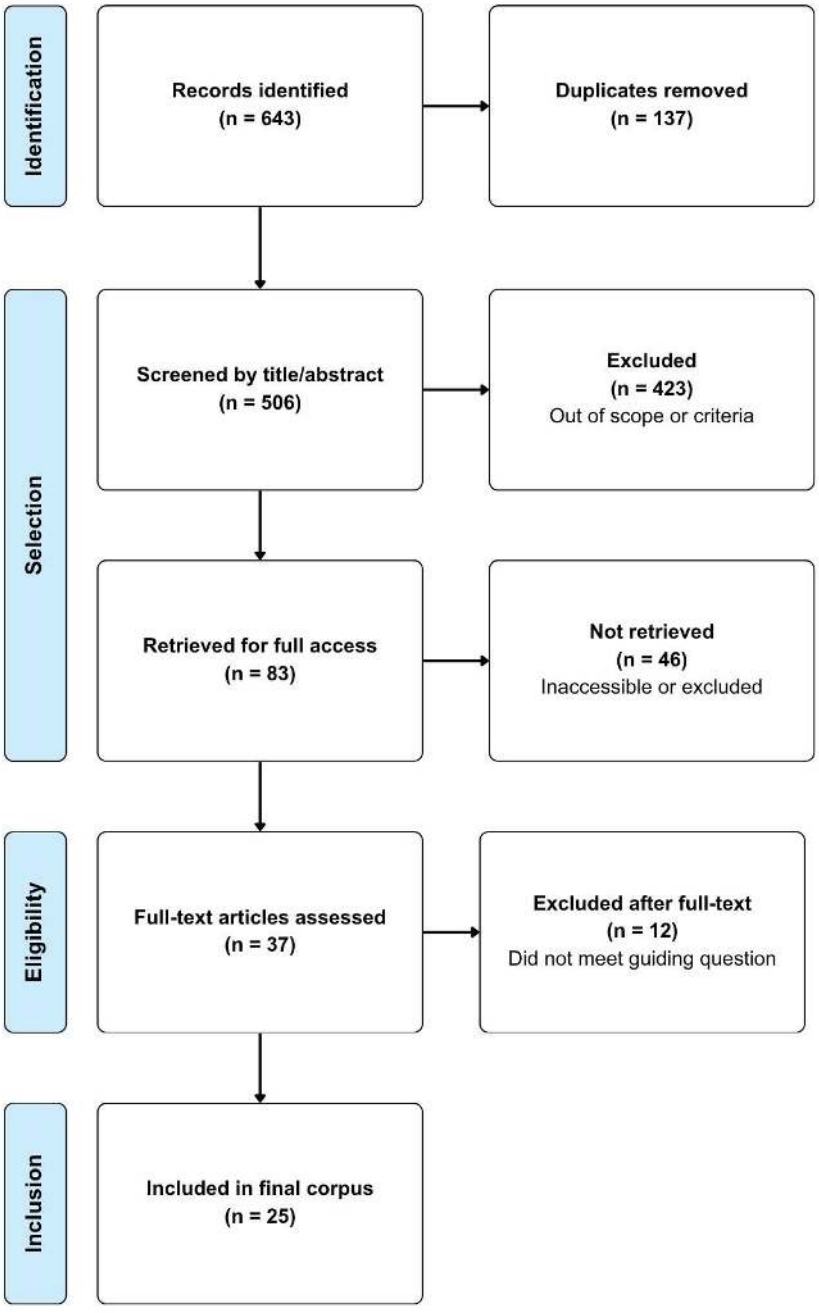
Data were organized into three analytical axes: (I) sex bias in preclinical animal research and methodological approaches to sex in experimental design; (II) ethical implications of female exclusion in light of the 3Rs principles; and (III) consequences of sex bias for the quality, applicability, and generalizability of scientific findings, with emphasis on health inequities. This structure guided the analysis and synthesis of evidence pertaining to the central bioethical questions of the review (Rosaneli; Fischer, 2024). A qualitative content analysis approach was applied, encompassing pre-analysis, material exploration, and interpretation of results. The process identified convergences, divergences, and gaps across studies and mapped findings onto the predefined analytical categories. The discussion drew on the selected studies and the theoretical framework, consistent with guidelines for integrative reviews in Bioethics (Sousa; Bezerra; Egypto, 2023; Rosaneli; Fischer, 2024).

The search retrieved a total of 643 records across all databases. Following the removal of 137 duplicates, 506 records were screened by title and abstract, of which 423 were excluded for failing to meet eligibility criteria. The remaining 83 documents were submitted for full-text retrieval; of these, 46 were discarded due to inaccessibility or incompatibility with the inclusion criteria. The remaining 37 texts were read in full, and 12 were excluded for not adequately addressing the guiding question. The final analytical corpus comprised 25 documents, the full selection process for which is summarized in Figure 1.

ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

Figure 1. Study selection flowchart (adapted from Page *et al*., 2021).



Source: elaborated by the authors.

For critical source appraisal, documents were classified by study design (systematic review, integrative review, narrative review, observational study, critical essay, commentary/reply, and institutional document), as identified in the

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

"Methodology" column of the synthesis tables. This classification guides the reading of findings: claims supported by systematic reviews or large-scale observational analyses carry greater evidentiary weight than those derived from critical essays or normative documents — a distinction explicitly signaled in the discussion whenever relevant.

The final analytical corpus of 25 documents was distributed across the three thematic axes defined by the guiding question. To ensure methodological transparency and enable readers to assess the relative weight of the evidence mobilized, Table 1 presents the full characterization of included studies, identifying document type and the analytical axis to which each work was assigned. This classification is particularly relevant given the epistemologically heterogeneous nature of the corpus, which spans systematic reviews, bibliometric analyses, critical essays, commentaries, narrative reviews, and institutional documents — sources of distinct epistemic standing whose argumentative weight is differentiated throughout the discussion.

Table 1. Characterization of the analytical corpus of the integrative review.

Authors (Year)	Study type	Analytical axis
Beery; Zucker, 2011	Narrative review	I
Yoon *et al.*, 2014	Cross-sectional observational study	I
Flórez-Vargas *et al.*, 2016	Bibliometric analysis	I
Ramirez *et al.*, 2017	Systematic review	I
Will *et al.*, 2017	Bibliometric analysis	I
Coiro; Pollak, 2019	Narrative review	I
Wheeler *et al.*, 2020	Systematic review	I
Mercel *et al.*, 2021	Longitudinal observational study	I
Tieu *et al.*, 2021	Narrative review	I
Becegato; Silva, 2022	Narrative review	I
Poulin *et al.*, 2023	Systematic review	I

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

NC3Rs, 2024	Institutional document	I
Karp *et al*., 2025	Methodological article	I
Karp, 2025	Critical essay	I
Fischer; Rodrigues, 2018	Documentary analysis	II
Karp; Reavey, 2018	Critical essay	II
Plevkova *et al*., 2020	Narrative review	II
Nunamaker; Turner, 2023	Narrative review/ Critical essay	II
Eliot; Richardson, 2016	Critical essay	III
Mazure, 2016	Commentary / Reply	III
Zakiniaeiz *et al*., 2016	Narrative review	III
Barlek *et al*., 2022	Cross-sectional observational study	III
Allegra *et al*., 2023	Narrative review	III
Gualtierotti, 2025	Narrative review	III
Warren; Garrett; Frame, 2025	Narrative review	III

Source: elaborated by the authors. **Axis legend:** I = Methodological approaches to sex in experimental design; II = Ethical implications in light of the 3Rs; III = Consequences for quality, applicability, and health equity.

3. Results and Discussion

3.1. Sex bias in preclinical animal research: methodological approaches to sex in experimental design

This axis encompasses studies that quantify the prevalence of sex bias across biomedical disciplines and critically examine methodological failures in experimental design (Table 2).

Table 2. Synthesis of studies on methodological bias and prevalence.

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

Authors (Year)	Objectives	Methodology	Main Findings	Contributions
Beery; Zucker, 2011	Quantify sex bias across 10 biomedical fields and analyze historical precedents.	Narrative review.	Male bias predominant in 8 of 10 fields. Frequent failure to analyze data by sex.	Demonstrates the historical and systemic exclusion of females from basic research and the resulting analytical inadequacies.
Yoon *et al.*, 2014	Investigate sex bias in basic and translational surgical research.	Cross-sectional observational study.	80% of animal studies used exclusively male subjects; 22% did not report sex at all.	Reveals pervasive methodological negligence within the surgical research discipline.
Flórez-Vargas *et al.*, 2016	Assess reporting of sex and age variables in mouse models.	Bibliometric / observational analysis.	Only approximately 50% of articles reported sex and age in 2014. Male bias pronounced in cardiology; female bias in infectious disease research.	Exposes persistent non-adherence to methodological reporting guidelines (ARRIVE).

Source: elaborated by the authors.

The endemic nature of male bias in preclinical experimentation is well established. Studies across neuroscience (Beery; Zucker, 2011; Will *et al.*, 2017), cardiology (Ramirez *et al.*, 2017), surgery (Yoon *et al.*, 2014; Mercel *et al.*, 2021), and parasitology (Poulin *et al.*, 2023) consistently demonstrate a systematic preference for the male normative model. A further concern is the phenomenon of "superficial compliance" with inclusion guidelines: both sexes are nominally enrolled in experimental designs, yet data are pooled without testing for sex-by-treatment interaction effects, or separate analyses are conducted in ways that introduce systematic analytical errors (Karp, 2025). Additionally, invasive methodological procedures — such as vaginal lavage in female rodents for estrous cycle monitoring — introduce stress-related confounders that are subsequently misattributed as inherent female characteristics, while male subjects are not subjected to comparable stressors (Becegato; Silva, 2022). Notably, recent NIH mandates have failed to

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

meaningfully alter these entrenched practices, underscoring the depth of institutional and cultural resistance (Wheeler *et al*., 2020; Mercel *et al*., 2021).

The rationale for female exclusion rests on the premise that the estrous cycle increases data variability and necessitates doubling of sample sizes; however, this assumption has been empirically refuted, as the biological variability of female subjects is statistically comparable to that of males (Beery; Zucker, 2011; Karp, 2025). Factorial experimental designs — such as two-way ANOVA — enable the sharing of statistical power across sexes without increasing the total number of animals, provided that the analysis includes testing for a sex-by-treatment interaction (Karp, 2025). The Sex Inclusive Research Framework (SIRF) provides a structured tool for training ethics committees and funding agency reviewers, ensuring that justifications grounded in statistical fallacies are not accepted as valid grounds for sex-restricted studies (NC3Rs, 2024; Karp *et al*., 2025).

This systemic male dominance manifests not only through the exclusive use of male subjects but also through the routine omission of sex from experimental reports (Beery; Zucker, 2011; Will *et al*., 2017; Poulin *et al*., 2023). Even when both sexes are included, inadequate statistical literacy frequently results in data pooling without sex disaggregation or interaction analysis (Karp, 2025). It bears emphasis that claims regarding the superior variability of females due to the estrous cycle have been empirically refuted: the intrinsic biological variability of female subjects does not exceed that of males (Beery; Zucker, 2011; Karp *et al*., 2025).

3.2. Ethical implications of female exclusion in light of the 3Rs principles

This axis consolidates studies addressing the ethical consequences of the under-representation of female subjects, examining issues of scientific integrity, the 3Rs principles (Replacement, Reduction, and Refinement) as originally formulated by Russell and Burch (1959), and the downstream effects on animal welfare (Table 3).

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

Table 3. Synthesis of studies on ethical implications and the 3Rs.

Authors (Year)	Objectives	Methodology	Main Findings	Contributions
Fischer; Rodrigues, 2018	Evaluate research planning in animal studies as a primary parameter of scientific integrity.	Documentary analysis / observational study.	Research design is frequently shaped by utilitarian bias. CEUAs must assume an active bioethical educational role to prevent protocols from becoming mere bureaucratic formalities.	Highlights that genuine researcher accountability and meaningful adherence to the 3Rs are essential to preventing ethical vulnerabilities.
Karp; Reavey, 2018	Explore strategies to overcome institutional inertia in the inclusion of sex as a biological variable.	Critical essay.	Resistance is rooted in misconceptions about the 3Rs — specifically, the erroneous belief that Reduction prohibits the inclusion of females due to increased sample size requirements.	Reframes Reduction as the promotion of robust, reproducible, and genuinely translatable experimental designs.
Plevkova *et al*., 2020	Analyze dimensions of sex and gender bias across different research models.	Narrative review.	Diseases predominantly affecting females (e.g., multiple sclerosis) generate a "reverse bias" in which male subjects are inadequately modeled. The estrous cycle is identified as a false pretext for exclusion.	Demonstrates that sampling imbalances compromise the welfare of human populations by generating translational failures.
Nunamaker; Turner, 2023	Reveal the adverse impacts of sex bias on animal welfare in experimental settings.	Narrative review / Critical essay.	Sex bias generates "biological surplus" — the systematic culling of the excluded sex — and results in suboptimal pain management due to sex-specific pharmacodynamic	Directly links biased experimental design to flagrant violations of animal welfare standards and the unnecessary loss of animal lives.

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

			differences in analgesic response.	

Source: elaborated by the authors.

The reviewed studies reveal a profound ethical paradox in the prevailing interpretation of the 3Rs principles. Under the guise of Reduction — which seeks to minimize the number of experimental animals — investigators have systematically justified single-sex study designs (Karp; Reavey, 2018). However, the preferential use of males requires animal facilities to breed subjects at scale only to select a fraction of each litter, thereby generating an extensive "biological surplus" that culminates in the euthanasia of healthy females — a substantial and unnecessary loss of animal life (Nunamaker; Turner, 2023). Furthermore, the absence of female subjects in experimental paradigms has hindered the accurate characterization of sex differences in nociceptive pathways and analgesic metabolism, resulting in the systematic under-treatment of pain in female experimental animals and a consequent compromise of their welfare (Nunamaker; Turner, 2023).

The reviewed evidence indicates that the Reduction principle has been routinely misappropriated to justify single-sex designs under the pretext of avoiding sample size inflation (Karp; Reavey, 2018). This interpretation is, however, methodologically untenable: modern factorial designs permit the allocation of the sample across sexes without sacrificing statistical power (Karp, 2025). Moreover, as documented by Nunamaker and Turner (2023), the systematic preference for males results in the routine production and disposal of healthy females in breeding facilities — the so-called "biological surplus" — constituting a direct violation of the very principle ostensibly being honored. These findings support the inference that Animal Ethics Committees (CEUAs) have a central role to play in correcting this institutionalized misconception; however, their effective action necessarily depends on parallel developments in methodological training, revision of funding and publication policies, and broader change in laboratory culture — dimensions that exceed their specific regulatory mandate.

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

The ethical implications of sex bias are bidirectional: they compromise human welfare by producing irreproducible science and simultaneously violate the welfare of the animals themselves, as highlighted by Karp and Reavey (2018) and Plevkova *et al*. (2020). Reduction, properly understood, does not sanction undersampling or the exclusion of fundamental biological variables; it demands the design of experiments with adequate internal and external validity, ensuring that the use of animal lives generates reliable and transferable knowledge. The mass culling of females driven by experimental bias nullifies any claim to "animal economy" under the 3Rs, making bioethical literacy within research institutions an indispensable condition for overcoming the entrenched perception that including both sexes constitutes a bureaucratic or financial burden.

3.3. Consequences of sex bias for the quality, applicability, and generalizability of scientific findings, with emphasis on health inequities

This axis consolidates studies examining the translational impact of sex bias, specifically, the systemic failure to transfer preclinical findings to clinical practice — and its contribution to health disparities (Table 4).

Table 4. Synthesis of studies on scientific validity and health equity.

Authors (Year)	Objectives	Methodology	Main Findings	Contributions
Eliot; Richardson, 2016	Critically examine the biological determinism arising from the imposition of SABV policy on animal models.	Critical essay.	Animal models are incapable of capturing the sociocultural dimensions of human gender. Mandating sex analysis in all preclinical studies may impose undue burden and divert scientific focus.	Introduces the debate on the limitations of animal models in simulating the complexity of gender.

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

Mazure, 2016	Refute criticisms of sex inclusion in preclinical research and defend its translational value.	Commentary / Reply.	Preclinical research is not intended to replicate human biology precisely but to provide a fundamental biological substrate. Ignoring sex corrupts the foundational understanding of physiology.	Reaffirms that biological sex — independent of gender — shapes pathophysiology and is essential to avoiding the repetition of past scientific failures.
Zakiniaeiz *et al.*, 2016	Assess sex differences in preclinical research and alert to the clinical consequences of female exclusion.	Narrative review.	Female exclusion has contributed to pharmacological disasters, including thalidomide and zolpidem overdose toxicity. Translational failures disproportionately expose women to adverse drug reactions.	Documents the urgency of aligning preclinical rigor with drug regulatory approval standards.
Barlek *et al.*, 2022	Determine the prevalence of sex-inclusive enrollment in high-impact clinical trials.	Cross-sectional observational study.	Women remain underrepresented (44% vs. 56% male). Industry-funded trials exclude more women relative to NIH-funded studies.	Demonstrates that the translational gap created by sex bias propagates to the apex of the clinical evidence hierarchy.
Allegra *et al.*, 2023	Review sex differences in pharmacology with respect to pharmacokinetics and pharmacodynamics.	Narrative review.	Differences in drug distribution, enzymatic metabolism, and excretion produce distinct efficacy and toxicity profiles between sexes. Extrapolation of male-derived data systematically	Maps the biological mechanisms underpinning the requirement for sex-disaggregated drug safety testing.

ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

			fails in female populations.	
Gualtierotti, 2025	Promote the integration of sex and gender into clinical research to improve health outcomes in internal medicine.	Narrative review.	Reliance on male anatomical standards impairs diagnostic accuracy (e.g., myocardial infarction). Inclusive guidelines are needed from the *in vitro* phase through epidemiology.	Provides a translational cycle integrating systematic reviews, clinical guidelines, gender diversity considerations, and terminological clarity.
Warren; Garrett; Frame, 2025	Examine disparities in women's health from a translational science perspective.	Narrative review.	Failures occur across all translational stages (T0–T4). Conditions such as fibromyalgia face gender stigmatization, while cardiovascular pathology in women remains systematically underrecognized.	Establishes a translational framework requiring intersectional approaches and systematic sex-disaggregated data collection.

Source: elaborated by the authors.

The reviewed literature suggests that the translational reliance on data derived predominantly from male organisms may contribute to adverse clinical outcomes in women, even as the translational chain involves multiple regulatory, epidemiological, and methodological mediations between the bench and the bedside. At the level of fundamental biological mechanisms, evidence consistently reveals substantial sex differences in pharmacokinetics and pharmacodynamics, mediated not only by hormonal factors but also by differential gene expression and enzymatic activity (Allegra *et al.*, 2023). Preclinical neglect of these differences contributed directly to the development of drugs with disproportionate toxicity in women — as evidenced by the zolpidem overdose crisis and the thalidomide disaster (Zakiniaeiz *et al.*, 2016).

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

This disproportion extends beyond the preclinical phase: large-scale, industry-funded clinical trials continue to enroll women at inequitable rates (Barlek *et al*., 2022), thereby perpetuating health inequities that originate in the preclinical stage. While animal models have inherent limitations in capturing the sociocultural complexity of human gender, the integration of sex as a biological variable (SABV) remains essential for elucidating mechanistic pathways. The resulting knowledge gap perpetuates underdiagnosis and suboptimal treatment in conditions that disproportionately affect women.

More broadly, translational failures traverse the entire scientific development continuum (stages T0–T4), compromising diagnostic accuracy in cardiovascular and psychiatric diseases (Warren; Garrett; Frame, 2025). Autoimmune conditions, which are highly prevalent among women, are frequently subject to gender bias — chronically underfunded and underrecognized as a result of being categorized as "women's diseases" (Warren; Garrett; Frame, 2025). The ongoing debate regarding the validity of animal models for translational medicine is instructive in this regard: while Eliot and Richardson (2016) caution that animal models fail to capture the psychosocial constructs of human gender — an important limitation particularly for behavioral and pain-related pathologies — Mazure (2016) and Gualtierotti (2025) argue compellingly that foundational biology constitutes an irreplaceable substrate for mechanistic inquiry.

The rigorous distinction between "sex" (a biological variable) and "gender" (a sociocultural construct) is a cornerstone of methodological integrity. When preclinical research excludes female subjects, the mechanistic basis of pathophysiology is systematically compromised, perpetuating diagnostic and therapeutic guidelines calibrated to male physiology — with consequences extending from drug dosing to cardiovascular prognosis (Gualtierotti, 2025). The systematic adoption of sex-disaggregated data collection (Gualtierotti, 2025; Warren; Garrett; Frame, 2025) and the enforcement of cross-sectoral accountability — from funding approval through

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

publication (Barlek *et al*., 2022) — are non-negotiable prerequisites for advancing health equity and establishing a truly generalizable, personalized medicine.

4. Concluding Remarks

Sex bias in preclinical animal research is the product of a historically entrenched default to male models. This practice has compromised scientific validity, reproducibility, and the safety of female patients for decades. The bias manifests not only through the exclusive use of male subjects, but also through the systematic omission of sex as a reportable variable and the absence of appropriate statistical analyses when both sexes are nominally included.

The Reduction principle of the 3Rs — conceived to ensure scientific rigor and economy in the use of animals — has been routinely co-opted to justify single-sex experimental designs. Factorial designs, however, enable the inclusion of both sexes without a proportional increase in sample size. The exclusion of females lacks methodological justification and contradicts the ethical foundations the Reduction principle is meant to uphold. Animal Ethics Committees bear institutional responsibility for correcting this deeply embedded misconception.

This review is subject to limitations that merit transparent acknowledgment. With respect to the literature analyzed, recurring terminological imprecision between "sex" and "gender" was observed across a subset of included studies, alongside considerable variation in the analytical depth of individual reviews, and the still-restricted scope of tools such as SIRF, whose application is currently confined to the evaluation of research proposals within funding agencies and does not extend to ongoing laboratory practice. With respect to the review itself, the primary limitation is the epistemological heterogeneity of the corpus, which encompasses systematic reviews, observational analyses, critical essays, commentaries, and institutional documents — sources of markedly different evidentiary standing that, while treated differentially throughout the analysis, collectively warrant caution in the generalization of conclusions.

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

Acknowledging these limitations does not invalidate the findings of this study; rather, it situates them within their legitimate methodological scope. Overcoming sex bias in preclinical research demands, as the reviewed literature consistently demonstrates, a coordinated agenda integrating statistical literacy, reform of funding and publication policies, and the critical and sustained training of researchers — a transformation that is, above all, cultural.

References

ALLEGRA, S. *et al*. Evaluation of sex differences in preclinical pharmacology research: how far is left to go?. **Pharmaceuticals**, v. 16, n. 6, p. 786, 2023. Available at: https://www.mdpi.com/1424-8247/16/6/786. Accessed: Jan. 27, 2026.

BARLEK, M. H. *et al*. The persistence of sex bias in high-impact clinical research. **Journal of Surgical Research**, v. 278, p. 364-374, 2022. Available at: https://doi.org/10.1016/j.jss.2022.04.077. Accessed: Jan. 27, 2026.

BECEGATO, M.; SILVA, R. H. Object recognition tasks in rats: does sex matter?. **Frontiers In Behavioral Neuroscience**, [S.L.], v. 16, p. 1-11, Aug. 12, 2022. Frontiers Media SA. Available at: https://doi.org/10.3389/fnbeh.2022.970452. Accessed: Dec. 16, 2025.

BEERY, A. K.; ZUCKER, I. Sex bias in neuroscience and biomedical research. **Neuroscience & Biobehavioral Reviews**, [S.L.], v. 35, n. 3, p. 565-572, Jan. 2011. Elsevier BV. Available at: https://doi.org/10.1016/j.neubiorev.2010.07.002. Accessed: Dec. 16, 2025.

COIRO, P.; POLLAK, D. D. Sex and gender bias in the experimental neurosciences: the case of the maternal immune activation model. **Translational Psychiatry**, v. 9, n. 1, p. 90, 2019. Available at: https://www.nature.com/articles/s41398-019-0423-8. Accessed: Mar. 9, 2026.

ELIOT, L.; RICHARDSON, S. S. Sex in context: limitations of animal studies for addressing human sex/gender neurobehavioral health disparities. **Journal of Neuroscience**, v. 36, n. 47, p. 11823-11830, 2016. Available at: https://www.jneurosci.org/content/36/47/11823. Accessed: Jan. 30, 2026.

FENWICK, N.; GRIFFIN, G.; GAUTHIER, C. The welfare of animals used in science: How the "Three Rs" ethic guides improvements. **The Canadian Veterinary Journal**, v. 50, n. 5, p. 523, 2009. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC2671878/. Accessed: Jan. 30, 2026.

FISCHER, M. L.; RODRIGUES, G. S. Planejamento e divulgação da pesquisa com

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

animais como parâmetro de integridade. **Revista Bioética**, v. 26, n. 4, p. 543-555, 2018. Available at: https://doi.org/10.1590/1983-80422018264273. Accessed: Feb. 12, 2026.

FLÓREZ-VARGAS, O. *et al*. Bias in the reporting of sex and age in biomedical research on mouse models. **eLife**, v. 5, p. e13615, 2016. Available at: https://elifesciences.org/articles/13615. Accessed: Mar. 9, 2026.

GUALTIEROTTI, R. Bridging the gap: Time to integrate sex and gender differences into research and clinical practice for improved health outcomes. **European Journal of Internal Medicine**, v. 134, p. 9-16, 2025. Available at: https://doi.org/10.1016/j.ejim.2025.01.030. Accessed: Jan. 27, 2026.

KARP, N. A. *et al*. The Sex Inclusive Research Framework to address sex bias in preclinical research proposals. **Nature Communications**, v. 16, n. 1, p. 3763, 2025. Available at: https://www.nature.com/articles/s41467-025-58560-5. Accessed: Mar. 16, 2026.

KARP, N. A. Navigating the paradigm shift of sex inclusive preclinical research and lessons learnt. **Communications Biology**, v. 8, n. 1, p. 681, 2025. Available at: https://www.nature.com/articles/s42003-025-08118-4. Accessed: Feb. 12, 2026.

KARP, N. A.; REAVEY, N. Sex bias in preclinical research and an exploration of how to change the status quo. **British Journal of Pharmacology**, v. 176, n. 21, p. 4107-4118, 2018. Available at: https://doi.org/10.1111/bph.14539. Accessed: Feb. 12, 2026.

MAZURE, C. M. Our evolving science: studying the influence of sex in preclinical research. **Biology of Sex Differences**, v. 7, n. 1, p. 15, 2016. Available at: https://link.springer.com/article/10.1186/s13293-016-0068-8. Accessed: Jan. 30, 2026.

MERCEL, A. *et al*. Sex bias persists in surgical research: a 5-year follow-up study. **Surgery**, v. 170, n. 2, p. 354-361, 2021. Available at: https://doi.org/10.1016/j.surg.2021.02.041. Accessed: Mar. 9, 2026.

NATIONAL CENTRE FOR THE REPLACEMENT REFINEMENT AND REDUCTION OF ANIMALS IN RESEARCH — NC3Rs (United Kingdom). **Sex-inclusive research framework: challenging misconceptions and fostering change**. London, Jul. 10, 2024. Available at: https://nc3rs.org.uk/news/sex-inclusive-research-framework-challenging-misconceptions-and-fostering-change. Accessed: Mar. 14, 2026.

NATIONAL INSTITUTES OF HEALTH (NIH). **Notice Number NOT-OD-15-102: Consideration of sex as a biological variable in NIH-funded research**. Bethesda: NIH, 2015. Available at: https://grants.nih.gov/grants/guide/notice-files/not-od-15-102.html. Accessed: Dec. 16, 2025.

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

NUNAMAKER, E. A.; TURNER, P. V. Unmasking the adverse impacts of sex bias on science and research animal welfare. **Animals**, v. 13, n. 17, p. 2792, 2023. Available at: https://www.mdpi.com/2076-2615/13/17/2792. Accessed: Feb. 12, 2026.

PAGE, M. J. *et al*. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. **BMJ**, v. 372, n. 160, 2021. Available at: https://doi.org/10.1136/bmj.n160. Accessed: Feb. 12, 2026.

PLEVKOVA, J. *et al*. Various aspects of sex and gender bias in biomedical research. **Physiological Research**, v. 69, n. Suppl 3, p. S367, 2020. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC8603716/. Accessed: Feb. 12, 2026.

POULIN, R. *et al*. Battle of the sexes: analysis of sex bias in host use and reporting practices in parasitological experiments. **International Journal for Parasitology**, v. 53, n. 7, p. 381-389, 2023. Available at: https://doi.org/10.1016/j.ijpara.2023.03.002. Accessed: Feb. 25, 2026.

RAMIREZ, F. D. *et al*. Sex bias is increasingly prevalent in preclinical cardiovascular research: implications for translational medicine and health equity for women: a systematic assessment of leading cardiovascular journals over a 10-year period. **Circulation**, v. 135, n. 6, p. 625-626, 2017. Available at: https://doi.org/10.1161/CIRCULATIONAHA.116.026668. Accessed: Mar. 9, 2026.

ROSANELI, C. F.; FISCHER, M. L. A revisão integrativa como ferramenta para educação profissional e tecnológica em Bioética. **Revista Brasileira da Educação Profissional e Tecnológica**, v. 2, n. 24, p. e17809-e17809, 2024. Available at: https://doi.org/10.15628/rbept.2024.17809. Accessed: Mar. 16, 2026.

RUSSELL, W.; BURCH, L. **The principles of humane experimental technique**. London: Methuen, 1959.

SOUSA, M. N. A.; BEZERRA, A. L. D.; EGYPTO, I. A. S. Trilhando o caminho do conhecimento: o método de revisão integrativa para análise e síntese da literatura científica. **Observatorio de la Economía Latinoamericana**, v. 21, n. 10, p. 18448-18483, 2023. Available at: https://doi.org/10.55905/oelv21n10-212. Accessed: Mar. 16, 2026.

TANNENBAUM, J.; BENNETT, B. T. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. **Journal of the American Association for Laboratory Animal Science**, v. 54, n. 2, p. 120-132, 2015. Available at: https://aalas.kglmeridian.com/view/journals/72010024/54/2/article-p120.xml. Accessed: Mar. 16, 2026.

TIEU, P. *et al*. Sex bias in animal models of thrombosis research. **Canadian Journal of Cardiology**, v. 37, n. 2, p. e16, 2021. Available at: https://onlinecjc.ca/article/S0828-282X(20)30119-7/abstract. Accessed: Feb. 25,

RSV
ISSN 2674-8584

Received: 13/01/2026 - **Accepted:** 22/04/2026
Vol: 08.02
DOI: 10.66104/tgq2sn41
Pages: 1-23

2026.

WARREN, A.; GARRETT, K.; FRAME, L. A. Disparities in women's health and clinical considerations from a translational science perspective: A narrative review and framework for future directions. **Women's Health**, v. 21, p. 17455057251399009, 2025. Available at: https://journals.sagepub.com/doi/full/10.1177/17455057251399009. Accessed: Jan. 27, 2026.

WHEELER, J. J. *et al*. A systematic review of animal models and sex as a variable in itch research. **Itch**, v. 5, n. 3, p. e40, 2020. Available at: https://journals.lww.com/itch/fulltext/2020/07010/A_systematic_review_of_animal_models_and_sex_as_a.5.aspx?context=LatestArticles. Accessed: Mar. 9, 2026.

WHITEHEAD, M. The concepts and principles of equity and health. **International Journal of Health Services**, Westport, v. 22, n. 3, p. 429-445, 1992. Available at: https://doi.org/10.2190/986L-LHQ6-2VTE-YRRN. Accessed: Mar. 9, 2026.

WILL, T. R. *et al*. Problems and progress regarding sex bias and omission in neuroscience research. **eNeuro**, v. 4, n. 6, 2017. Available at: https://www.eneuro.org/content/4/6/ENEURO.0278-17.2017.short. Accessed: Mar. 9, 2026.

YOON, D. Y. *et al*. Sex bias exists in basic science and translational surgical research. **Surgery**, v. 156, n. 3, p. 508-516, 2014. Available at: https://www.sciencedirect.com/science/article/pii/S0039606014004255. Accessed: Mar. 16, 2026.

ZAKINIAEIZ, Y. *et al*. Balance of the sexes: addressing sex differences in preclinical research. **The Yale Journal of Biology and Medicine**, v. 89, n. 2, p. 255, 2016. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC4918870/. Accessed: Jan. 27, 2026.

ZUCKER, I.; BEERY, A. K. Males still dominate animal studies. **Nature**, [S.L.], v. 465, n. 7299, p. 690-690, Jun. 9, 2010. Springer Science and Business Media LLC. Available at: https://doi.org/10.1038/465690a. Accessed: Dec. 16, 2025.