

Reporting Laboratory and Animal Research in ANESTHESIOLOGY

The Importance of Sex as a Biologic Variable

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Biologic differences between the two sexes have been naturally acknowledged from the dawn of humanity. Laboratory, clinical, and epidemiologic data now provide ample evidence for sex-specific differences in both disease and drug responses,¹ and perioperative medicine is not exempt. For example, sexual dimorphism in response to ischemic brain, cardiac, and renal injury has been repeatedly reported.²⁻⁴ The incidence of postoperative cognitive dysfunction is higher in men, and male and female rodents differ in vulnerability to developmental anesthesia neurotoxicity.^{5,6} Chronic pain is more frequently reported in women, although the effects of sex on postoperative pain are unclear.⁷ Sex-specific differences have been demonstrated in morphine-induced analgesia and opioid-related side effects.^{8,9} Postoperative nausea and vomiting are more common in women, and attendant clinical protocols are sex-specific.

Despite this dimorphism, women have long been studied less often in biomedical research. In 1993, the National Institutes of Health Revitalization Act required the inclusion of women in clinical studies, and further amendments of this document advocated for justification on how sex is factored into research design and analysis.¹⁰⁻¹² Full implementation of these recommendations, however, is incomplete. While clinical studies, when applicable, now do systematically include women and men, only a small proportion of them report outcome by sex or include both sexes as a covariate.¹³ This limitation can be partially explained by the fact that the Consolidated Standards of Reporting Trials



“Consideration of sex as a biologic variable in conducting laboratory animal research—and inclusion of both sexes—is strongly encouraged.”

whole investigation. Indeed, most basic science research in mammals is conducted in a single sex, predominantly in males.¹⁶ This is due at least in part to the longstanding belief that females are more variable than males, due to estrous cycles.^{17,18} For example, in the field of neuroscience, less than 20% of investigations used both sexes and, disturbingly, a quarter of the investigations did not specify the sex of research animals.¹⁶ Moreover, analysis of results with consideration of sex as a variable remains infrequent.¹⁹ The potentially confounding effects of this overt sex bias on the meaning of single-sex experimental data are, however, rarely considered. This is worrisome because, in light of the evolutionary well conserved sex-specific differences in biology, erroneous conclusions may be drawn when extrapolating

(CONSORT) statement for randomized controlled trials does not include reporting results by sex.¹⁴ It is notable, however, that sex-specific pathology and pharmacology in perioperative medicine and calls for sex-specific reporting have been known and advocated for more than a decade.¹⁵

Laboratory and animal research is often a predecessor and driving force behind clinical investigation. It may also ensue from clinical observation. In contrast to the real-life complexity of clinical research, laboratory and animal research is often highly reductionist in nature in an attempt to understand specific mechanisms underlying physiology and disease. One common and traditional approach to presumably reducing data variability in the laboratory is to include only one sex in an experiment or

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outcome data from one sex to another.^{16,20} Therefore, sex bias in preclinical research can have important translational implications because it may contribute to the frustrating gap between laboratory and clinical advances.

Advocacy, over the past few years, for better attention to sex as a biologic variable in laboratory studies and reformation of single-sex science ignited intense debates among researchers and policymakers.^{21–23} Some countered that including both females and males in research would increase variability and consequently necessitate greater numbers of animals with attendant increased costs of laboratory studies.²² However, the canard of greater variability in female than male animals has been discredited across many species.^{17,18} Moreover, when research is specifically preclinical, intending translation to clinical investigation, is it worthwhile to conduct single-sex experiments when results may be explicitly flawed in their applicability to humans? Indeed, the costs of failed clinical trials, and the consequences of therapies inappropriate to women (or men), far outweigh purported cost-saving benefits of ignoring sex as a biologic variable in the laboratory. Studying both animal sexes may be more expensive *per se* yet may enhance translation and be more cost-effective overall. Although it remains unknown whether simply including both sexes in laboratory studies will be salutary, given the many failures in translating laboratory results to human therapeutics and the costs and consequences of failed clinical trials, the axiomatic inclusion of both sexes does appear justified. Additionally, even if translation to clinical investigation is not the immediate goal of animal research, sex differences can inform on the mechanism of underlying biology, pathophysiology, or pharmacology.

In 2015, the National Institutes of Health implemented new expectations on considering sex as a biologic variable in cell and animal investigations, as part of a rigor and transparency initiative.²⁴ The National Institutes of Health perspective was that (1) sex as a biologic variable is frequently ignored in animal studies, leading to incomplete understanding of potential sex-based differences in basic biologic function, disease processes, and treatment response; (2) overreliance on males in basic and preclinical research may obscure understanding of key sex influences on health processes and outcomes; and (3) accounting for sex as a biologic variable includes research questions, study

design, data collection, data analysis, and reporting of findings, because sex may be critical to interpretation, validation, and generalizability. The National Institutes of Health “expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data, or other relevant considerations must be provided for applications proposing to study only one sex.”²⁴ Similar statements have been subsequently issued by other international funding agencies, such as the European Commission and the Canadian Institutes of Health Research, and echoed in scientific articles.^{25,26} A recent survey noted an important increase, although still incomplete, in appreciation of these requirements by grant reviewers.²⁷ As with clinical research, however, it remains to be seen how well this will lead to improvement in the conduct of laboratory research.

Changes in ANESTHESIOLOGY

ANESTHESIOLOGY supports the above principles and encourages the consideration of sex as a biologic variable in laboratory as well as clinical research. Adequate consideration of sex by investigators in the formulation of research questions, study design, experimentation, and collection and analysis of data is important and should be considered by sponsors in their funding decisions. Investigators must decide whether to simply include both sexes in research or to deliberately power studies to detect sex differences and make sex-specific conclusions.

The adequate consideration of sex in research *reporting* is also the subject of guidelines^{24,28} and is directly in the purview of journals.²⁹ For example, the Sex and Gender Equity in Research guidelines provide a comprehensive description for reporting of sex in study design, data analysis, and interpretation.²⁶ Journal editors, editorial policies, and the careful scrutiny of peer reviewers will play a major role in ensuring adequate reporting of sex as a biologic variable, and ANESTHESIOLOGY embraces these principles. Furthermore, consideration of sex as a biologic variable is the purview of journals, including ANESTHESIOLOGY, as it pertains to peer review and the validity of results and conclusions. The reporting of sex as a biologic variable in

Box 1. Sex and a Biologic Variable in Laboratory Research: ANESTHESIOLOGY Reporting Guidelines

1. Consideration of sex as a biologic variable in conducting laboratory animal research, and inclusion of both sexes, is strongly encouraged.
2. Consideration of sex as a biologic variable in reporting laboratory animal research is required by ANESTHESIOLOGY.
3. The term “sex” rather than “gender” should be used when referring to biologic/genetic differences.
4. A clear indication of the sex(es) of animals included in the research should be included in the Abstract and Methods sections of a manuscript. If only one sex was studied, specification in the title may be appropriate.
5. Data may be reported and analyzed individually by sex, whether showing sex differences or not, where appropriate.
6. The Discussion section should discuss potential implications of sex on results and conclusions, where appropriate.
7. If research or data analysis by sex was not conducted, the Discussion section should provide the rationale and discuss any implications for the interpretation of results.

Note: Although this addresses laboratory and animal research, it continues to apply also to clinical research.

laboratory/animal research (box 1) has already undergone increased attention and scrutiny in the ANESTHESIOLOGY peer review process over the last 2 yr but has been somewhat informal and variable.

Therefore, after discussion and agreement among the Editorial Board, ANESTHESIOLOGY will attend to sex as a biologic variable more thoroughly and henceforth require greater reporting transparency in submitted and published manuscripts. We will continue to insist on the correct usage of the terms sex (a biologic variable based on chromosomal assignment) and gender (a constellation of sociologic processes that interact with and have the potential to influence human biology).²⁹ We will evaluate the inclusion of both sexes in research, as relevant to results and conclusions. We will require that if there is only one sex in an animal or human study (excluding studies relevant to only one sex), the Abstract must specify the sex. Inclusion of the single sex in the title is also encouraged, but it should not mistakenly conflate a single-sex study population with results and conclusions that were found specific to only one sex. The Methods section should report the sexes of cells or tissues (if known), animals, and humans studied, justify reasons for any exclusion, and whether/how sex was considered in study design. The Results section should report the sex composition of the final study population. Data may be reported and/or analyzed without disaggregation by sex, individually by sex using descriptive statistics to simply communicate results separately for each sex, or individually by sex where experiments were formally designed to detect effect modification by sex (*i.e.*, an effect \times sex interaction) using *a priori* statistical power considerations to properly ground such analyses, as appropriate. Authors are encouraged to report results disaggregated by sex, whether showing sex differences or not, in the main article or supplement, as appropriate. The Discussion section should discuss potential implications of sex on results and conclusions, where appropriate. If research or data analysis by sex was not conducted, the Discussion section should provide the rationale and discuss any implications for the interpretation of results. For convenience, these new reporting standards are summarized in box 1. Although this Editorial and the Guidelines are written to address laboratory and animal research, they have applied and will continue to apply also to clinical research.

It is intended and hoped that these reporting requirements will increase the transparency, rigor, and value of the research published in ANESTHESIOLOGY, strengthen the evidence, and enhance the translation of discovery to practice.

Competing Interests

Dr. Vutskits served as consultant for Primex (Zug, Switzerland) and Regeneron (Tarrytown, New York). Dr. Kharasch is the Editor-in-Chief of ANESTHESIOLOGY, and his institution receives salary support from the American

Society of Anesthesiologists (Schaumburg, Illinois) for this position. The other authors declare no competing interests.

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