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VIEWPOINT

Sex Differences Series

# Sex biases in infectious diseases research

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**Reporting the distribution and inclusion of both males and females in immunology and infectious diseases research is improving, but rigorous analyses of differential outcomes between males and females, including mechanistic inquiries into the causes of sex differences, still lags behind.**

In 2016, the U.S. National Institutes of Health implemented the policy that sex as a biological variable (SABV) be factored into the research design, analyses, and reporting in vertebrate animal and human studies (Clayton, 2018). The policy did not require that researchers utilize methods, double sample sizes, or power studies to detect sex differences. The policy merely asked that investigators know the existing data, balance the sexes in experimental design, and consider SABV. The policy has been met with mixed results across disciplines, with some disciplines, including immunology, making significant progress in reporting use of both sexes in animal studies (Woitowich et al., 2020). In this viewpoint, we seek to illustrate why it is imperative to consider SABV as a means for promoting rigor and reproducibility as well as equity and inclusion in all disciplines, but our focus will be on immunology and infectious diseases research. We will distinguish consideration of SABV from rigorously studying sex differences, and also illustrate the richness of the data from studies of sex differences in immunology and infectious diseases research at population, clinical, organismal, and cellular levels of analyses (Fig. 1).

The pandemic raised awareness about the value of SABV in epidemiological and clinical studies. Around the world, rates of mortality from COVID-19 have been greater for males than females (Scully et al., 2020), with consistent observations of male-biased inflammatory responses (Scully et al., 2021) and female-biased $CD8^+$ T cell responses (Takahashi et al., 2020). Animal models provide further insight into the male-biased disease burden by showing that inflammation and pulmonary tissue damage are greater in male hamsters, but mucosal immune responses are greater in females (Dhakal et al., 2021). Among humans, although males suffer greater disease burden during acute SARS-CoV-2 infection, females appear more likely to suffer long-term symptoms of post-acute sequalae of SARS-CoV-2 (Fernández-de-las-Peñas et al., 2022), with no mechanistic studies addressing how brain fog, fatigue, anosmia, and other symptoms are more likely to linger in females than males. As vaccines became readily available using novel platforms, like mRNAs, manufacturers did not consider SABV in the reporting of dependent measures or evaluate sex differences in vaccine outcomes. Analyses of subsequent clinical data are revealing that females tend to mount greater antibody responses and report more adverse events to the SARS-CoV-2 vaccines than males (Uwamino et al., 2022). Whether the durability of vaccine-induced immunity or responses to SARS-CoV-2 variants of concern are also different between the sexes has not yet been reported.

Historically, biomedical research animal studies were more likely to include male than female rodents (Woitowich et al., 2020). Arguments about increased variability among female rodents caused by reproductive cycles have been used to justify the lack of female inclusion, but have been rigorously refuted (Prendergast et al., 2014). We have used animal models to provide mechanistic insights about sex differences in influenza disease processes and efficacy of vaccines. Epidemiological studies suggest that females of reproductive ages are more likely to be hospitalized with severe influenza than males, and mouse models further show that inflammatory immune responses and pulmonary tissue damage are worse in influenza A virus (IAV)–infected female than male mice (Vermillion et al., 2018). Female mice also develop greater adaptive immune responses, including effector T and B cell activity, than males during IAV infection (Fink et al., 2018). Following influenza vaccination, female mice develop greater influenza vaccine–induced antibody responses and protection against live IAV challenge than males, which is mediated by epigenetic regulation of *Tlr7*, an X-linked gene, in B cells as well as estrogenic effects

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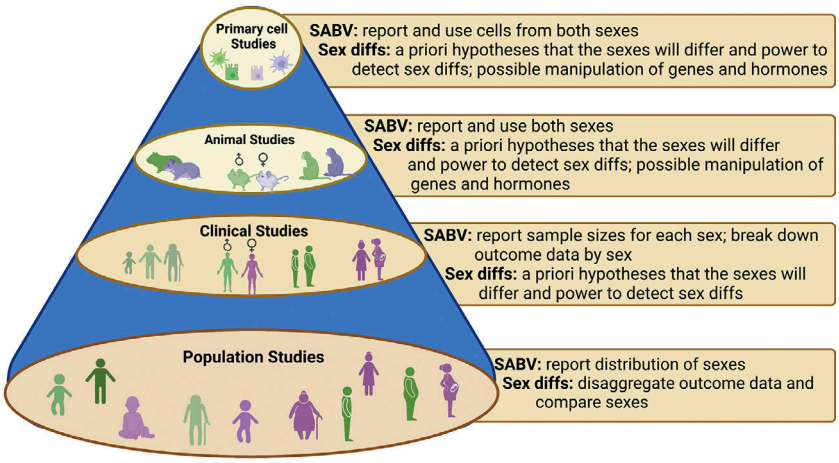


Figure 1. **Consideration of SABV refers to the consideration and reporting of the sex of participants, animals, and hosts from which primary cell cultures are derived.** The study of sex differences (sex diffs) refers to the rigorous experimentation associated with mechanistically and statistically comparing biological outcome data between males (green) and females (purple), with manipulation of the causes of these sex differences where possible (e.g., in animal and primary cell culture studies). Image generated using Biorender.

(Fink et al., 2018; Potluri et al., 2019). With growing interest in novel vaccine platforms for protection against influenza, especially among older adults, we have shown that the age-associated decline in protective immunity following vaccination with a stalk-based universal influenza vaccine occurs to a greater extent in female than male mice, suggesting that animal models might provide insight into sex-specific effects of aging on immunity (Dhakal et al., 2020; Potluri et al., 2019). A literature search for influenza vaccine articles published in the year 2021 in PubMed, using keywords "universal influenza vaccine," yielded 85 studies in murine models, of which, 81% (69/85) mentioned the sex of the animals and 19% (16/85) did not. Most of these studies used females only (59/69, 86%), with 6% using males only (4/69), 9% (6/69) including both sexes, and only 6% (4/69) comparing outcome data by sex. While consideration of SABV is on the rise, there is room for improvement in the study of sex differences in vaccine outcomes in animal studies.

Reporting of SABV in primary cell culture studies is considerably less common than among animal studies, although equally important (Potluri et al., 2017). Cells from males and females respond differently to microbes and drug treatments, with cellular phenotypes determined by sex chromosome complement and sex-specific epigenetic factors. In vitro studies are a research tool to generate mechanistic data about sex differences in responses to viruses, including HIV (Meier et al., 2009). HIV infection results in sex differential disease manifestation, with females experiencing worse disease outcomes, characterized by faster disease progression from similar viral loads, than males (Rechtien and Altfeld, 2019). Results from in vitro experiments using peripheral blood mononuclear cells (PBMCs) from healthy males and females, treated with HIV-1–derived *Tlr7/8* ligands, show greater frequencies of IFN-α producing plasmacytoid dendritic cells in samples from females, which contribute to greater activation of $CD8^+$ T cells in females than males (Meier et al., 2009). Using a primary PBMC model of HIV latency, replication of HIV-1 in PBMCs is age dependent in cells from females but not males (Macedo et al., 2018). Sex differences in the establishment of HIV latency in vitro and its reactivation are not observed. The incorporation of SABV into primary cell models of HIV infection and latency should be used for developing prophylactic and therapeutic strategies for HIV. While the field of HIV has embraced the value of accounting for the sex of primary cells, other fields within immunology and infectious diseases have not.

If rigor and reproducibility is the shared goal of our research, then reporting sex, gender, or both in clinical and preclinical research is imperative for immunology and infectious diseases research (Clayton and Tannenbaum, 2016). While basic immunology animal studies are doing a better job reporting sex (Woitowich et al., 2020), reporting is significantly more likely to occur if the first or senior author is female, with these papers often published in less-impactful journals (Sugimoto et al., 2019). If we are to promote diversity, equity, and inclusion in biomedical science, then we must address these biases in our research, with not only considering SABV, but also how SABV intersects with social determinants of health to affect disease outcomes as we have observed in the COVID-19 pandemic (Shapiro et al., 2021). This will only be accomplished if we collectively embrace this task and promote SABV in our research models and data analyses.

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