Let's talk about (biological) sex

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You spent years investigating your beloved protein. You generated mouse models, found a phenotype and fixed it with a drug. You even used human cells to reveal a conserved mechanism. But a prestigious journal has rejected your manuscript because you did not include females. Here is why you should not be mad at them.

Why sex? Why now?

Many aspects of human physiology differ between the sexes. Most common diseases show some degree of sex difference in their incidence, progression and/or response to treatment[1–3]. This should provide opportunities for targeted therapeutics, yet clinical studies often fail to include sex as a variable. For instance, only 4% of COVID-19 clinical studies planned on doing so, despite evidence of sex differences in infection, mortality and drug responses[4].

Failure to analyse sex can be fatal. A case in point is the male bias of the 'textbook' symptoms of cardiac arrest, which has led to delayed diagnosis and treatment of women[3,5]. Assessment of drug responses only in males has also led to inappropriate and potentially dangerous dosing guidelines[1–3].

Sex differences are relevant to animal models, too. Systematic phenotyping of mice revealed that many physiological and behavioural traits are sexually dimorphic, as are the effects of most genetic mutations[1,2]. Yet many studies still use single-sex (typically male) animals. This is partly because female data had been regarded as more variable as a result of the reproductive cycle; comparable variability in males owing to testosterone fluctuations and other factors recently dispelled this assumption[1,3].

This failure to include sex as a biological variable has created a major knowledge gap: a situation that both funding agencies and scientific journals are now seeking to rectify by demanding inclusion of both sexes in experimental design and analysis.

Sex differences beyond the gonad

Browse through any anatomy book and the only organs you are likely to see segregated by sex are the gonads. However, most non-reproductive organs differ anatomically and/or transcriptionally between the sexes[2,6]. The brain is no exception: without overstating its implications, there is evidence in both rodents and humans of sex differences in the volume of specific brain sub-regions, neuronal gene expression and physiology[2,5,6].

Historically, sex differences in both rodent and human non-reproductive organs were exclusively attributed to the effects of hormones. We now know that cell-intrinsic, sex chromosome-mediated mechanisms also play important yet under-investigated roles[6,7] (Supplementary Figure 1a). Our organs may therefore know their sex independently of gonadal hormones.

Conversely, there is also increasing realization that hormonal mechanisms are at play in animal models such as *Drosophila*, in which sex differences were once thought to arise from exclusively cell-intrinsic mechanisms[2]. This finding provides an opportunity to leverage sophisticated genetic tools in flies to investigate how cells integrate both intrinsic and extrinsic signals to acquire, maintain and potentially modulate their sexually dimorphic characteristics.

The sex of cells and molecules

If intrinsic mechanisms contribute to sex differences in non-reproductive organs, then even cells in vitro, and any processes within them, may 'have a sex'. Intriguingly, sex differences in gene expression, proliferation and/or behaviour have been reported for primary cell cultures, organoids, embryonic and induced pluripotent stem cells (iPSCs)[6,7]. Other aspects of intracellular physiology may also differ between the sexes; you may want to keep this in mind next time you examine biomolecular condensates, cytoskeletal dynamics, organelle communication or mechanisms of viral infection.

The mechanisms underlying these sex differences remain to be established and, in the case of iPSCs, are somewhat controversial[7,8]. There is evidence of contributions from both X- and Y-specific genes and different epigenetic mechanisms (Supplementary Figure 1a).

Whether looking at cells, organs or animals, lack of previous evidence of sex differences is not a good reason to exclude either sex in future experiments. A cell line or animal model may not exhibit sex differences to start with, but your particular treatment may reveal some. Furthermore, an identical phenotype in males and females may result from sexually dimorphic mechanisms. For example, although both male and female mice can experience pain, chronic pain processing is more dependent on microglia in males and T cells in females[9]. These sex-dependent mechanisms

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have obvious implications when considering genetic susceptibility to — and treatment of — human disease[2,3].

Those of you concerned with purely molecular studies may feel relieved to have left this sexually dimorphic cellular environment behind, but you might still need to incorporate sex as a variable in any future applications: the target and/or clearance of your engineered drug may differ between the sexes[1,3]. You may also need to consider your own sex: for example, in mice and rats, exposure to male but not female experimenters causes stress and reduces pain perception[3,9].

Why ONLY sex?

What is special about sex? If the goal is to develop more tailored and effective therapies, should we not also consider factors such as age, reproductive status, ethnicity or socioeconomic status? If experimental considerations allow, it is of course good practice to consider all relevant variables. I would argue that there are three good reasons to start with sex, however.

Firstly, considering only one sex disregards a major source of variation with potential impact on nearly half of the world's population. Secondly, uncovering a sex difference provides an entry point into future stratification. For example, women may differ in their response to a treatment because they have been pregnant; a second experiment can now be designed to compare them to women who have not born children. Finally, consideration of sex should raise awareness of the importance of experimental design and analysis. Doing so may make us consider whether the model that we will be using in our next experiment is likely to recapitulate the biology of, for example, a disease with late-age onset or one more prevalent in an underrepresented group.

Sex as well as gender

A pet peeve of many researchers with an interest in sex differences is the incorrect use of sex and gender — in particular, the use of the latter as a synonym for the former. Sex refers to biological attributes that distinguish organisms as male, female, intersex and hermaphrodite. Gender is a social construct, encompassing various psychological and social characteristics that collectively define individuals as men, women, non-binary or trans[3,10].

It is important to recognize that both apply to humans: gender does not negate or override sex differences, but, equally, not all differences between the sexes in humans are socially constructed. Perhaps more surprisingly, both sex and gender can independently or synergistically modulate specific traits (for example, pain) and act as independent risk factors for disease (for example, 'female' gender roles are associated with higher risk of cardiovascular disease independently of sex effects)[3].

But is it going to cost me?

Another common assumption is that incorporating sex as an experimental variable doubles research costs. In some cases, it may simply entail disaggregating (that is, not pooling) mixed-sex data, as well as choosing the right experimental design/analysis[1,3]. This choice is admittedly not trivial — many of us fail to analyse and/or interpret our data appropriately, which has likely led to both over-reporting of sex-specific effects and under-recognition of key sex differences in pooled datasets[10]. Factorial designs, in which sex is considered along other variables of interest, such as genotype and/or treatment, are often a suitable choice[1,10].

An apparently noisy dataset can be reanalysed to reveal statistically significant sex differences in, for example, responses to a specific drug or genetic manipulation (Supplementary Figure 1b). Hence, inclusion of sex is not only clinically relevant and socially responsible: it might increase your chances of detecting meaningful and reproducible effects, which could even reduce the total number of experiments/animals required.

Are you persuaded, yet still confused about how to incorporate sex as a variable in your research? Several recent resources provide useful tips on how to design, conduct, analyse and report your experiments[1,3,10]. If still in doubt, please do get in touch!

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Supplementary information

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