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Module 1, Video 1: Introductory Video to Sex as a Biological Variable and the Video Series

Up until about 1993, almost all health-related research in humans was conducted on men. Women were actively excluded from clinical trials because of the fear of liability associated with birth defects in pregnant women and the misconception that no fundamental sex differences existed between males and females outside of reproduction.

This common bias was carried over to preclinical research. Researchers consistently argued that results from male animals, cells and tissues would apply equally to females. They also argued that hormone cycles would decrease the homogeneity of study populations, leading to uninterpretable data variability. As a result, most preclinical research was similarly conducted in males only, with a 2016 meta-analysis showing that only 21% of animals studies reported the sex of the subjects [1].

The assumption that male data could be easily extrapolated to females set a dangerous precedent across the research spectrum. As a result, many important fundamental differences between males and females went largely overlooked.

Fortunately, by the end of the 20th century, this perspective began to shift. Congress passed the National Institutes of Health Revitalization Act of 1993, mandating the inclusion of women and minorities in NIH-funded clinical trials. That same year, the FDA changed its policies to require that women also be included in safety and dosing studies.

It would take over 20 years for similar guidance on female inclusion to be applied to preclinical research. The antiquated myth that females are “messy” and make research more difficult persisted, despite no evidence to support their exclusion. As preclinical studies began to examine female inclusion in research, it became clear that females were not more inherently variable than males. As a result, beginning in 2015, the NIH enacted a policy that investigators must account for sex as a basic biological variable in NIH-funded preclinical research.

This NIH policy impacts every investigator who plans to seek future federal research funding. Most importantly, this mandate aims to drastically improve the translational relevance and reproducibility of preclinical science. Ultimately, the policy is expected to improve the safety and efficacy of treatments for human disorders.

It is important to note that considering sex as a biological variable is not the same as looking for a sex difference. In fact, the guidelines do not explicitly state that sex differences must be examined. In contrast, the policy requires investigators to consider the inclusion of both male

and female animals and to observe and report sex-based data. Strong justification must be provided if only one sex is proposed.

So how will the inclusion of sex as a biological variable enhance preclinical AND clinical research?

First, at the experimental design stage, intentional study design at the outset to determine whether there are sex differences in a particular area of study will allow researchers to develop hypotheses and randomize and balance the sexes across experimental groups.

Second, studying the outcome measures separately in each sex could lead to better interpretation of treatment effects more broadly and inform the design of additional tests and tools that consider fundamental differences between males and females.

Third, in the analysis stage, disaggregating data by sex reveals sex differences that are hidden when pooling data from males and females to establish whether there is a sex difference from a treatment [2].

Fourth, at the reporting stage, improved reporting of the sex of the animals and cells used in research will inform others in their research and allow more researchers to pursue fruitful avenues of sex differences research.

As a result of the 2015 NIH policy, a culture shift has slowly begun to spread across the preclinical research ecosystem. The consideration of sex as a biological variable in biomedical research is increasing. But there is still much work to be done.

Additionally, a knowledge gap has emerged, with many researchers left confused as to how to navigate the new policy effectively. Many researchers have long-standing projects and are now trying to understand how their results in males apply to females. Historical literature is biased towards males, and so researchers must embark on new research projects without knowing HOW or IF their findings will be observed in females or if a true sex difference exists.

This is the major goal of this video series. Across the next seventeen videos, we will help researchers that are new to the topic of sex as a biological variable navigate the inclusion of females into their research. We will demonstrate why it is important, how it will open new areas of research and why it will improve the reproducibility of preclinical research.

The content of these training videos will be distributed across three modules, each designed to cover the main topics needed to abide by the NIH policy.

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In Module 1, we will cover the fundamentals of sex differences in brain and behavior.

In Module 2, we will cover practical recommendations for all areas of study design, from housing considerations, to when and how to track hormones, to statistical analysis and study design.

Finally, Module 3 will cover sex differences in pharmacology.

As you watch these videos, there are a few final points too keep in mind. First, while sex and gender are often used interchangeably, these constructs are very different. Sex is a biological designation that originates from an organism's sex chromosome complement, represented as XY or XX, whereas gender is a social construct that is only applied to humans. Since this video series is restricted to preclinical research, the term "sex" will refer to a biological distinction. Additionally, throughout the videos, we use the term sex hormones to refer to steroid hormones made in the gonads, and steroid hormones made in the brain are called neurosteroids. Finally, because approximately 85% of biomedical studies use only rodent models, we focused on rodents throughout this series [1].

We thank you for watching this educational series. While no group of videos on sex as a biological variable can be inclusive, we hope that these videos serve as a guide for practically approaching the NIH's policy into your own research program.

References

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2. Clayton, J.A., *Applying the new SABV (sex as a biological variable) policy to research and clinical care.* Physiol Behav, 2018. **187**: p. 2-5.DOI