Sex matters at the NIH

By Lauren Martz, Staff Writer

For decades, the norm in preclinical research has been to use male animals for *in vivo* studies and ignore the sex of cells in *in vitro* studies for reasons of cost and convenience. The NIH wants to make the inclusion of both sexes the default in all preclinical studies and thinks the best way to alter entrenched habits is by focusing on grants and publications.

Last month, the NIH announced an investment of \$10.1 million to supplement 82 existing projects and enable them to add sex as a variable. The funding adds to \$4.6 million committed in 2013 by the NIH's Office of Research on Women's Health (ORWH). In addition, the NIH is evaluating a range of policy changes and training modules to encourage routine inclusion of both sexes in cell-based, tissue-based and animal studies.

"These awards help stimulate researchers to add sex as a biological variable to existing research and increase awareness of the importance of studying sex in preclinical research," Juliana Blome, associate director for science policy, planning and analytics at ORWH, told *SciBX*. "Nonetheless, we want scientists to incorporate sex as a biological variable into all research projects moving forward."

Clinical trials have included equal representation of men and women since the NIH Revitalization Act of 1993, which requires the inclusion of females in NIH-funded clinical trials. Last year, more than half the patients enrolled in clinical trials in the U.S. were female.¹

However, preclinical studies have no such guidelines, and most researchers not directly addressing questions related to sex do not consider it in their experimental design. But because preclinical experiments are the most common way of learning about drug mechanisms, the risk of performing them in only one sex is that important mechanistic differences that could translate to humans go unrecognized.

Farida Sohrabji, a professor and associate chair in the Department of Neuroscience and Experimental Therapeutics at **Texas A&M Health Science Center**, said, "If you are only studying male cell and animal models, then the first indication of how the drug will perform in females is determined by patient testing. This is putting women at an unequal and elevated risk."

"It is surprising that investigators wouldn't report the sex. They report the reagents used. They report where they got those reagents. Why is that information more important than the biological sex of the test subjects?"

—Gillian Einstein,
University of Toronto

She added, "We have known about the issue for a long time, but it has bubbled to the forefront now because we have been uncovering clinical problems. We are just now really seeing the impacts that failing to study sex differences have on drug efficacy and safety."

According to Kathryn Sandberg, the issue has received renewed attention because of a study last year on **Sanofi's** insomnia therapy Ambien zolpidem that showed blood levels of the drug remained high enough in women the morning after taking it to impair driving or other activities that require alertness. Based on those results, the **FDA** mandated that doses of zolpidem-containing insomnia drugs be halved for women.

Lawrence Cahill added, "Ambien received a lot of attention, but do you think that Ambien is the only drug out there with potential different effects in men and women?"

Indeed, 8 of the 10 drugs taken off the market by the FDA between 1997 and 2000 had more serious adverse events in women than men.²

Sandberg is a professor and vice chair of research in the Department of Medicine, director of graduate studies in physiology and director of the Center for the Study of Sex Differences in Health, Aging and Disease at **Georgetown University**. Cahill is a professor of neurobiology and behavior at the **University of California, Irvine**.

Avoiding the issue

Several researchers who spoke with *SciBX* said that most scientists include only one sex in preclinical studies because it is generally assumed that using both sexes will not add any relevant data if the mechanism being studied is not directly related to male or female systems.

There is also a disincentive to include an extra arm in a study with sex as a variable because it adds complexity and cost to experiments.

"When William Harvey made the first Western scientific discovery that blood goes in a circle, no one asked if this was also true in women," Cahill said. "When Watson and Crick later identified the double helix, no one asked if it might be a triple helix in women. It was just assumed that the sex variable is not important. Now in the last 10–20 years, data from all over the place are showing that the sexes are both similar and different and that the differences have been understudied."

According to Sandberg, male cells and animals have been the default in preclinical studies because of a long-standing assumption that studying males is simpler. "There is a myth that you need to control for the hormone fluctuations from the estrous cycle if you choose to study females," she said. "If you are following the stages of the cycle, this adds variables to the study, requires more groups of animals and requires that you sync the cycles in your animals."

Cahill added that "Irving Zucker blew this notion out of the water when he actually studied variability of males and naturally cycling females and found no variability."

Zucker did a meta-analysis of 293 papers to study the behavioral, morphological, physiological and molecular traits in male mice versus

female mice in each of the four stages of the estrous cycle. His team found that variability was not significantly greater in the females for any of the traits tested.³

Sohrabji told *SciBX* that although including both sexes increases costs in an environment in which researchers are already fighting for funds, that is not a justification for avoiding the issue.

“Budgetary concerns are not a good reason to exclude one of the sexes,” she said. “If you find in initial studies that there is no difference between the sexes in your experiment, you can group males and females into a single cohort and you don’t need an overall larger group of animals for statistical significance. If you do find an apparent difference, the scientific concerns should outweigh the budgetary concerns because the differences may translate to the human population.”

She noted that one way to control costs while considering sex differences is to study differences at the cellular level first. “It is just as important to pay attention to the sex of cells as of animals,” said Sohrabji. “Every cell has a sex, which sometimes affects the kinds of proteins expressed and the cellular processes that occur. It is less expensive to identify differences that affect a mechanism of interest at the cell level than once the research has progressed to animals or people.”

Beyond the budget

The grant supplements are only a first step in the NIH’s plans to change routine clinical practice. The agency’s next steps include plans to alter requirements in grant applications and discussions with journal editors to influence how data are published.

“Large ships in the ocean don’t turn very fast, and we are talking about a huge, 500-year-old ship. It is going to be hard to change the way that sex is considered in preclinical research, and we need to roll out a plan carefully,” said Cahill.

According to a commentary published in *Nature* earlier this year from NIH director Francis Collins and associate director of ORWH Janine Clayton, the goal is to implement policies at the NIH that will require all grant applicants to report plans for studying and balancing the sexes in their work.¹

Sandberg thinks that starting with grant requirements could be an effective strategy. “Obtaining grants is very competitive,” he said. “If the NIH required us to say which sex or sexes of an animal we plan to study, the prevalence of the disease that we are studying in each sex and

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—Juliana Blome,
National Institutes of Health

an explanation of why we have chosen to look at each sex, that may have an impact.”

He added, “If this information were made as a scorable point in grant applications, it would go a long way to addressing the problem.”

Changing reporting practices for publication of preclinical work would also incentivize researchers

to include sex as a variable. “Inadequate inclusion of female cells and animals, and the failure to specify sex used in experiments, affects our ability to verify and duplicate scientific results,” said Blome.

Sandberg said that journals should take it upon themselves to require that researchers report the sex of animals when they publish their studies.

Gillian Einstein agreed. “Journals have a lot of power, and it is important for them to take an editorial position on this,” she said. “If researchers can’t get their papers published in the journal that they want, they will do what needs to be done.”

She added, “It is surprising that investigators wouldn’t report the sex. They report the reagents used. They report where they got those reagents. Why is that information more important than the biological sex of the test subjects?” Einstein is an associate professor of psychology and director of the Collaborative Graduate Program in Women’s Health at the **University of Toronto**.

Cahill agreed that adding requirements at the level of grants and publications could help change practices. He added that it could be an easier sell if it were put in the context of personalized medicine, a hot topic among researchers. “We are also focusing so much effort on this idea of personalized medicine,” he said. “If you think about it, personalizing for sex should be one of the first things that we do.”

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COMPANIES AND INSTITUTIONS MENTIONED

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