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K. Ranganathan and Manish Kumar Goyal are in the Department of Civil Engineering, Indian Institute of Technology, Guwahati 781 039, India.*
**e-mail: ranganathan@iitg.ernet.in*

Subject selection bias in animal studies

Mihir Parikh

Animal studies have always shown bias in selection of particular sex. Female subject numbers may be becoming substantial in clinical trials after law enforcement but sadly due to no such mandates, female animals are often left out. Because of multiple cited reasons male animals are still the preferred choice in the preclinical studies. The report highlights this issue and ways to overcome bias by suggesting steps that can be taken.

Several preclinical or biomedical studies have proven time over that male and female animals behave differently to drugs and devices. They show disparity in terms of safety, efficacy or subtle differences in pharmacokinetic and pharmacodynamics profiles which, if not given importance at basic research level, will amplify in clinical studies and may prove to be a waste of resources, let alone abject disaster for human health.

Realization of the fact that research with no to little female participants is egregious for science by the National Institutes of Health (NIH), USA, saw the establishment of the Office of Research on Women's Health (ORWH). The NIH Revitalization Act passed on 10 June 1993 by legislators exhorted and mandated to enrol more female participants, if not equal to male numbers, in all government control and supported phase III clinical trials. This drove change leading to increased enrolment of women in clinical studies in USA, the European Union and Australia. But no such compulsion has been made for basic biomedical research to include both sexes. Most experimental pharmacological studies show preferences for male rodents.

Biological fields and bias

From basic science to biotechnological, preclinical to translational research, all show male bias. According to an editorial in *Nature*[1], males dominated most of the animal studies as male biases were found in 8 out of 10 biological fields with ratios of male-only against female-only studies: 5.5 in neuroscience, 5 in pharmacology and 3.7 in physiology. It was also reported that 75% articles in reputed immunology journals did not indicate the sex of animals used in the study.

Reliance on existing limited knowledge

For generations researchers and scientists have only used male animals. Publications also do not have sufficient sex-related data, which may help researchers in selecting appropriate animal gender. Referees of manuscripts ignore significance of the subject's sex. Even if both sexes are found to be studied in some journal articles, they fail to perform differential data analysis. Many ostensible reasons are cited for the male bias in research: literature search and adhering to the same protocol, convenience, cost, experimental simplicity, belief that sex difference is of no major concern beyond the reproductive system – results in data from male study extrapolated error free to predict for the female population with ease, etc. The most arguable, valid and contentious reason deals with the repro-

ductive oestrous cycle in females. Scientists surmise that oestrous cycle makes females inherently more variable than males – confounding the result data. To address this quasi-notion, it was thought that reliability from female studies can be achieved if female animals are monitored throughout their four stages of oestrous cycle, with daily vaginal cytology; but this exponentially adds to the cost of the project. When it comes to cell or tissue line studies, it is even rare to find from which sex they were procured, despite the fact that mammalian cells have unique chemistry, and structure depending upon the sex of the species. This is proved from a study[2] that demonstrated the gene responsible for difference in cells: XY chromosomes (as in male mice) had more neurodegeneration in the central nervous system than XX chromosomes (as in female mice). It is also now known that neurons from male animals are more sensitive to reactive oxygen species-induced stress, while those of female origin respond more sensitively to stimuli that leads to apoptosis. Therefore, sex of *in vitro* cell lines should not be ignored in the studies.

Female diseases and male subjects

More dreadful condition arises when male animals are chosen in the experiments to study female-dominant diseases. Anxiety disorders are diagnosed 2.25 times more in women than in men, but only 45% of animal studies use female subjects. With poor functional outcomes accompanied with more depression, stroke events are more prevalent in females compared to males, but female animals are used in only 38% of studies. Hypertension is found to be equally present in both sexes, but 65% of scientific reports have males as the study subjects. Surgical research is also not free from animal bias. It has been reported that 80% of the studies in surgical journals used male animals. Women are 1.5 times more susceptible to pain than men and women show higher adverse drug reactions, but still studies use males exclusively, which resulted in withdrawal of 8 out of 10 prescription drugs from the US market as side effects manifested differently in both sexes[3].

Empirical evidence to use female animals

Dearth of evidence against oestrous cycle interference does not make females unsuitable experimental models. With the purpose to find solution for the issue of oestrous cycle which hinders researchers in using females in their study, more than 8000 nociceptive-related observations were made. The outcome was that females tested, irrespective of their four-stage oestrous cycle, showed no more variations in data compared to male animals[4]. The conclusion drawn in nociceptive experiment that females depict no significant variations compared to males needs to be verified in other diseases or conditions.

Conclusion

To correct the bias in animal research, reforms should be made by many and at myriad levels. Legislative strictness to include both sexes in the studies is welcomed. Government agencies such as CPCSEA that overlook the process of approving animal studies protocol should encourage and ask for equal sex participations in projects. In October 2014, NIH launched a new policy wherein investigators will have to disclose their preclinical study plans that include both sexes. It is comprehensible that to carry out studies on both sexes for an experimental unknown drug would incur a great amount of monetary burden; but if promising results appear in one sex study, then the project should extend to include the other sex. GenderBasic of the European Union, German Society of Epidemiology, and Canadian Institutes of Health have been making changes since many years[5].

Authors should report unambiguously the data obtained from both sexes to facilitate reproduction and replication of the study using same sex or both sexes. On the other hand, journals should ask their editors and reviewers to mandatorily check if the animal sex has been mentioned in the manuscript by the authors. Frequent revisions can be asked till the authors disclose the sex of the animals used.

The driving force behind research studies is the fund. So the funding agencies should not consider the preclinical research proposals that do not properly state the animal sex to be used and should encourage the utilization of both sexes and proper analysis of data.

If properly planned and executed, preclinical research will be soon free from bias and would be of worth for the benefit and betterment of humans.

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Mihir Parikh is in the Pharmacology Department, Anand Pharmacy College, Anand 388 001, India.
e-mail: parikhmihirp@yahoo.co.in