Reporting Sex and Sex Differences in Preclinical Studies

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ATVB publishes research work that advances scientific fields in a rigorous and reproducible manner. We have implemented multiple approaches to follow the National Institutes of Health guidelines for rigor and reproducibility. In 2013, *ATVB* developed a checklist in the peer review process to facilitate comments on multiple technical requirements, including the sex of animals used in preclinical studies. We have also emphasized the National Institutes of Health guidelines that encourage researchers to study both sexes in preclinical animal models. These include publishing a review[1] entitled "Sex Differences in the Development of Cardiovascular Diseases" and an ATVB Council statement[2] to encourage authors to consider sex differences in designing and reporting experimental arterial pathology studies that detail the mode by which *ATVB* complies with the National Institutes of Health guidelines.[3] The journal appointed a technical review editor, Dr Hong Lu, who assumed the role in September 2017 to assess the many elements required for adherence to the National Institutes of Health guidelines. These include issues such as the rigor of statistical analyses and animal background strain, age, and sex. The designation of sex of origin in studies of primary cells derived from cell culture is not as common as it is in animal studies. This is probably because of the unproven assumption that the lack of the hormonal environment in cell culture eliminates the need for designation of sex. However, because the differences of X and Y sex chromosomes and possibly sex-related differences in genomic imprinting are preserved in cultured cells, these cells could theoretically retain the ability to respond in a sex-dependent manner.[4] Therefore, we also encourage and will monitor the reporting of sex in primary cell isolation and culture.

In a recent letter to the *ATVB* editors, Ramirez and Hibbert[5] performed a comprehensive literature search and detailed statistical analysis on articles focusing on preclinical research of atherosclerosis and aneurysms from 2006 through 2016. They concluded that publication of guidelines and statements alone is not sufficient to assure the reporting of sex and sex differences in preclinical studies. To more objectively assess whether and how sex information in preclinical research has been reported, the *ATVB* editors reviewed 332 basic science research articles published in *ATVB* between 2016 and 2017.[6–337] After excluding those that studied only human samples, human cells, cell lines, or computational models, 159 articles published in 2016 and 136 articles published in 2017 were analyzed (Tables 1 through 5), which reported studies in animal models or primary cells isolated from animals.[6–300] There were 7 species reported in these articles (Table 1), including zebrafish, mouse, rat, rabbit, dog, pig, and nonhuman primates. Among these 295 articles analyzed, 79% and 92% of those from 2016 to 2017, respectively, provided sex information for in vivo models. However, only 11% of the articles in 2016 and 21% in 2017 reported results from both males and females. One hundred and thirty-three of the 295 articles reported primary cells isolated from mouse, rat, cow, or pig. Twenty-eight percent of the articles in 2016 and 27% in 2017 provided sex information, whereas only 3 articles studied cells from both male and female animals.

In this article, we provide the results of analysis of reported data on sex from animal models (Tables 2 through 5) and primary animal cell cultures among basic science articles published in *ATVB* between 2016 and 2017.[6–300] We also discuss what we have already implemented and what we will do in the future to encourage authors to report findings from both sexes in preclinical studies. We will continue to monitor and document the analytic results of reporting sex and sex differences in *ATVB* on an annual basis.

Zebrafish

Zebrafish have become an increasingly valuable model to study cardiovascular development and functions. Zebrafish breed prodigiously and generate large numbers of offspring rapidly. Their larvae are transparent, which make many anatomic features easily visible during development. Zebrafish have similar genetic structure and organ distributions to humans and share ≈70% of their genome with humans.[338] The popularity and value of zebrafish research have also been reflected by the number of research articles published recently in *ATVB*. In 2016 and 2017, 8 articles reported zebrafish models,[6–13] involving studies of lipoprotein signaling, endothelial development

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Table 1. All Animal Species Reported in Preclinical Studies

Species	No. of Articles		References
	2016	2017	
Zebrafish	5	3	6–13
Mouse	134	117	14–264
Rat	10	11	265–285
Rabbit	2	4	286–291
Dog	1	0	263
Pig	2	4	11,292,293,295–297
Primate	3	0	298–300

and functions, angiogenesis, and lymphangiogenesis. These articles studied zebrafish at stages during embryonic or larval development when the sex cannot be identified.

Sex determination is more complicated compared with mammalian organisms because zebrafish do not have sex chromosomes.[339,340] However, there are multiple features that can help identify male and female zebrafish at the adult stage. Because sexual dimorphism has been noted in zebrafish (in a few examples[341–344]), the *ATVB* editors encourage authors to study both sexes and provide the sex identification information if adult zebrafish are studied. If the sex cannot be identified before the adult stage, we encourage the authors to briefly state this potential limitation in the Methods section.

Rodent Models

Rodents are the most common species used to study cardiovascular functions and diseases. The most popular species are mice and rats. Among the 295 articles analyzed, 251 (85%) articles reported mouse models,[14–264] 114 (39%) reported results on primary cells isolated from mice, 21 (7%) reported rat models,[265–285] and 14 (5%) reported results on primary cells isolated from rats. For in vivo studies, 84% of the articles reported sex in mouse models, and 95% reported sex in rat models after exclusion of those that only used embryos or neonates. The most commonly studied sex is male in both mice and rats (Tables 3 and 4). In adult mice and rats, the estrous cycle is ≈4 to 5 days, which leads to striking changes in sex hormones during this brief interval.[345,346] In addition, some cardiovascular diseases have milder phenotypes in females than in males.[1,3] For these reasons, many researchers elect to focus their studies on male rodents, especially in mice.

Table 2. Reporting Sex of Animals in Preclinical Studies

Species	Articles Reporting Sex, %	
	2016	2017
Mouse	79	91
Rat	100	91
Rabbit	0	100
Dog	100	…
Pig	50	100
Primate	100	…

Table 3. Percentage of Articles Reporting Sex in Mouse Studies

Reporting Sex	Year of Publication	
	2016	2017
Male, %	60	53
Female, %	7	15
Both male and female, %	11	23

For articles that studied rodent primary cell cultures, after excluding those that used cells from neonates, sex was reported in ≈25% and 40% of the studies in mice and rats, respectively. Many studies included both in vivo studies and primary cell cultures from the rodent model. Most articles specified the sex of animals in the Methods section but did not provide sex information for primary cell isolation and culture. Although it is very possible that same sex was used for both in vivo and in vitro studies, our analysis only counted the articles that clearly stated sex information in the primary cell isolation and culture sections.

ATVB publishes articles that cover a spectrum of research areas on lipoprotein metabolism, atherosclerosis, thrombosis, and vascular biology, and related diseases. Sex differences are an important feature of these cardiovascular physiological and pathological states. One example is angiotensin II-induced abdominal aortic aneurysm that was discussed in the recent ATVB Council statement.[2] The incidence of angiotensin II-induced abdominal aortic aneurysm is ≈80% to 100% in male hypercholesterolemic mice but only ≈10% in female mice with the same genetic background. Although the first publication in apolipoprotein E-deficient mice used females,[347] the vast majority of subsequent studies evaluated only male mice,[348] except for a few articles that have studied sex differences of the disease in this mouse model. Therefore, if a strong sexual dimorphism has already been identified and recognized by the research community, the *ATVB* editors recommend monitoring this specific issue and suggest that the authors provide a succinct justification in their manuscripts as to why a specific sex was studied. Otherwise, we recommend that the authors study both male and female rodent models.

Large Animal Models

Large animals are more expensive to maintain and study than rodent models, and thus data are frequently derived from relatively small sample sizes. However, these larger models may have more relevance to human physiological and pathophysiological conditions.[349] For example, atherosclerotic plaque rupture—a potentially fatal clinical condition—does not occur in the most commonly used mouse models of atherosclerosis.[349] In addition, a common and disease-relevant location of

Table 4. Percentage of Articles Reporting Sex in Rat Studies

Reporting Sex	Year of Publication	
	2016	2017
Male, %	90	91
Female, %	10	0
Both male and female, %	0	0

Table 5. No. of Articles Reporting Sex in Large Animals

Species	Articles Reporting Sex/Total No. of Articles		
	Male	Female	Both
Rabbit	2/6	0/6	2/6
Dog	…	…	1/1
Pig	4/6	1/6	0/6
Primate	3/3	…	…

human atherosclerosis is the coronary arteries, but atherosclerosis does not occur in coronary artery branches of mice.[350] Large animals, especially pigs and nonhuman primates, not only mimic multiple features of human diseases but also are valuable to test drug toxicology and potency. Therefore, the editors acknowledge the importance of and encourage cardiovascular research studies using large animals.

In articles published between 2016 and 2017 in *ATVB*, 6 studied rabbit models,[286–291] 1 studied a dog model,[263] 6 studied pig models,[11,292,293,295–297] and 3 studied nonhuman primate studies.[298–300] Among these 16 articles, 81% reported sex, 2 articles studied both sexes in rabbits, and 1 article reported both sexes in dogs (Table 5). There are multiple issues that limit large animal studies, such as sample size, the cost, and study duration. Although the journal does not require that both sexes should be evaluated in large animal studies, it is suggested that authors state clearly the sex of animals, provide a necessary justification as to why a specific sex was studied, and discuss the potential limitation if only 1 sex was studied.

Perspectives

ATVB implemented the technical review mechanism in September 2017, which did not impact reporting sex in the articles that were reviewed and analyzed between 2016 and 2017 (Tables 1 through 5). However, we note that more articles have reported both male and female mouse data (26 articles) in 2017, compared with publications (15 articles) in 2016 (Table 3). For those that only studied a single sex, reporting of female mice has also increased (17 articles in 2017 versus 9 articles in 2016; Table 3). The editors expect that this recently instituted technical review process will lead to a further increase in the reporting of sex and sex differences in *ATVB*.

The *ATVB* editors will evaluate each original research article with the following specific requests for reporting sex:

1. Sex of in vivo animal models and ex vivo primary cell culture studies must be clearly stated in the Methods section, Results section, tables, and figure legends.
2. If the authors studied only 1 sex, the authors will be asked to provide a justification for the selection of this specific sex.

We also recommend that the authors state the potential limitations of studying a single sex in either the Methods or Discussion section. The *ATVB* editors appreciate the responsiveness of authors to study sex differences in preclinical models more frequently. We hope that this continued effort from both the editors and authors will help the research community to enhance understanding and exploring sex differences of cardiovascular functions and diseases.

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Disclosures

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