Sex Bias in Basic Science and Translational Otolaryngology Research

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**Objectives/Hypothesis:** Recent studies revealed sex bias in surgical research. Although many diseases exhibit sex-based clinically relevant differences, otolaryngology research has not been evaluated for sex reporting and sex-based analysis. We postulate that a similar bias is prevalent in otolaryngology literature.

**Study Design:** Literature review.

**Methods:** Articles published from 2016 to 2017 in *The Laryngoscope, Otolaryngology–Head and Neck Surgery,* and *JAMA Otolaryngology–Head and Neck Surgery* were reviewed. Articles with animal subjects, human subject cells, or commercial cell lines were included. Data collected included study type, cell/animal sex, and sex-based data analysis.

**Results:** One hundred forty-four basic/translational research articles were identified. Sixty-nine (47.9%) of those lacked sex reporting. Of 75 studies that reported sex, 22 (29.3%) included both sexes, and 11 (14.7%) analyzed data by sex. One hundred five (72.9%) used animal subjects, of which 54 (51.9%) lacked sex breakdown. Among animal studies, 48/105 included only one sex, and three articles analyzed data by sex. Fifty-four studies used commercial cell lines (N = 23) or human/animal subject cells (N = 31). Among cell groups, 28/54 (51.9%) were of unknown sex, and seven were single sex. Eight (14.8%) studies included data analysis by sex. Domestic studies exhibited a lower rate of sex reporting in both animal and cell studies, and a lower rate of sex-based analysis in cell studies.

**Conclusions:** Sex may influence outcomes but is underreported and underanalyzed in basic/translational otolaryngology research. Because this research frequently lays the groundwork for clinical trials and standards of care, future research must address these sex-based discrepancies.

**Key Words:** Gender bias, biomedical research, humans, animals, cells, sexism, male, female, research design/standards.

**Level of Evidence:** NA

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**INTRODUCTION**

Well-known differences exist between the male and female sexes in the healthcare realm. The sexes differ in prevalence of certain diseases, drug metabolism, and response to various treatment modalities. The current era of rapid medical discovery and advances in clinical care has been accompanied by an increasing focus on personalized medicine, with clinical and treatment decisions based on biological differences among sexes, races, exposures, and genetic markers, to name a few. Sex may be the most fundamentally different biological variable. Thus, sex deserves paramount attention in clinical and preclinical research endeavors to best inform clinical guidelines.1

Prior studies have demonstrated the importance of including sex as a biological variable in medical research. In addition to well-studied differences between the sexes, more subtle yet still clinically significant variability in pharmacokinetics has been unveiled, further highlighting the need for sex-based research reporting and analysis. After accounting for body weight, many drugs still exhibit sex-based differences in metabolism and toxicity.2 In general, women have a higher risk of adverse drug reactions (ADRs) than men.3 Between 1997 and 2001, eight of 10 prescription drugs withdrawn from the market were withdrawn due to an elevated risk of life-threatening ADRs in women.1,4 Perhaps the most widely known example is zolpidem tartrate, which was removed from the market after multiple women were involved in fatal motor vehicle accidents in the morning postadministration. The initial market dosage was identical for both sexes despite zolpidem clinical trial data demonstrating that females metabolize the drug more slowly than men. Following the ADRs, the Food and Drug Administration later halved the dose for women.5,6 Because these
metabolic differences are in part due to cellular and subcellular processes, disclosure of sex in cell research is equally important.

**Guidelines Surrounding Sex in Research**

Despite known sex differences, female subjects have often been excluded from clinical and preclinical research studies due to concerns for potential physiologic confounders such as the estrous cycle, easier access to male animals or a single-sex animal group, and/or simple obliviousness to the importance of sex. In response to early recognition that women were unequally included in clinical research, the National Institutes of Health (NIH) Revitalization Act of 1993 was passed, requiring all NIH-funded clinical studies to include female and minority participants. Other foreign governmental agencies subsequently adopted and issued similar guidelines governing international clinical research. Since the early 1990s, sex bias and sex reporting in clinical research has shown mild yet insufficient improvement, and many health journals still lack sex reporting requirements for their articles.

Of note, the 1993 Revitalization Act covered female participation in clinical trials only. Thus, basic science and translational research involving animal and cell subjects remained without sex-related mandates and have failed to show even the minimal improvement that clinical research has demonstrated with regard to female inclusion and sex reporting. Beery et al. analyzed rodent studies across multiple biology disciplines and demonstrated that 80% of studies were still using only male animals in 2009, a statistic nearly identical to animal studies in 1990. A 2014 review of five surgery journals—the largest published analysis of sex bias in basic science literature to date—revealed that 32% of articles did not report animal- or cell-subject sex. Furthermore, those reporting sex were 80% male-only studies, and only 3% used both animal sexes. Among studies analyzing female-prevalent pathologies, a striking 12% actually included female animal/cell subjects.

The recent 2016 NIH mandate requiring that “sex as a biological variable will be factored into research designs, analyses and reporting in vertebrate animal and human studies” extended sex reporting requirements to animal studies in all NIH-funded human and animal studies, in addition to requiring sex-based data analysis. Currently there are no published analyses of the impact of these new requirements in post-2016 research. In addition, it is important to note that cell research was not included in this 2016 mandate, thus leaving the growing cohort of basic and translational cell-based research without regulations to prevent sex bias or enforce sex reporting.

**Sex Bias in Otolaryngology Research**

There are known sex-based differences in diseases of the head and neck. Thyroid disease is more common in females, whereas males have a higher risk of certain head and neck cancers. Females are more likely to suffer from benign paroxysmal positional vertigo. There are notable differences in nasal airflow between the sexes, among numerous other examples. However, despite this variability, limited data exist regarding sex bias in otolaryngology research. A single otology study is the only published analysis of sex bias in otolaryngology literature to date. This study analyzed 210 basic science and preclinical publications on noise-induced hearing loss from 2010 to 2015, and showed that 33% of studies did not report the total number of animal subjects, almost two-thirds used only male animals, and only 25% studied both sexes.

Given numerous well-known sex-based differences driving many clinical and pharmaceutical guidelines, female subjects must be included in future human, animal, and cell research to investigate potential sex differences with clinical significance. Failure to include both sexes, perform sex-based analysis, and publish outcomes by sex may contribute to rising irreproducibility in preclinical biomedical research and implementation of unsafe practices. Otolaryngology research is not exempt from the potentially hazardous implications of failure to comply with these mandates. To date, the general otolaryngology literature has not been analyzed for sex bias and reporting practices.

**Objective**

The goal of this study was to determine if sex bias exists in the current general otolaryngology basic science and translational research by analyzing the prevalence of sex-based reporting and statistical analysis.

**MATERIALS AND METHODS**

**Data Collection**

All published original articles in all 2016 to 2017 issues of *The Laryngoscope, Otolaryngology–Head and Neck Surgery,* and *JAMA Otolaryngology–Head and Neck Surgery* were reviewed (Fig. 1). These three peer-reviewed journals were selected because they are the highest impact factor, general otolaryngology journals with broad readership. Articles with animal subjects, human subject cells, or commercial cell lines were included. One of two reviewers performed data extraction for each article. Text, figures, tables, and appendices in each article were reviewed for data collection. Data collected included study type, cell/animal sex, and sex-based data analysis. Cell group and commercial cell line sex were investigated using online search tools when cell sex was not provided in the article.

**Variables**

Variables collected for each article included study type (basic science vs. translational, human vs. animal, primary cells vs. commercial cell lines), study location (domestic vs. international), single versus multicenter or database study, randomized controlled trial, and subject demographics. Studies were examined for reporting of cell or animal gender, providing sex breakdown, sex-based statistical analysis, and presence of any statistically significant sex differences in results. Sex-based statistical analysis was defined as presentation of results separately for each sex in the article text or in table/figure format.
Statistical Analysis

The sex variable was analyzed for overall reporting in addition to a subanalysis by subject type and study location. Differences in sex distribution and reporting were assessed using the $\chi^2$ and Student t tests where appropriate. Statistical analysis was performed with Stata 14.0 software (StataCorp, College Station, TX).

RESULTS

All Studies

Of all articles in the 2016 to 2017 issues of the three journals, 144 basic/translational research articles were identified (Fig. 1). Ninety (62.5%) articles utilized animal subjects only, 39 (27.1%) used cell or commercial cell line subjects only, and 15 (10.4%) used both animal and cell subjects. One hundred eight were published in The Laryngoscope, 30 in Otolaryngology–Head and Neck Surgery, and six in JAMA Otolaryngology–Head and Neck Surgery. Of these, 47.9% (69/144) lacked sex breakdown (Table I). Of 75 studies that reported sex, 22 (29.3%) included both sexes, and 11 (14.7%) analyzed data by sex (Fig. 2).

Animal Studies

One hundred five (72.9%) articles used animal subjects, of which 54 (51.9%) lacked sex reporting (Table I). Of 50 studies that reported sex, animals of a single sex were used in 48 (96.0%) studies, and only three (6.0%) analyzed data by sex. Six (5.7%) animal research articles did not report the total number of animals studied.

Cell Studies

A total of 54 studies used commercial cell lines (N = 23) or human/animal subject cells (N = 31) (Table II). The sex of the commercial cell lines was provided in 5/23 (21.7%) articles. Cell sex was provided in 11/31 (35.5%) articles. Among all 54 cell groups, 26 (48.1%) reported sex, and seven (26.9%) of those studies used a single sex. Data analysis by sex was included in 8/54 (14.8%) articles.

Study Location

Among animal studies, 36/67 (53.7%) of domestic and 18/37 (48.6%) of international studies lacked sex reporting (Table III). Domestic studies were also less likely than international studies to report cell sex: 65.6% versus 40.9% lacked sex breakdown, respectively, and 44.8% domestic and 48.6% international articles studied a single-sex animal. Sex-based data analysis was performed in 3.0% versus 2.7% animal studies and 12.5% versus 18.2% cell studies in domestic and international articles, respectively.

DISCUSSION

Although the 1993 and 2016 legislation regarding sex bias and sex-based reporting of outcomes in NIH-funded research have initiated a movement toward improved research practices, the current literature review highlights a significant deficiency in sex reporting and sex-based data analysis, particularly within basic science and translational research. However, otolaryngology research was not included in past sex bias analyses of basic science literature; this is the first study to highlight the extent of this problem in otolaryngology.
This analysis of peer-reviewed otolaryngology journals, encompassing general otolaryngology and all subspecialties, reveals significant sex underreporting and strikingly low rates of sex-based analysis. Overall, the majority of these measures of sex bias are unfortunately more frequent in otolaryngology animal research compared to analyses of other surgical literature. In animal research, sex was unspecified in 51.9% of otolaryngology and 22% of general surgery articles.

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TABLE II. Demographics and Sex Reporting for 54 Studies Involving Human/Animal Subject Cells or Commercial Cell Lines.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Sex Not Reported</th>
<th>Single-Sex Cells</th>
<th>Both-Sexes Cells</th>
<th>Sex-Based Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial cell lines, N = 23</td>
<td>18/23 (78.3%)</td>
<td>2/23 (8.7%)</td>
<td>2/23 (8.7%)</td>
<td>2/23 (8.7%)</td>
</tr>
<tr>
<td>Human subject cells, N = 31</td>
<td>11/31 (35.5%)</td>
<td>4/31 (12.9%)</td>
<td>16/31 (51.6%)</td>
<td>6/31 (19.4%)</td>
</tr>
<tr>
<td>Total (all cell groups), N = 54</td>
<td>28/54 (51.9%)</td>
<td>7/54 (13.0%)</td>
<td>18/54 (33.3%)</td>
<td>8/54 (14.8%)</td>
</tr>
</tbody>
</table>

TABLE III. Demographics and Sex Reporting Based on Study Location for Animal and Cell Studies.

<table>
<thead>
<tr>
<th>Study Location</th>
<th>Sex Not Reported</th>
<th>Subjects of Single Sex</th>
<th>Subjects of Both Sexes</th>
<th>Sex-Based Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Domestic, N = 67</td>
<td>36/67 (53.7%)</td>
<td>30/67 (44.8%)</td>
<td>5/67 (7.5%)</td>
<td>2/67 (3.0%)</td>
</tr>
<tr>
<td>International, N = 37</td>
<td>18/37 (48.6%)</td>
<td>18/37 (48.6%)</td>
<td>2/37 (5.4%)</td>
<td>1/37 (2.7%)</td>
</tr>
<tr>
<td>Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domestic, N = 32</td>
<td>21/32 (65.6%)</td>
<td>5/32 (15.6%)</td>
<td>7/32 (21.9%)</td>
<td>4/32 (12.5%)</td>
</tr>
<tr>
<td>International, N = 22</td>
<td>9/22 (40.9%)</td>
<td>2/22 (9.1%)</td>
<td>11/22 (50.0%)</td>
<td>4/22 (18.2%)</td>
</tr>
</tbody>
</table>

Three of seven (42.9%) otolaryngology and 7/13 (53.8%) general surgery articles that used animals of both sexes reported outcomes by sex. However, sex bias is less prevalent in otolaryngology cell research, as 48.1% otolaryngology and 24% general surgery articles specified cell sex. Among 18/54 (33.3%) cell groups in otolaryngology that used both cell sexes, eight (44.4%) performed sex-based data analysis, whereas no general surgery study using cells included sex-based reporting.17

This study also reveals a disparity in sex reporting and analysis when comparing domestic and international otolaryngology cell research. Domestic cell studies appear to exhibit overall lower rates of both sex reporting and sex-based analysis compared to international studies. Domestic animal studies also exhibit slightly lower rates of sex reporting, but similar rates of sex-based data analysis. This difference may partially be due to a greater awareness of better sex reporting and analysis practices among international authors, because many international journals have mandatory sex-based reporting/analysis requirements.23

Despite NIH-initiated attempts to address the sex bias that is rampant within basic science research across all medical and surgical fields, otolaryngology research continues to exhibit significant sex bias in animal and cell research that is just as poor, if not worse, than other surgical literature. There are well-established sex-based differences in many otolaryngology diseases, and likely additional, currently undiscovered differences in disease risk, course of illness, and drug metabolism. We strongly emphasize the importance of enacting and following more stringent sex-related reporting and analysis guidelines in all otolaryngology basic science research. Furthermore, as the 1993 and 2016 NIH mandates do not cover cell research, establishment of similar widespread guidelines for research involving cell subjects is imperative, as these studies often provide the foundation for future animal studies and clinical trials.24,25 Without these steps we consciously limit our ability to conduct quality research and discover the sex differences that permit safer, evidence-based, individualized patient care.

CONCLUSION

Although sex may have a significant impact on otolaryngology outcomes, it is underreported and underanalyzed in basic science and translational otolaryngology research involving animal and cell subjects. Future research in otolaryngology must address these sex-based discrepancies in both demographics and sex-based statistical analysis to provide a sound foundation for subsequent clinical trials and most accurately guide evidence-based, patient-centered clinical guidelines.

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BIBLIOGRAPHY


