38th Advisory Committee on Research on Women’s Health

Sex Differences in Basic Research: Part II: Sex Inclusion Toolkit

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Part I: Fostering an Increased Awareness of the Importance of Sex Differences
Hypothesis

The next generation of biomedical advances that improves the lives of all people - drugs, devices, and best medical practices - requires fundamental discovery research that includes sex as a variable.
Personalized Medicine Begins with Basic Research

Factors important to medical care vary by individual:

✓ Sex
✓ Age
✓ Environment – housing, husbandry, birth order

Gender
Ethnicity
Race
Socio-economic status

Future of medical treatment involves “personalized medicine” based on all of these factors.
Messing

- Hard to disseminate to public efficiently
- Tough to generate political capital
- Difficult to “boil down” to one sentence

At its core, the message of sex-based studies is simple: Females and males differ in their experience of health, dx and tx of disease and sign posts of age.
Intellectual
The biology of sex matters to the health of our nations people.

Economic
The study of sex-based basic biology provides a cost-effective way to increase the quality and reduce costs of healthcare.

Personal
There must be something more than having my baby and then waiting for my cancer!
Why have male animals become the model for all?

- There is a long accepted tradition of over reliance on male models
- No estrous cycles in males
- Researchers unaware of the magnitude of sex differences that are due to non-reproductive factors
- Reviewers are not looking for sex differences at the preclinical stage of the research trajectory
- Despite calls to action for sex inclusion in research by NIH, little focus and change on animal and cell research….
- ....UNTIL NOW!
Barriers to Dual Sex Inclusion in Animal Research

- Cost (debatable)
- Complexity for female hormones (estrous cycle)
- Lack of institutional/IRB guidance on sex inclusion
- Reviewer lack of knowledge regarding sex variables in experimental design
- Traditional over-reliance of male animals and cells as accurate models
- No buy-in from stakeholders (reviewers/funders/journals)
- Lack of knowledge about magnitude of sex effects
- Misuse of “sex vs. gender” adds complexity and excuses to the issue
- Publishers neglect sex consideration in cell and animal research.
Has sex disparity in surgical research improved or worsened over time?

Sex bias exists in basic science and translational surgical research.
Yoon DY ... Kibbe MR. Surgery. 2014 Sep;156(3):508-16.
Consequences of not including females

• Over reliance on males obscures sex differences that may have clinical consequences
• Increased number of adverse effects in women from drugs and devices
• Missed opportunities to better understand the sex-biology needed to develop more personalized diagnostics, interventions and treatment
• Exacerbation of the real and alarming rise in US female mortality*
• Increased long term cost associated with finding sex differences late in the clinical stage
• Inadequate inclusion may contribute to irreproducibility in preclinical biomedical research

(Kindiz & Cheng. Health Aff. 2013)
Benefits of including XX and XY early

• Increases knowledge about sex-skewed conditions
• Accelerates the translational process from preclinical to clinical outcomes
• More rigor in scientific sex-based knowledge
• First steps toward personalized medicine
• Reporting sex prevents inappropriate generalization and helps facilitate better meta analysis
Steps toward sex equity in research

- Require researchers to report sex of subject & outcomes by sex
- Train researchers on experimental design that includes sex variables
- Educate reviewers to evaluate sex inclusion design in proposals
- Monitor policy compliance better
- Encourage institutional research “cores” to explore sex inclusion language and ways to help with experimental design.
- Develop FAQs for stakeholders on inclusive design
- Educate journal editors on the importance sex based outcome reporting
- Increase awareness about sex equity to consumers, physicians, researchers, etc.
- Add sex inclusion modules to medical school curriculum.
So what is the right guidance to NIH and to our fellow investigators?

1. The absence of sex as a reported variable in basic science and clinical medicine must change
2. The reliance on a single sex in most studies must also change
3. The assumption that cost should limit our move toward sex inclusion is not in the best interest of science
4. NIH funds basic science discovery – if the pipeline is more costly or is slower than doing single sex studies, then that is the cost and pace of good research
5. The public assumes that our work is done in their best interests, not in the best interests of our careers, publications or the next grant – therefore, it is in the public’s best interest and in they are paying for it – that we do the right studies
6. Basic scientists are therefore guided to assess efficacy in both sexes (Case 2) and then move toward Case 3, reporting negative data from Case 1 in journals that now have sex inclusion policies that are supportive of data indicating ‘no effect’ in single sex.

What tools can enable the community to engage in this research area
Experimental Design & Analysis Tools

- Gonadectomized animals
- Fore-Core-Genotype Models (help distinguish gene vs hormone effects)
- Animal Cycling and Breeding Instructions
- Stanford Gendered Innovations
Strategy for discerning the basis of a sex difference starting with adult difference in the activational actions of testicular versus ovarian hormones.

**Strategy One: Adult**

Gonadectomize adult males and females

- Equalize hormones in males and females
- Deprive hormones in males and females
- Compare males to males or females to females with and without hormone replacement

Are they different?

- Yes
  - Conclude sex difference is hormonally modulated in adulthood
  - Consider sex chromosome effects
- No
  - Consider developmental hormonal effects

Four Core Genotype Mouse Model

Sex chromosome

Females

Males

XX

XXSry

XY

XY-Sry

Gonadal Sex

Slide courtesy of Sabra Klein, Hopkins

Aronold, AP 2014 Exp Neurol
Sex chromosomal complement influences the outcome of some but not all viruses

**Coxsackievirus B3**

**Influenza**

\[ p = 0.056 \]

Slide courtesy of Sabra Klein, Hopkins

Robinson et al. 2011 Biology of Sex Differences 2:8
Putting Women First—In health, science and education!

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Website: http://womenshealth.northwestern.edu/programs/research-toolbox

Academic Resources

- Publications on Inclusion of Sex and Gender Differences
- Research on Sex and Gender Differences by Disease/Topic

Online Resources for Sex and Gender Inclusion

- The Organization for the Study of Sex Differences (OSSD)
- NIH Office of Research on Women's Health (ORWH)
- Draft Guidance for Industry and Food and Drug Administration Staff - Evaluation of Sex Differences in Medical Device Clinical Studies

Tools from Gendered Innovations at Stanford University

- Rethinking Concepts and Theories
- Not Considering Sex Differences as a Problem
- Steps to Sex and Gender Inclusion

Animal Research

Tools from Woodruff Lab at Northwestern University

Animal Cycling and Breeding Instructions

- Vaginal Cytology (Smearing) Protocol - Courtesy of the Woodruff Laboratory
- Reproductive Biology of Breeding Mice - Courtesy of The Jackson Laboratory

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Patient Recruitment

- **Illinois Women’s Health Registry**
  The Women’s Health Research Institute created the Illinois Women’s Health Registry in order to address the underrepresentation of women in scientific and clinical research studies.
  For **women who join the Registry**, we offer you the opportunity to participate in important research impacting women’s health. Click here to learn more.
  For **investigators**, we facilitate the recruitment of women for approved studies. Click here for more information.

- **Shared & Core Facilities at Northwestern University**

  - **Skin Disease Research Core Center (SDRC) at Northwestern University**
    Almost 100% of what is known about normal epidermal skin cell (keratinocyte) function today has been studied in males --because foreskin has been the source of normal keratinocytes for studies. But clearly there are differences between the function and responses of male and female keratinocytes. For example, Irina Budunova, MD, PhD has recently found that glucocorticoid receptor expression in epidermis is higher in males than in females and that male mice are more sensitive to the side effects of topical steroids than female mice.

    The Skin Disease Research Core Center (SDRC) at Northwestern, one of only 6 NIH-funded SDRCs, includes as one of its goals sex-sensitive research. SDRC faculty recognize that chromosomal, hormonal and structural differences between male and female cells may translate into altered function, and that discovery of differences at the basic level will ultimately save time and money in later clinical trials.

    To that end, the SDRC is developing libraries for use by Northwestern faculty of cultured skin cells and skin tissue of different sex, age and race/ethnicity in its cell and tissue repository. Importantly, the SDRC recently specifically requested proposals that address sex disparities in skin cell and tissue responses in its latest cycle of Pilot and Feasibility seed grant funding.

    Click here to visit the SDRC website, and learn more!
In the past, the research community assumed that beyond the reproductive system, differences between men and women simply did not exist or were not relevant. Some of the reasons researchers have preferred male subjects include: the cost of using both sexes, a sense of having to protect vulnerable women and/or a potential fetus, uniformity, avoiding the “complications” of the menstrual cycle, and perceived complexity of recruitment. However, the truth, as outlined in Dr. Teresa Woodruff’s 2010 *Nature* editorial, is that sex- and gender-based approaches to research and medicine frame important questions about the differences and similarities in men’s and women’s normal biological, behavioral and social function in combination with their experience of the same diseases. Consequently, the Women's Health Research Institute established the Bench Research Integration Leadership Committee to ensure sex-specific research tools and core services are available to all investigators throughout Northwestern University. The resources listed here are designed to support investigators, as they engage in sex-specific research.

**CURRENT NEWS**

- NIH Policies to ensure preclinical research considers females and males- *Nature* May, 2014
- Stephen Colbert talks about sex and gender research- May 2014

**Academic Resources**

- Publications on Inclusion of Sex and Gender Differences
- Research on Sex and Gender Differences by Disease/Topic

**Website:**

[http://womenshealth.northwestern.edu/programs/research-toolbox](http://womenshealth.northwestern.edu/programs/research-toolbox)
The Gender Lens Tool

The Gender Lens Tool can be used to identify gaps and differences in health information from a gender perspective. By using a Gender Lens, we can examine any area of the medical literature with gender in mind and can create a health-based framework to consider how the multiple [biopsychosocial] spheres of influence contribute to a woman’s health:

- biology
- social structure
- education
- economic factors

Incidence and prevalence, diagnosis, risk factors, treatment efficacy, and disease progression are all influenced by gender. Like using a microscope to exam paper, the Gender Lens Tool helps form an understanding of gender-specific characteristics in health-related areas.

Website:
http://womenshealth.northwestern.edu/programs/research-toolbox
Introduction to Gender-Based Analysis Plus

Website:
http://womenshealth.northwestern.edu/programs/research-toolbox
Gender Awakening Tool
Bibliography: Sex and Gender in Research

2007
The copyright of this product lies with
Linda Nieuwenhoven
Madelief Bertens
Ineke Klinge (i.klinge@zw.unimaas.nl)

Maastricht University
Faculty of Health, Medicine & Life sciences
Metamedica / Health, Ethics & Society

Center for Gender and Diversity
Maastricht

And was designed as a part of

GenderBasic

a project in the 6th Framework Programme.

Website:
http://womenshealth.northwestern.edu/programs/research-toolbox
Step 1 – Relevance check
Are gender and/or sex issues relevant for your subject of research?

Step 2. Literature search
Was sex or gender addressed in previous studies? Compare your research efforts to existing literature with the help of the table below to assess how you are dealing with sex and gender issues:

Step 3. Research questions & hypotheses
If you are addressing sex/gender issues:
- detail the research questions so that it reflects the population under investigation (and avoid unspecified terms like ‘adult’ or ‘patient’);
- preferably ask a question that aims at investigating the possible difference between men and women;

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http://womenshealth.northwestern.edu/programs/research-toolbox
Step 4. Research methods and design

Describe how sex and/or gender issues are taken into account in the research design and methodology.
- Is substantiated why women or men (or both) are included (or excluded)?
- Is it necessary to collect sex disaggregated data?
- Is it possible to collect sex disaggregated data?

Step 5. Data analysis and interpretation

Website:
http://womenshealth.northwestern.edu/programs/research-toolbox
Step 6. Report

Go through your findings with a ‘gender lens’ and make sure that:

- The pitfalls (over-generalization, gender insensitivity, double standards) are avoided
- The found sex and/or gender differences are visualized in the used tables, figures and conclusions.
- Results and conclusions regarding gender and sex issues are reported.
- It is considered if the results will differently affect

Step 7. Conclusions & recommendations

- Is it necessary that future research on this topic pays attention to gender and/or sex issues?

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<table>
<thead>
<tr>
<th>Item</th>
<th>Recommendation (Kilkenny et al., 2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TITLE</strong></td>
<td>Provide as accurate and concise a description of the content of the article as possible.</td>
</tr>
<tr>
<td><strong>ABSTRACT</strong></td>
<td>Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.</td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>3</td>
</tr>
</tbody>
</table>
| Background | a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.  
| | b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology. |
| **Objectives** | 4 |
| | Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. |
| **METHODS** | 5 |
| Ethical statement | Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research. |
| **Study design** | 6 |
| | For each experiment, give brief details of the study design including:  
| | a. The number of experimental and control groups.  
| | b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when).  
| | c. The experimental unit (e.g. a single animal, group or cage of animals).  
| | A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out. |
| **Experimental procedures** | 7 |
| | For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example:  
| | a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s).  
| | b. When (e.g. time of day).  
| | c. Where (e.g. home cage, laboratory, water maze).  
| | d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used). |

Methods
Provided for specific facets of sex & gender research

Rethinking Research Priorities and Outcomes
Rethinking Concepts and Theories
Formulating Research Questions
Analyzing Sex
Analyzing Gender
Analyzing how Sex and Gender Interact
Analyzing Factors Intersecting with Sex and Gender
Engineering Innovation Processes
Designing Health & Biomedical Research
Participatory Research and Design
Rethinking Standards and Reference Models
Rethinking Language and Visual Representations

Terms
To appropriately address misconceived words part of gender-based research

Sex and Gender are Distinct Terms
Sex
Gender
Interactions between Sex and Gender
Gender Dimension
Not Considering Sex Differences as a Problem
Overemphasizing Sex Differences as a Problem
Women & Men
Femininities & Masculinities
Race & Ethnicity
Stereotypes
Feminisms

Check lists

Engineering
Health & Medicine
Tissues & Cells
Urban Planning & Design

Health & Medicine Checklist

Step 1: Determining Relevance
Step 2: Literature Search
Step 3: Establishing Research Questions and Hypothesis
Step 4: Establishing Research Methods and Planning Data Analysis
Step 5: Interpreting Data through Concepts and Theoretical Models
Step 6: Reporting Findings
Step 7: Establishing Conclusions and Making Recommendations

Website: http://genderedinnovations.stanford.edu
• Require researchers to report sex of subjects and outcomes by sex

• Train researchers on experimental design that includes sex variables

• Educate proposal reviewers on sex inclusion design elements

• Monitor inclusion policy compliance better

• Encourage institutional research center cores to identify services that focus on sex variables

• Develop FAQs for stakeholders on sex inclusion

• Educate journal editors on the importance of sex based reporting

• Add sex variables to medical school curriculum

• Encourage professional societies to develop and disperse sex based clinical guidelines

• Increase awareness on the importance of sex in medical research to consumers
Hypothesis

The next generation of biomedical advances that improves the lives of all people - drugs, devices, and best medical practices - requires fundamental discovery research that includes sex as a variable.
Thank You

Knowing is not enough; we must apply. Willing is not enough; we must do.

Goethe

I don’t want to be your other half. I believe one and one make two.

Alanis Morissette

http://womenshealth.northwestern.edu/programs/research-toolbox