Statistical Considerations for Sex Inclusion in Basic Science Research

Denise M. Scholtens, Ph.D.
Associate Professor, Department of Preventive Medicine
Associate Director, Division of Biostatistics
dscholtens@northwestern.edu
BCC: Biostatistics Collaboration Center

Who We Are

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BCC Director

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Asst. Professor

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Asst. Professor

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Assoc. Professor

Alfred Rademaker, PhD
Professor

Masha Kocherginsky, PhD
Assoc. Professor

Gerald Rouleau, MS
Stat. Analyst

Amy Yang, MS
Senior Stat. Analyst

Hannah L. Palac, MS
Senior Stat. Analyst

Not Pictured:
1. David A. Aaby, MS
   Senior Stat. Analyst

2. Tameka L. Brannon
   Financial | Research Administrator
Our mission is to support FSM investigators in the conduct of high-quality, innovative health-related research by providing expertise in biostatistics, statistical programming, and data management.
BCC: Biostatistics Collaboration Center

How We Do It

Are you writing a grant?

YES

We provide:
- Study Design
- Analysis Plan
- Power Sample Size

BCC faculty serve as Co-Investigators; analysts serve as biostatisticians.

NO

Short or long term collaboration?

Short

Recharge Model (hourly rate)

Long

Subscription Model (salary support)

The BCC recommends requesting grant support at least **6-8 weeks** before submission deadline

Every investigator is provided a **FREE** initial consultation of up to 2 hours with BCC faculty or staff
BCC: Biostatistics Collaboration Center

Contact Us

• Request an Appointment
  - http://www.feinberg.northwestern.edu/sites/bcc/contact-us/request-form.html

• General Inquiries
  - bcc@northwestern.edu
  - 312.503.2288

• Visit Our Website
  - http://www.feinberg.northwestern.edu/sites/bcc/index.html
Topic for today:

Sex as a variable in basic science research
‘Crucial experimental design elements that are all too frequently ignored include blinding, randomization, replication, sample-size calculation and the effect of sex differences.’

Policy: NIH plans to enhance reproducibility

Francis S. Collins & Lawrence A. Tabak

27 January 2014

Francis S. Collins and Lawrence A. Tabak discuss initiatives that the US National Institutes of Health is exploring to restore the self-correcting nature of preclinical research.

Figure from Collins & Tabak, Nature (2014)
‘Furthermore, inadequate inclusion of female cells and animals in experiments and inadequate analysis of data by sex may well contribute to the troubling rise of irreproducibility in preclinical biomedical research, which the NIH is now actively working to address.’
From NOT-OD-14-128:
‘Although we have made major progress in achieving balance of sex in human studies — women now account for roughly half of the participants in NIH-funded clinical trials — we have not seen a similar pattern in biomedical research. Animal studies have typically focused on males, and investigators studying cell models have often not reported the sex of the individual from which the cells were obtained. Even if both sexes are included in a study design, resulting data may not be analyzed or disaggregated by sex. 

By developing a policy to ensure that sex is considered in NIH-funded studies, NIH will ensure that sex and sex differences are examined in all aspects of biomedical research. This will lead to a stronger foundation upon which to build clinical research and clinical trials.’
Sex as a Biological Variable

Recent History

Collins & Tabak
Nature Comment
Policy: NIH plans
to enhance
reproducibility

Clayton & Collins
Nature Comment
Policy: NIH to balance sex in cell
and animal studies

NOT-OD-14-128
RFI: Consideration
of Sex as a Biological Variable
in Biomedical Research

NOT-OD-15-102
Consideration
of Sex as a Biological Variable
in NIH-funded Research

NOT-OD-16-011
Implementing Rigor
and Transparency
in NIH & AHRQ
Research Grant Applications

January 2014
May 2014
September 2014
June 2015
October 2015
January 2016


Research Toolbox
Northwestern University’s Women’s Health Research Institute

https://www.womenshealth.northwestern.edu/sites/womenshealth/files/u926/
Statistical Considerations in Basic Science Sex Inclusive Research.pdf

5 Scenarios
pertaining to sex inclusion in basic science research

Sample size / experimental design
Statistical analysis plans
Grant applications / manuscripts

Gentle guide only
(i.e. contact the BCC)
Very simple experimental setting

• *Suppose an investigator wants to evaluate the effect of a treatment compared to control* in a basic science setting involving cell lines.

• *The investigator plans to measure a continuous outcome variable* $Z$ *in the cells.*

• ‘Female’ and ‘male’ refer to *chromosomal female (XX)* and *chromosomal male (XY), respectively.*
3 Questions resulting in 5 Scenarios

- Is there strong biological justification to study cells of only one sex?

- In the control condition, is the mean of $Z$ the same for males and females?

- Does treatment affect $Z$ in the same way for females and males?
### Scenario 1

<table>
<thead>
<tr>
<th>Scenario</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>No</td>
</tr>
</tbody>
</table>

**Sample size / experimental design:**

Sample size calculations can be performed without consideration of the sex variable

**Statistical analyses:**

Statistical analyses do not need to consider sex as a variable

**Grant applications / manuscripts:**

Include strong biological justification for studying only one sex
**Scenario 2**

<table>
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<tr>
<td>2</td>
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Scenario 2

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</tr>
</tbody>
</table>

Sample size / experimental design:

**IF** if it can be reasonably assumed (either based on preliminary data or published literature or both) that mean levels of Z in the control condition are similar for both sexes **AND** that treatment has the same effect in both sexes, **THEN** sample size calculations will be no different than calculations made in Setting 1.
Scenario 2

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</tbody>
</table>

Statistical analyses:

When analyzing collected data, the assumption that Z has the same mean in cells of both sexes in the control condition should be examined using descriptive statistics (i.e. means) and/or plots.

The similarity of the treatment’s effect on Z for cells of both sexes should also be examined using descriptive statistics and/or plots.

**IF assumptions ARE** met, it **MAY** be appropriate to analyze data from the female and male cells together, without statistical control for sex.
## Scenario 2

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</tbody>
</table>

**Statistical analyses con’t:**

**IF** assumptions **ARE NOT** met, move to Scenarios 3, 4 or 5.

**Grant applications / manuscripts:**

For grants, include strong justification for assuming equal means and treatment effects. Incorrect assumptions may result in experiments with too few samples.

For manuscripts, descriptive statistics or plots confirming assumptions of equal means and treatment effects (or lack thereof) should be reported.
### Scenario 3

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Is there strong biological justification to study cells of only one sex?</th>
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<td>3</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Graph:**
- **Y-axis:** Z
- **X-axis:**
  - **Female** (Control: purple, Treatment: green)
  - **Male** (Control: purple, Treatment: green)

**Legend:**
- **Female Control:** Purple filled circles
- **Female Treatment:** Green filled circles
- **Male Control:** Purple filled circles
- **Male Treatment:** Green filled circles

**Note:**
- The graph visually demonstrates the comparison between control and treatment groups for females and males, with sex being a significant variable as indicated by the diagram and the table.
Scenario 3

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</table>

**Sample size / experimental design:**

When planning the experiment, sample size requirements are likely to be higher than in studies of one sex only. Actual requirements will depend on the anticipated extent to which the mean of Z in the control condition differs for female and male cells.

Consult your statistician.
### Scenario 3

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<td>No</td>
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<td>Yes</td>
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</table>

**Statistical analyses:**

When analyzing collected data, sex should be included as a ‘covariate’ in all analyses, including regression.

The similarity of the treatment’s effect on Z for cells of both sexes should be examined using descriptive statistics and/or plots.

**IF** treatment effects **ARE** equal, it is appropriate to analyze data from the female and male cells together, including statistical adjustment for sex with a common treatment effect.
Scenario 3

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<thead>
<tr>
<th>Scenario</th>
<th>Is there strong biological justification to study cells of only one sex?</th>
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<td>No</td>
<td>Yes</td>
<td>Yes</td>
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</table>

Statistical analyses:

*IF treatment effects ARE NOT equal, move to Scenarios 4 or 5.*

Grant applications / manuscripts:

For grants, include strong justification for the assumption that treatment effects do not differ by sex.

For manuscripts, it may or may not be necessary to report results separately for both sexes. It will depend on the setting. However, descriptive statistics or plots confirming equal treatment effects should be reported.
Scenario 4

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Is there strong biological justification to study cells of only one sex?</th>
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<tr>
<td>4</td>
<td>No</td>
<td>Possibly</td>
<td>No, not of primary interest</td>
<td>Yes</td>
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</tbody>
</table>

![Graph](image_url)

- Z is measured for control and treatment groups for females and males.
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<td>No, not of primary interest</td>
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</table>

#### Sample size / experimental design:

When planning the experiment, sample size requirements will be higher than in studies of one sex only. Actual requirements will depend on the extent to which the mean of Z in the control condition and/or the treatment’s effect on Z are anticipated to differ for female and male cells.

Consult your statistician.
## Scenario 4

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### Statistical analyses:
When analyzing collected data, sex should be included as a ‘covariate’ in all analyses, including regression. An ‘interaction term’ between treatment and sex should also be included to account for the difference in treatment effect according to sex.

Descriptive statistics and/or plots will be helpful to demonstrate possible differences in means and treatment effects.
### Scenario 4

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**Grant applications / manuscripts:**

For grants, there should be justification for why it is not of primary interest to formally demonstrate differences in treatment effects for males and females.

For manuscripts, it is important to report means of Z in the control condition and after treatment separately for female and male cells.

In Scenario 4, formally showing that the treatment effect is statistically significantly different for males and females **WILL NOT** be a main point in the manuscript.
Scenario 5

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Is there strong biological justification to study cells of only one sex?</th>
<th>In the control condition, is the mean of Z the same for males and females?</th>
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<tbody>
<tr>
<td>5</td>
<td>No</td>
<td>Possibly</td>
<td>No, and of primary interest</td>
<td>Yes</td>
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</table>

<table>
<thead>
<tr>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
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Northwestern Medicine
Feinberg School of Medicine
Scenario 5

<table>
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Sample size / experimental design:
When planning the experiment, sample size requirements will be higher than in studies of one sex only. Specifically, the study should have adequate sample size to test whether the interaction term between treatment and sex is statistically significantly different from zero. Actual requirements will depend on the extent to which the mean of Z in the control condition and/or the treatment’s effect on Z are anticipated to differ for female and male cells.

Consult your statistician.
### Scenario 5

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**Statistical analyses:**

When analyzing collected data, sex should be included as a ‘covariate’ in all analyses, including regression. An ‘interaction term’ between treatment and sex should also be included to account for the difference in treatment effect according to sex.

Analyses should include formal statistical hypothesis testing on the interaction term.
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**Grant applications / manuscripts:**

For grants, it is important to justify why it is necessary to formally conclude that there are treatment differences for males and females. This will be crucial to justifying the additional expense for higher sample size.

For manuscripts, it is important to report means of Z in the control condition and after treatment separately for female and male cells.

In Scenario 5, formally showing that the treatment effect is statistically significantly different for males and females **WILL** be a main point in the manuscript.
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**Scenario 2**

![Graph showing Z values for control and treatment groups for females and males]

**Scenario 3**

![Graph showing Z values for control and treatment groups for females and males]

**Scenario 4/5**

![Graph showing Z values for control and treatment groups for females and males]
What if I’m not studying cell lines with a continuous outcome and not just treatment v. control?

Same story, different details

• Details that might differ:
  – Mice instead of cell lines
  – Categorical outcome
  – Multiple treatments
What if I’m not studying cell lines with a continuous outcome and not just treatment v. control?

Same story, different details

- If outcome levels and treatment effects are the same or highly similar for males and females (and this has been checked, not just assumed) then analyses can for the most part be carried out without worrying about sex as a variable.
- Sex is not adding variability in this case.
- Experiments can also be planned without worrying about needing higher sample size than a study of one sex only.
- Experiments should be planned so that both males and females are studied so that appropriate conclusions can be made.
What if I’m not studying cell lines with a continuous outcome and not just treatment v. control?

Same story, different details

• If outcome levels and/or treatment effects differ for males and females, then statistical analyses do need to account for sex as a variable.
• Sex is adding variability in this case.
• Experiments will in general require higher sample size.
• The highest sample size will typically be required for formally showing that treatment effects differ for males and females.
• Experiments should be planned so that both males and females are studied so that appropriate conclusions can be made.
What if I planned my experiment incorrectly?

Help!!

• What if I assumed Scenario 2 would apply, but descriptive statistics and/or plots suggest Scenarios 3, 4 or 5 are more applicable?

• Statistical modeling can still be performed, but sample size may limit statistical power for making correct conclusions.

• More samples may be required.

• Consult a statistician.
What if I planned my experiment incorrectly?

Help!!

• What if I assumed Scenario 4 or 5 would apply, but descriptive statistics and/or plots suggest Scenarios 2 or 3 are more applicable?

• You’re in good shape.

• If you designed your experiment with adequate sample size for Scenarios 4 or 5, you will have plenty of statistical power for Scenarios 2 or 3.
**BCC: Biostatistics Collaboration Center**

**How We Do It**

1. **Are you writing a grant?**
   - **YES**
     - We provide:
       - Study Design
       - Analysis Plan
       - Power Sample Size
     - BCC faculty serve as Co-Investigators; analysts serve as biostatisticians.
   - **NO**
     - Short or long term collaboration?
       - **Short**
         - Recharge Model (hourly rate)
       - **Long**
         - Subscription Model (salary support)

**The BCC recommends requesting grant support at least 6-8 weeks before submission deadline**

**Every investigator is provided a FREE initial consultation of up to 2 hours with BCC faculty of staff**
Statistically Speaking ...

What’s next?

Tuesday, October 11

**Statistical Considerations for Sex Inclusion in Basic Science Research**

Denise M. Scholtens, PhD, Associate Professor, Division of Biostatistics
Associate Director, Department of Preventive Medicine

Friday, October 14

**The Impact of Other Factors: Confounding, Mediation, and Effect Modification**

Amy Yang, MS, Sr. Statistical Analyst,
Division of Biostatistics, Department of Preventive Medicine

Tuesday, October 18

**Statistical Power and Sample Size: What You Need and How Much**

Mary Kwasny, ScD, Associate Professor, Division of Biostatistics,
Department of Preventive Medicine

Friday, October 21

**Clinical Trials: Highlights from Design to Conduct**

Masha Kocherginsky, PhD, Associate Professor, Division of Biostatistics,
Department of Preventive Medicine

Tuesday, October 25

**Finding Signals in Big Data**

Kwang-Youn A. Kim, PhD, Assistant Professor, Division of Biostatistics,
Department of Preventive Medicine

Friday, October 28

**Enhancing Rigor and Transparency in Research: Adopting Tools that Support Reproducible Research**

Leah J. Welty, PhD, BCC Director, Associate Professor, Division of Biostatistics,
Department of Preventive Medicine

All lectures will be held from noon to 1 pm in Hughes Auditorium, Robert H. Lurie Medical Research Center, 303 E. Superior St.