Rigor & Reproducibility: 
Implementation of NIH Policy for 
Sex As a Biological Variable (SABV)

Elena Gorodetsky, M.D., Ph.D.
Office of Research on Women’s Health
National Institutes of Health

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ORWH’s Mission

• Research strategies and priorities
• Inclusion of women in clinical trials
• Beyond inclusion – SABV Phase II: development & implementation
• Women in biomedical careers
The Reproducibility Challenge

Beware the creeping cracks of bias
Evidence is mounting that research is riddled with systematic errors. Left unchecked, this could erode public trust, warns Daniel Sarewitz.

Why animal research needs to improve
Many of the studies that use animals to model human diseases are too small and too prone to bias to be trusted, says Malcolm Macleod.

Believe it or not: how much can we rely on published data on potential drug targets?
Florian Prinz, Thomas Schirane and Klausre Assaadullah

False-Positive Psychology: Undisclosed Flexibility in Data Collection and Analysis Allows Presenting Anything as Significant
C. Glenn Begley and Lee M. Ellis propose how methods, publications and incentives must change if patients are to benefit.

Drug targets slip-sliding away
The starting point for many drug discovery programs is a published report on a new drug target. Assessing the reliability of such papers requires a nuanced view of the process of scientific discovery and publication.

Reforming Science: Methodological and Cultural Reforms
A call for transparent reporting to optimize the predictive value of preclinical research


The US National Institute of Neurological Disorders and Stroke convened major stakeholders in June 2012 to discuss how to improve the methodological reporting of animal studies in grant applications and publications. The main workshop recommendation is that at a minimum studies should report on sample-size estimation, whether and how animals were randomized, whether investigators were blind to the treatment, and the handling of data. We recognize that achieving a meaningful improvement in the quality of reporting will require a concerted effort by investigators, reviewers, funding agencies and journal editors. Requiring better reporting of animal studies will raise awareness of the importance of rigorous study design to accelerate scientific progress.
NIH plans to enhance reproducibility

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.

Preclinical problems
Reproducibility is potentially a problem in all scientific disciplines. However, human clinical trials seem to be less at risk because they are already governed by various regulations that stipulate rigorous design and independent oversight — including randomization, blinding, power estimates, pre-registration of outcome measures in standardized public databases such as ClinicalTrials.gov and oversight by institutional review boards and institutional review boards.

“Efforts by the NIH alone will not be sufficient to effect real change in this unhealthy environment.”
NIH to balance sex in cell and animal studies

Janine A. Clayton and Francis S. Collins unveil policies to ensure that preclinical research funded by the US National Institutes of Health considers females and males.

“Over the course of FY 2015, NIH plans to roll out policies that will require applicants to address inclusion of both sexes in biomedical research.”
<table>
<thead>
<tr>
<th>Scientific premise</th>
<th>To form the basis for the proposed research question, e.g., observations, preliminary data, published literature</th>
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<tr>
<td>Scientific rigor</td>
<td>To ensure robust and unbiased experimental design, methodology, analysis, interpretation, and transparent reporting of results</td>
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<tr>
<td>Consideration of relevant biological variables including sex</td>
<td>Sex, age, weight, and underlying health conditions are critical factors, where &quot;SEX&quot; is frequently ignored in animal studies</td>
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<tr>
<td>Authentication of key biological and/or chemical resources</td>
<td>Resource quality is critical to reproduce the results. NIH expects that these resources will be regularly authenticated to ensure identity and validity</td>
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*Released June 9, 2015, implemented Jan 26, 2016*
The NIH’s Rationale: Reporting Both Sexes Contributes to Rigor and Reproducibility

- **Rigorous experimental design**
- **Missing information for females will produce biased results.**
- **Lack of transparency → lack of reproducibility**
- **Lack of reproducibility of studies puts people at risk of being harmed**

“Scientific rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results. This includes full transparency in reporting experimental details so that others may reproduce and extend the findings.”

[https://grants.nih.gov/reproducibility/index.htm](https://grants.nih.gov/reproducibility/index.htm)
How did we get here?
- Overreliance on male animals and cells
- Inattention to sex effects
- Lack of transparency
- Inconsistent reporting of sex-specific findings in publications

End Result
- Incomplete knowledge base
- Risk of erroneous conclusions
- Does not maximize ROI
- Irreproducible results
- Toxicity surprises
- Erosion of public trust

"Science isn't science if it isn't reproducible"*

*Roth and Cox, AJP, January 2015
Under-reporting of sex is still an issue in animal studies

A text-mining analysis suggests that by 2014, 53% of experiments using mice recorded both the sex and age of the animals.

Male Animals Dominate Many Research Areas

Female Gap In Animal Models Of Human Disease

The human cost of not considering SABV

What is “default” biology?
- In the clinic: 70-kg male
- In the lab: male cells and animals

Fundamental is not only that which is shared
- Fundamental biology = same *and* different in males, females

Reproducibility and Transparency
- Every experiment is part of larger system, the quest to understand fundamental basic living systems
- Approaches and results should be consistent and free of bias at the outset

* 8/10 drugs withdrawn by the FDA had more adverse effects for women
Lessons Learned: Prescription Drugs Withdrawn From the United States Market, 1997–2000 Because of Inadequate Research on Women

Eight of the 10 prescription drugs withdrawn since January 1, 1997, posed greater health risks for women than for men.

For four of the withdrawn drugs, the greater health risk may have been due to a higher level of use among women.

Four other withdrawn drugs posed greater health risks for women even though they were widely prescribed to both women and men.

Most Drugs Withdrawn in Recent Years Had Greater Health Risks for Women. GAO-01-286R: Published: Jan 19, 2001.
Summary: Why Study SABV?

Mistakes made by not considering SABV---Drug recalls because of greater health risks and adverse events in women.

Deliver personalized care based on studies of both sexes.

To build a knowledge base that better informs the design of clinical research and trials in humans.
SABV Policy in a Nutshell

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

“NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies.”

*January 25, 2016 (effective date)

The biologic basis for this recommendation is incontrovertible:

- **XX XY**: Sex is established genetically at conception.
- Sexual differentiation ensues.
- Intrinsic existence and extrinsic interactions of an organism are mediated by sex throughout life.
The 4Cs of Studying Sex to Strengthen Science

**Consider**
Design studies that take sex into account, or explain why it isn’t incorporated

**Collect**
Tabulate sex-based data

**Characterize**
Analyze sex-based data

**Communicate**
Report and publish sex-based data
Considering SABV is not the same as looking for sex differences. In its efforts to enhance reproducibility and transparency by expecting investigators to consider SABV, NIH will not require any specific research design or method for accomplishing this goal. Rather, the existing state of knowledge in a particular scientific area and the specific research question under study will both affect how an investigator considers sex and other basic biological variables. On that note, it is important to point out that NIH
**Why Consider SABV?**

Historically, disease models have mainly used a single sex or sex unspecified animals

Based on assumption that disease mechanisms or treatment effects would be the same for both sexes

One sex may be preferred due to greater sensitivity to experimental manipulations in animal disease models

Use of only male animals

✓ Means of decreasing experimental variability caused by female hormone cycling

Use of only female animals

✓ Inter-male aggression in some species and strains makes co-housing difficult

*Courtesy of Dr. Stephanie J. Murphy, VMD, PhD, DACLAM Director, Division of Comparative Medicine ORIP/DPCPSI/OD/NIH*
Common Myths And Urban Legends

Myth: Estrous cycle renders ♀ vs. ♂ rodents intrinsically more variable

✓ Meta-analysis of 293 articles that monitored behavioral, morphological, physiological, and molecular traits of ♂ and ♀ mice
✓ Randomly cycling ♀ mice were no more variable than ♂ on any trait
✓ Variability was substantially greater in ♂ for several traits
✓ Group housing increased trait variability by 37% in both sexes
✓ Estrous cycle may need not be monitored when utilizing ♀ mice

No empirical basis for this belief


Courtesy of Dr. Stephanie J. Murphy
Summary: The new SABV requirements…

Provide transparency
Enhance rigor
Fill “gaps” in knowledge

Do not require specific methods
Do not require power to detect sex differences
Do not require you to double sample size
Considering Sex as a Biological Variable:

Getting Started

- Add sex, gender, male, female to literature search on the research topic of interest
- Sex-skewed disease prevalence may suggest underlying sex- or gender-based influences on physiological or pathological processes
- Include both females and males in test groups (factorial, randomized block designs)
- Report sex-based data and any identified sex-based influences
- Conduct pilot studies, such as adding a hormone treatment to tissue cultures
How to Apply SABV in Research?

**Researching**
- Review available literature on the influence of biological sex
- Formulate research questions, considering the influence of sex on study design
- Examine treatment or toxicity effects for each sex separately
- Incorporate both males and females into studies or articulate strong justification for a single-sex study
- Stratify randomization of males and females into experimental conditions

**Reporting**
- Describe all relevant variables, including sex
- Characterize study results for males and females
- Consider the influence of sex in the interpretation of results
- Generalize research findings appropriately

http://grants.nih.gov/reproducibility/faqs.htm
Impact of considering SABV

Accounting for SABV Is Not the Same as Powering for Sex Differences

Ways to account for SABV, but not by increasing sample size

- Consider sex chromosomes (XX vs. XY)
- Consider sex hormones (ovarian vs. testicular secretions): androgens, estrogens, pregnanes

- Choice of animal model, randomize subjects to study groups by sex
- Analyze disaggregated data separately
- Analyze estrous cycle as a mediator

- Control for sex as a confounding variable
- Sex differences in incidence vs. in results

Sex and Gender are not the same
Sex and Gender are Different

Every cell has a sex.

Sex begins in utero.

Sex: genetic, molecular, cellular, physiological

feminine, masculine

Sex affects behavior and perception.

Gender also affects behavior and perception.

Gender: behavioral, environmental, social, cultural

IOM Report, Exploring the Biological Contributions to Human Health: Does Sex Matter?
April 24, 2001
## Sex and Gender are Determinants of Health

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<th>Sex</th>
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<tr>
<td>Smoking</td>
<td>Nicotine metabolism</td>
<td>Sensory and social stimuli susceptibility</td>
</tr>
<tr>
<td>Heart Disease</td>
<td>Hormonal Influences</td>
<td>Stress</td>
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### Factors
- **Genetic, Molecular, Cellular, Physiological**
- **Behavioral, Environmental, Social, Cultural**

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**NIH National Institutes of Health**

**Office of Research on Women’s Health**
Sex/Gender and Human Diseases

- Women experience higher rates of adverse drug reactions than men
- Stroke incidence rates in men > women at younger ages but more women die of stroke each year
- Nearly 80% of people with autoimmune diseases are women
- ~70 to 75% of multiple sclerosis patients are women but men tend to have a worse prognosis than women
- Incidence of melanoma is higher in women but mortality is higher in male
- Women have half the mortality of men in acute renal failure
NIH in 2016: Beyond Inclusion
Sex/Gender Influences on Health and Disease

Preclinical studies | Toxicology | Phase I, II, III, IV Clinical Trials

Basic

Healthy Women, Men, Girls, Boys

Clinical

Cell, Animal Studies | Education | Sex-Specific Data Analyses

Health Care | Health Policy | Sex-Specific Reporting
MOVING INTO THE FUTURE:
A VISION OF SEX/GENDER-BASED RESEARCH FOR 2020

GOAL 1: Increase sex differences research in basic science studies

GOAL 2: Incorporate findings of sex/gender differences in the design and application of new technologies, medical devices, and therapeutic drugs

GOAL 3: Actualize personalized prevention, diagnostics, and therapeutics

Policy Implementation is a Team Effort

- Scientists
- Societies
- Industry
- Congress
- Journals
- Academia
- Public
- Non-Profits
- Media