



## SEX BIAS IN MOUSE-BASED RESEARCH – THE IMPACT ON SCIENTIFIC CONCLUSIONS AND ANIMAL WELFARE

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# TODAY'S PRESENTER



**Urte Jäh (Biologist)**

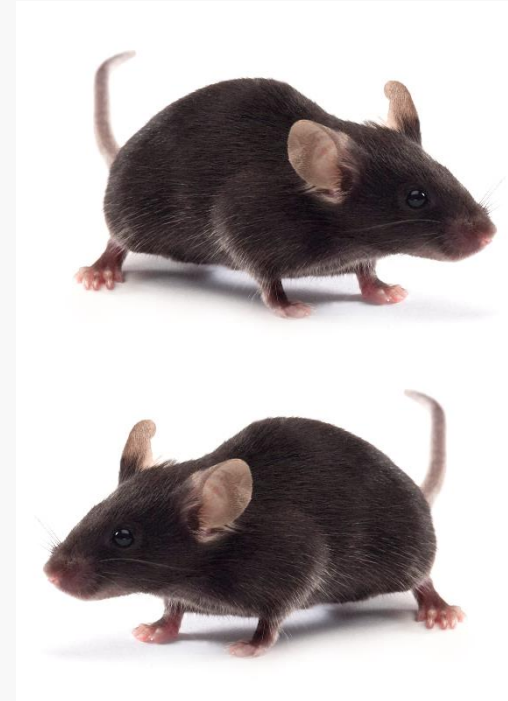
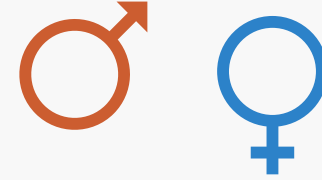
European Scientific Service Support Manager

*Charles River*

# TODAY'S PRESENTATION

## Sex Bias in Mouse-based Research- The Impact on Scientific Conclusions and Animal Welfare

- Why male or female mice are sometimes preferred
- The scientific impact sex bias has for multiple biological disciplines
- The significant effect of sex bias on animal welfare
- 3Rs guidelines and recommendations to eliminate sex bias in preclinical studies



# SEX BIAS IN PRECLINICAL RESEARCH

The Impact on Science

# JAX MISSION

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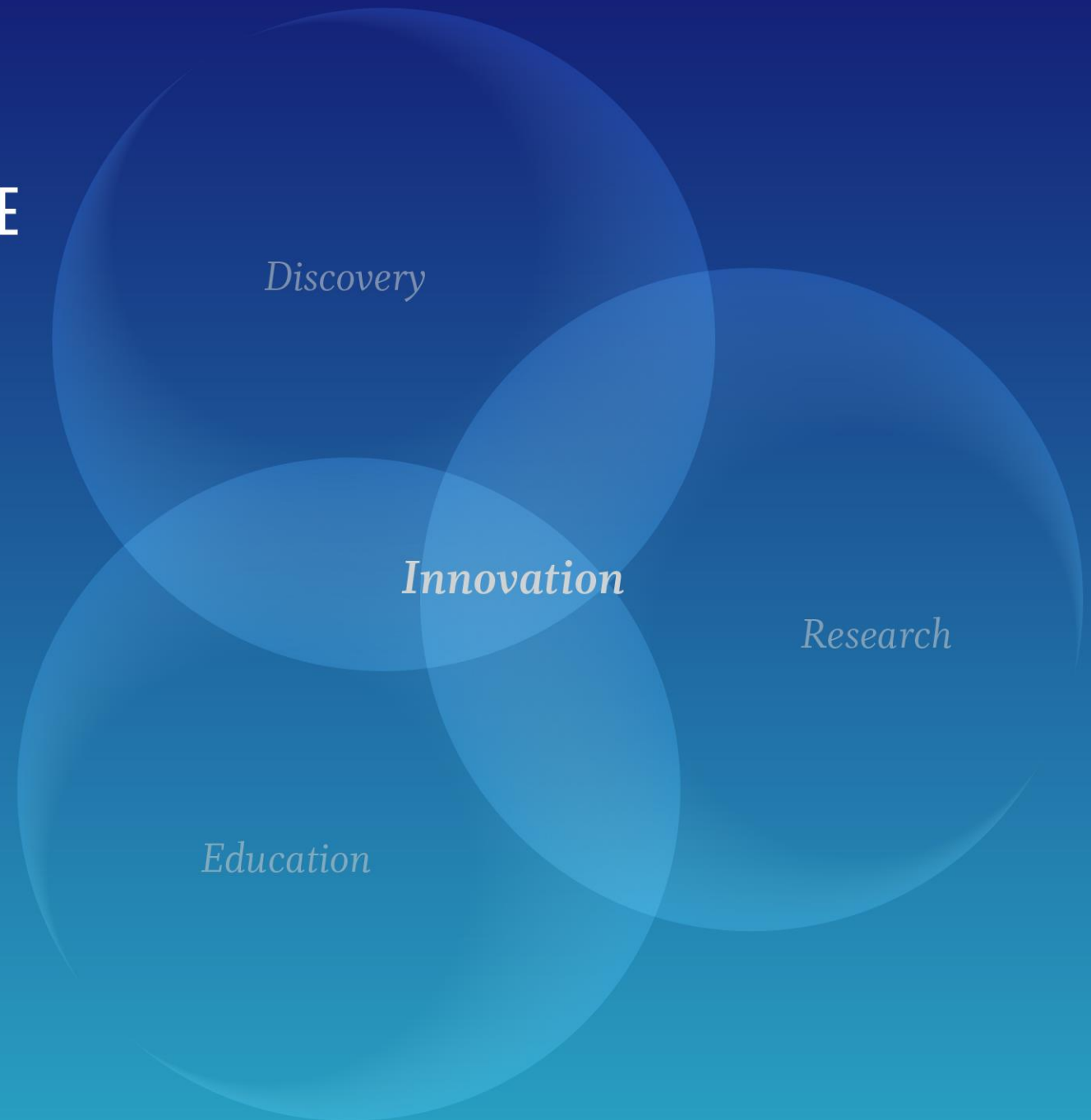
To discover precise genomic solutions for disease and empower the global biomedical community in the shared quest to improve human health.



# EMPOWERING SCIENTIFIC EXCELLENCE

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# Sex as a Biological Variable In Preclinical Study

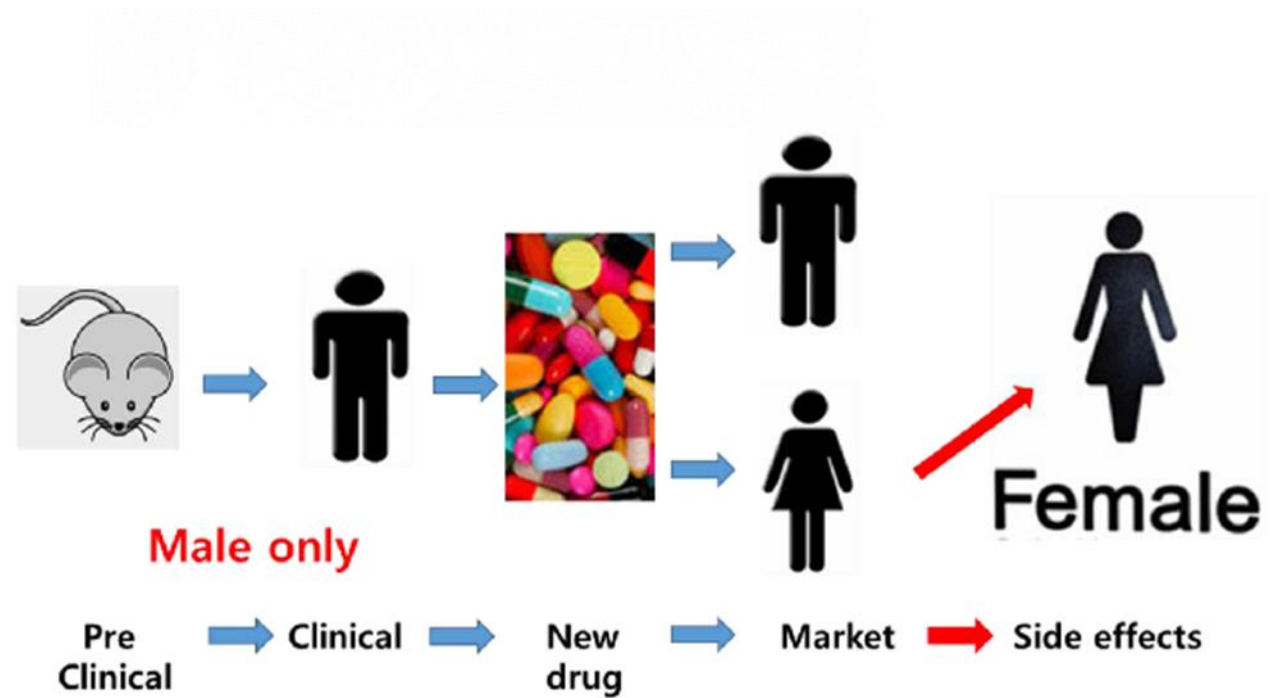
## Example of Ambien

- Ambien™ - Sleeping aid: Zolpidem
- Women reported “morning-after” effects including impaired alertness during driving
- FDA changed the dosage guidelines for women because of sex-related differences in clearance rates



# Learning Goals

- Understand the importance of selecting both male and female mice
- Discover the role of sex as a biological variable and see bias in multiple biological disciplines
- Appreciate the low percentage of papers reporting results by sex



DOI:[10.5483/BMBRep.2018.51.4.034](https://doi.org/10.5483/BMBRep.2018.51.4.034)

# Sex and Gender Definitions

## Sex

Classification derived from the chromosomal complement

Biological construct

## Gender

Person's self-representation as male or female

Cultural Concept

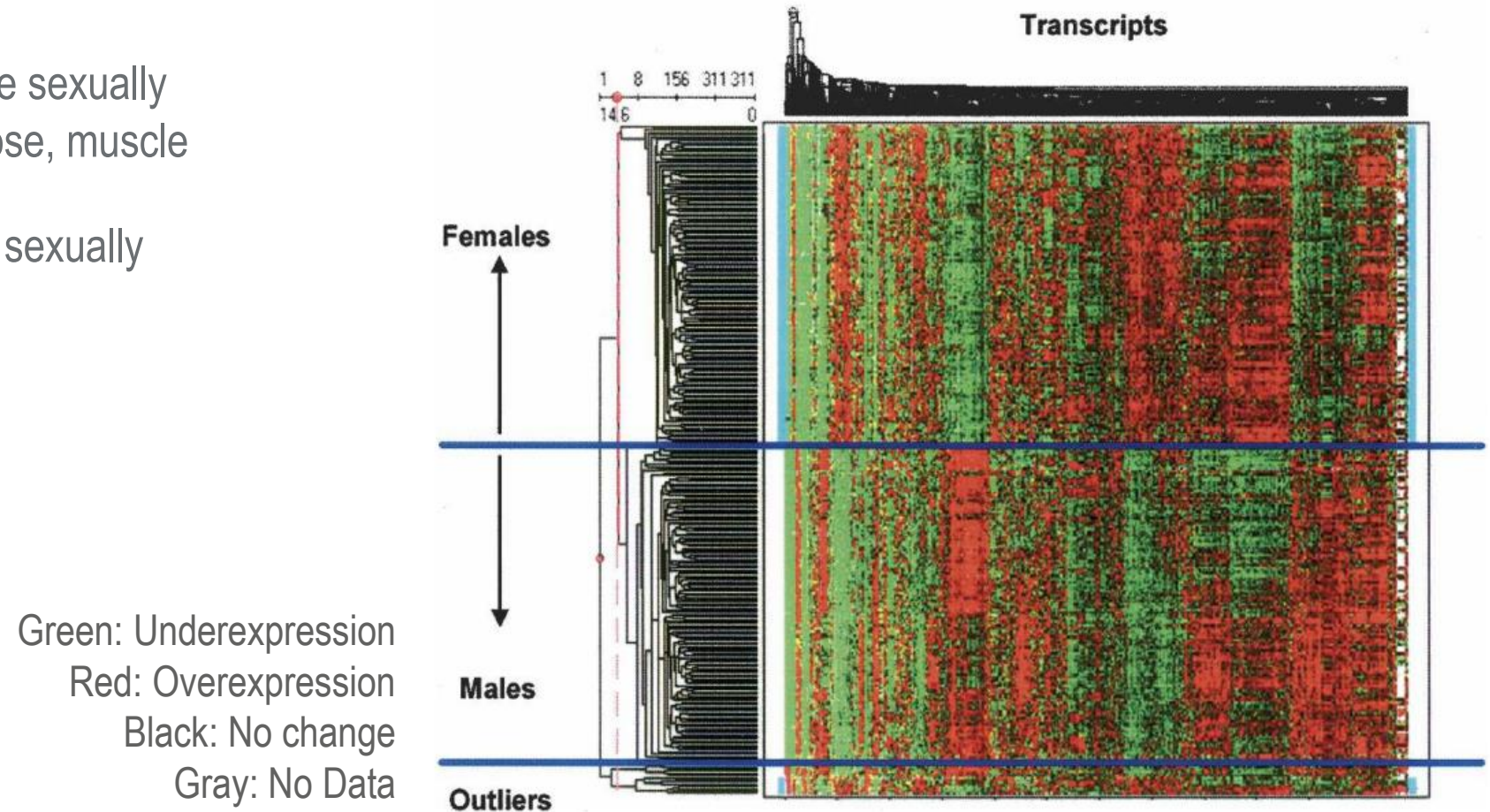
# Considerations for Sex as a Biological Factor

- Sex is frequently ignored in animal study designs and analyses
  - leads to an incomplete knowledge of biological function, disease, and treatment
- Sex should be factored into research designs, analyses, and reporting in vertebrate animal and human studies
- Strong justification from the scientific literature, preliminary data or other relevant considerations to study only one sex
- Don't forget about other biological variables such as age



# Gene Expression Differs in Male and Female Mice

- Thousands of genes are sexually dimorphic in liver, adipose, muscle
- Hundreds of genes are sexually dimorphic in brain

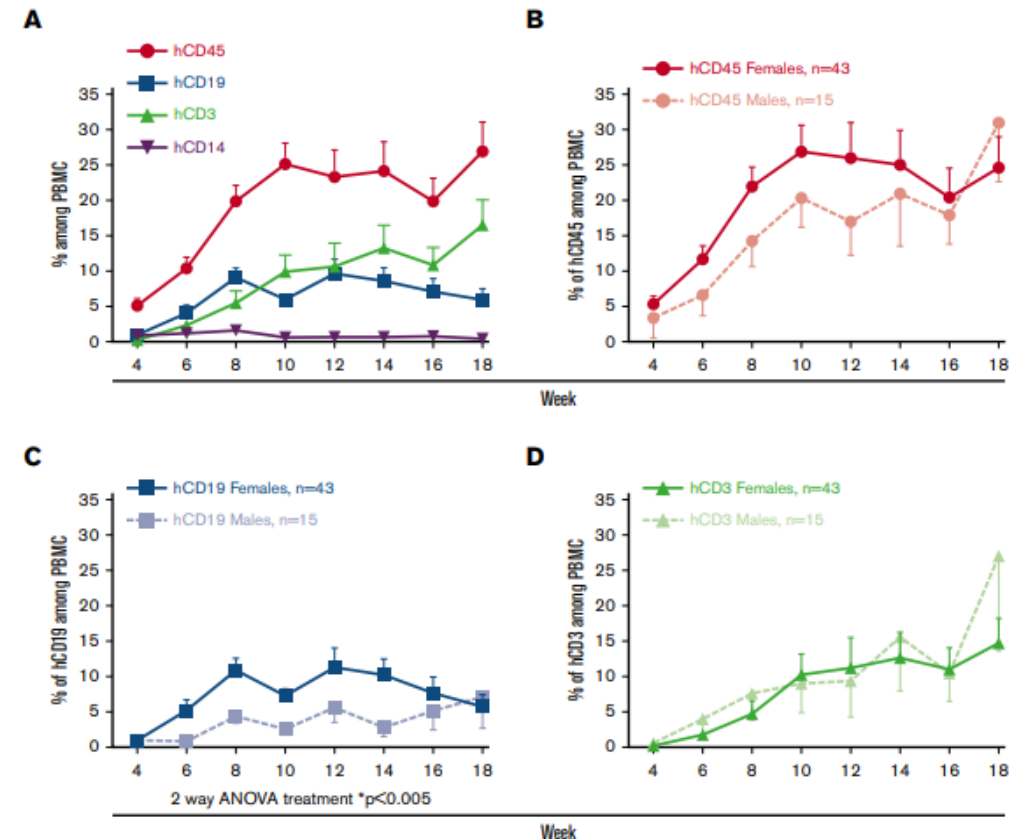


doi:10.1101/gr.5217506

# Choose The Mouse Depending on Your Study Design

## Example of Humanized NSG

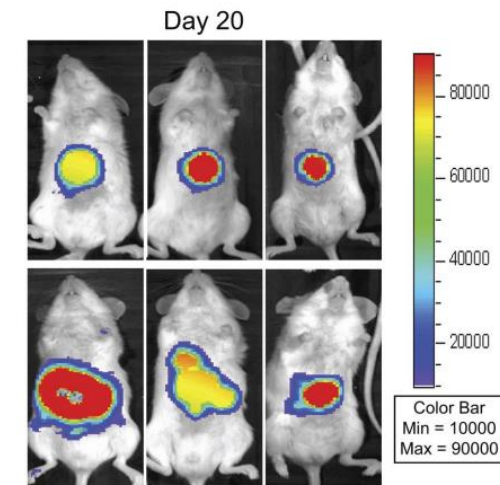
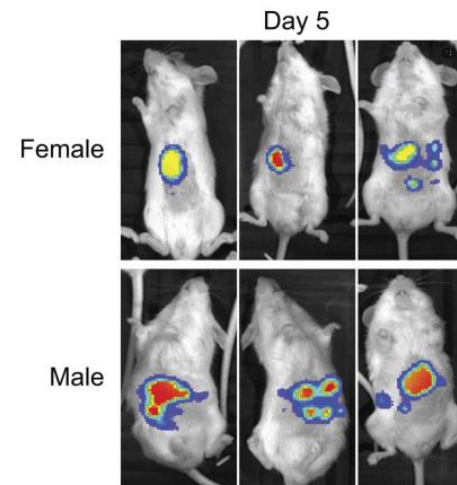
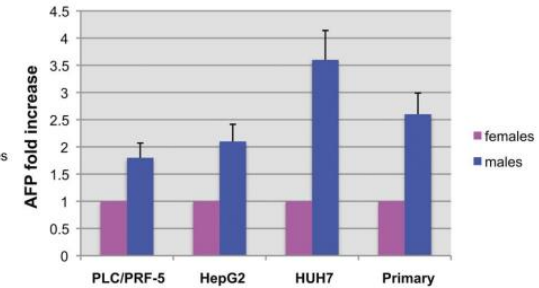
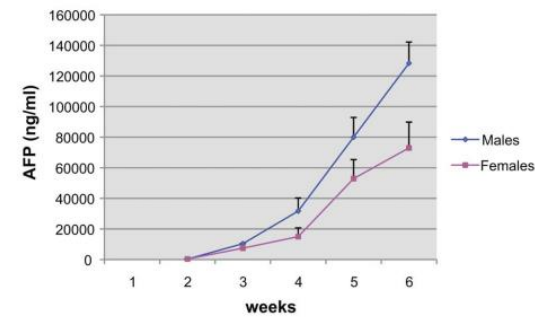
- BM CD34+ cells injected with fetal thymus implanted under the kidney capsule
- There are differences in the expansion kinetics of specific subpopulations of immune cells during expansion and maturation
- Select the sex of host mice depending on the study design



# Choose The Mouse Depending On Your Study Design

## Example Of PDX Growth Kinetics

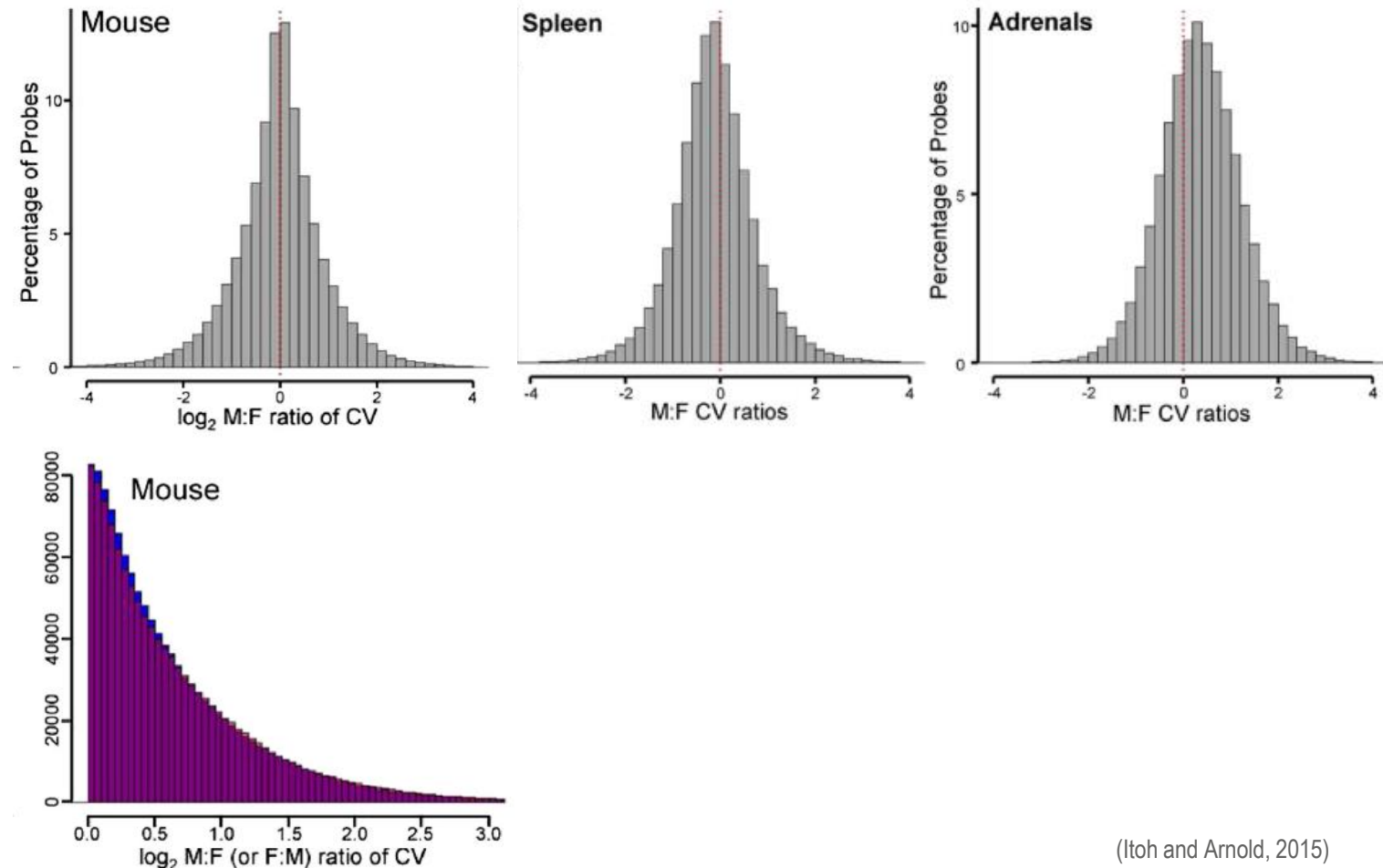
- 6 different hepatocellular carcinoma cells were injected into either male or female NSG mice
- The tumor progression was significantly larger and faster in male recipients



# Inconclusive Assumption:

## Experimental Results in Females are Intrinsically More Variable Than in Males

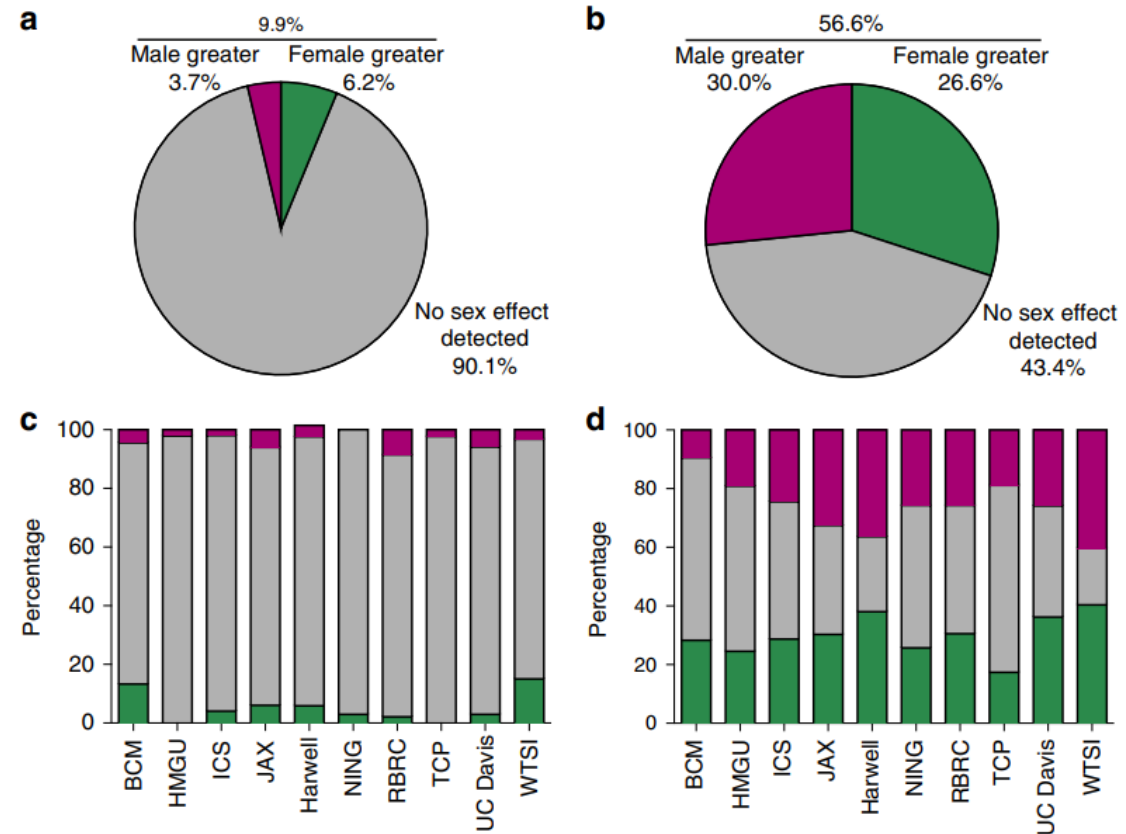
- Meta-analysis indicated that there is no overall greater phenotypic variability in females
- Slight male bias or sexual equivalence of Coefficients of variation
- In expression data from spleen, female mice had higher variation in gene expression, but from adrenals, males had higher variability



(Itoh and Arnold, 2015)

# Female And Male Mice Often Differ in Disease Manifestation and in Their Response to Treatments

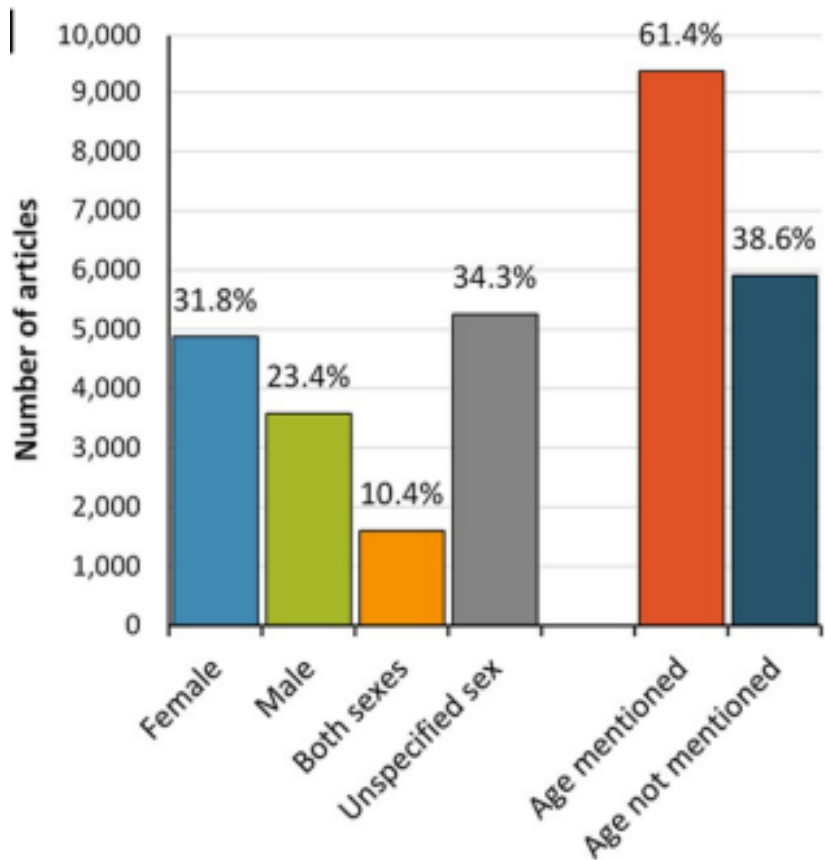
- Phenotypes were classified as either continuous or categorical
- Sexually dimorphic rate was analyzed by Institute



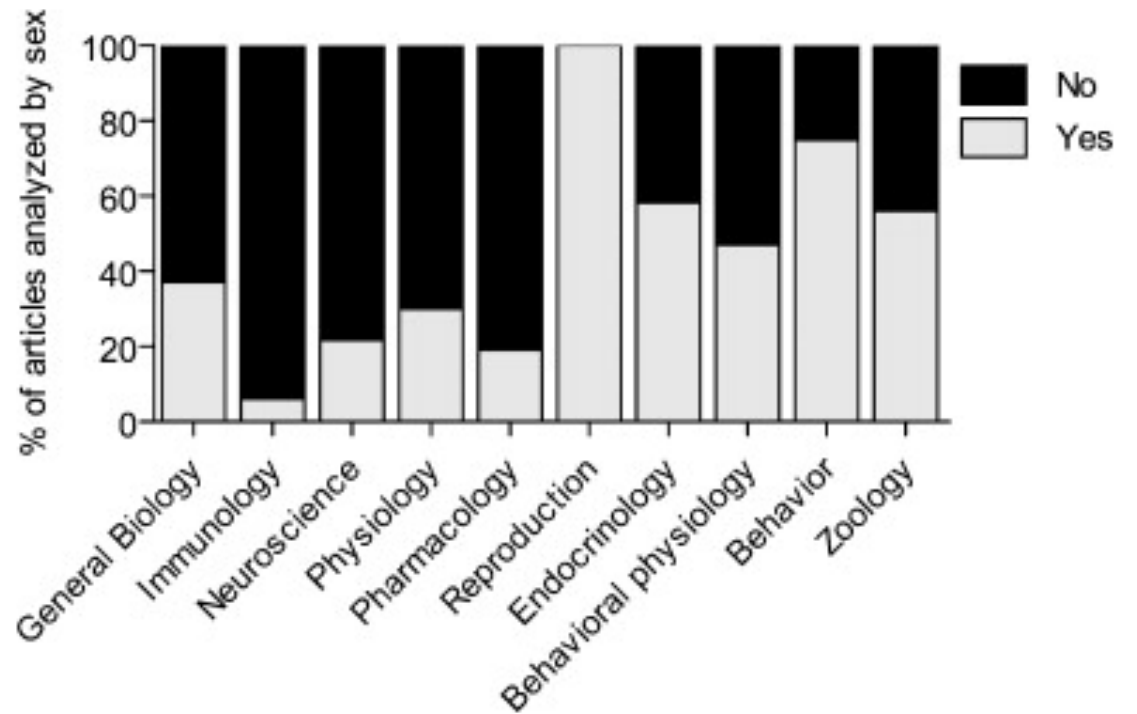
(Karp et al., 2017)

# Report and Analyze Research Results by Sex

- Number and percentage of articles reporting/not reporting of sex by sex



- Percent of articles that some portion of the results was analyzed by sex



(Beery and Zucker, 2011, Flórez-Vargas et al., 2016)

# Sex Bias in Multiple Biological Disciplines

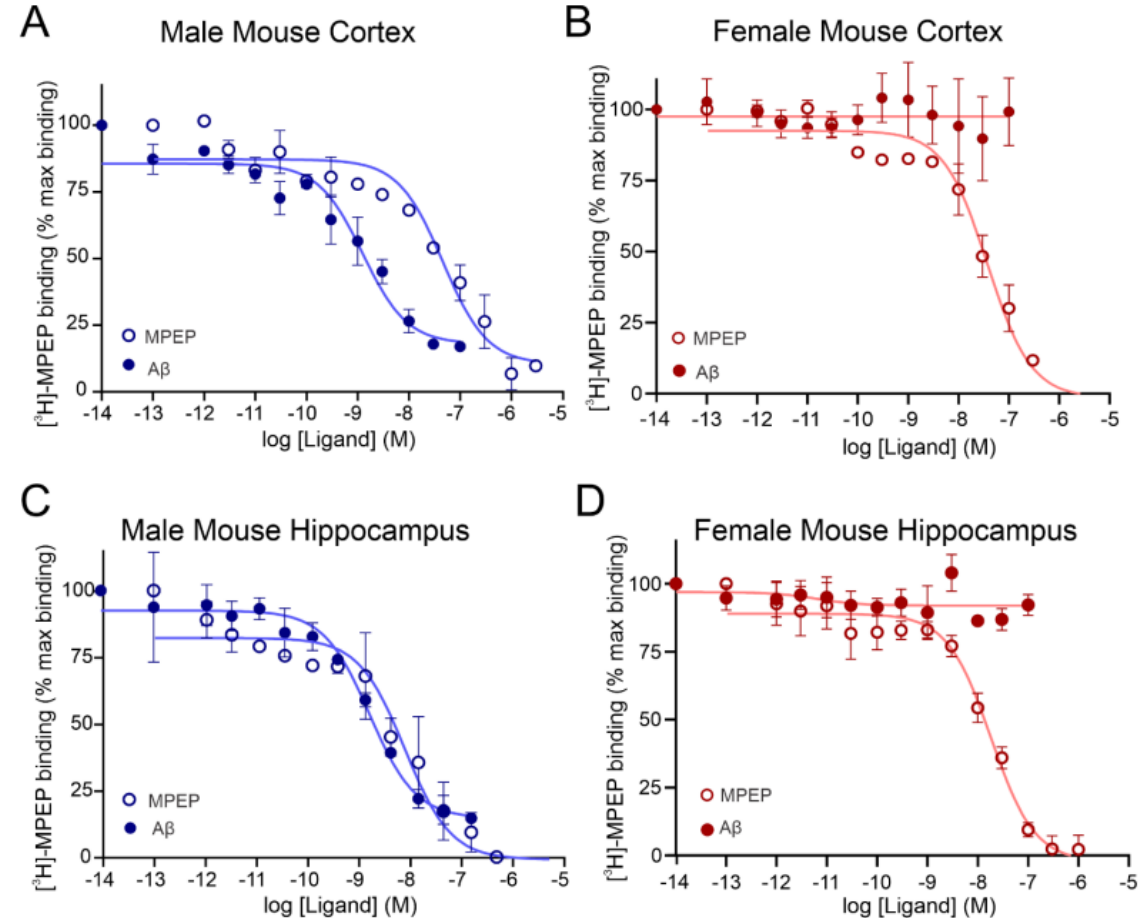
- Neuroscience
- Cardiovascular Disease
- Autoimmunity
- COVID-19
- And more disciplines



<https://www.nature.com/articles/509282a.pdf>

# Sex Differences in Neuroscience

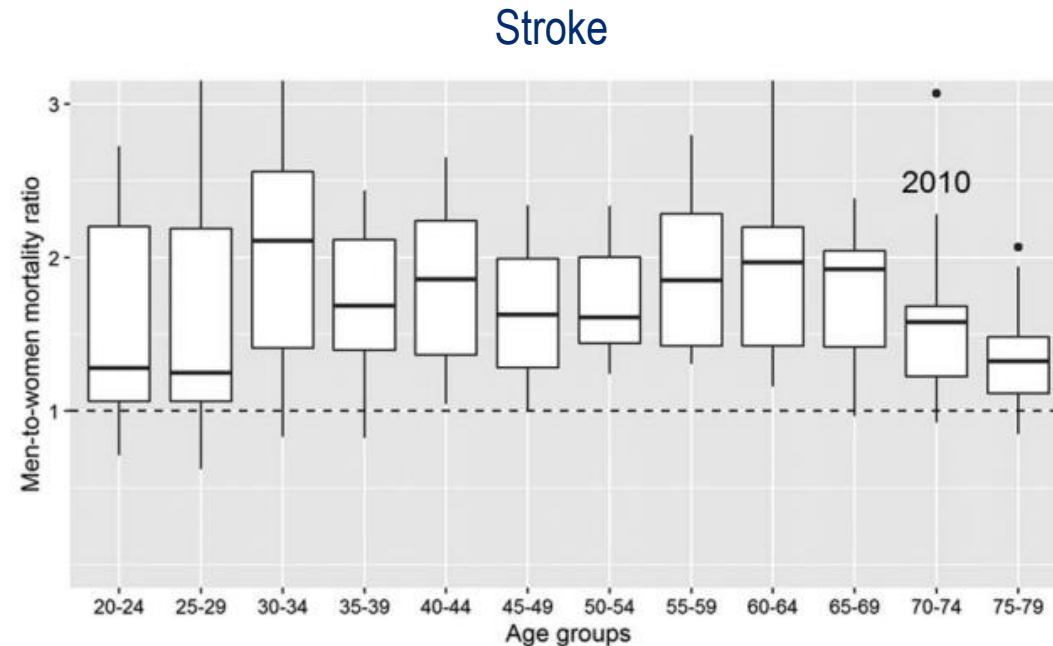
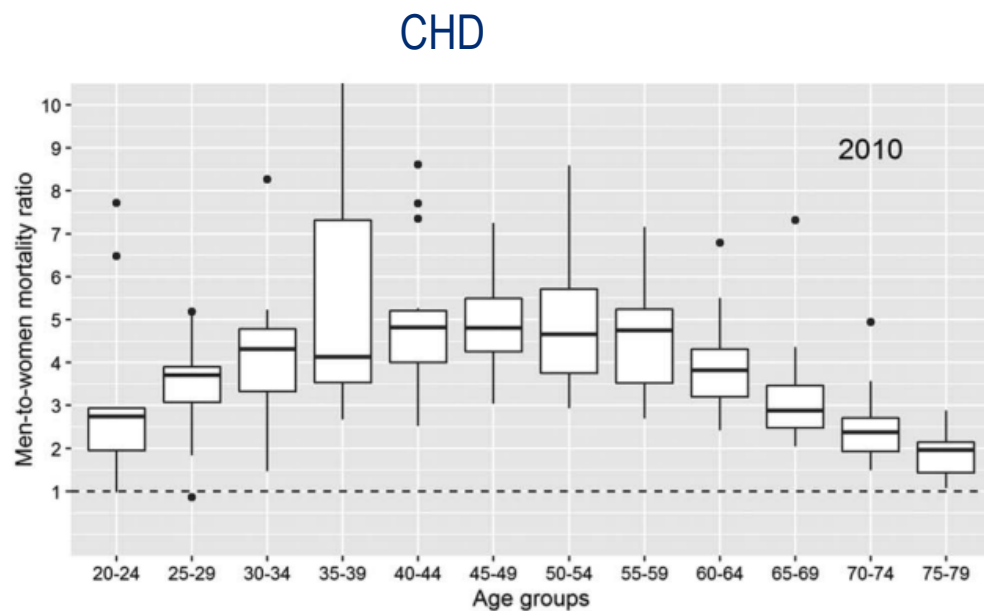
- A $\beta$  oligomers binds with high affinity to mGluR5 in both male mouse and human cortex but exhibits no affinity for mGluR5 in either female mouse or human brain tissue.



(Abd-Elrahman et al., 2020)

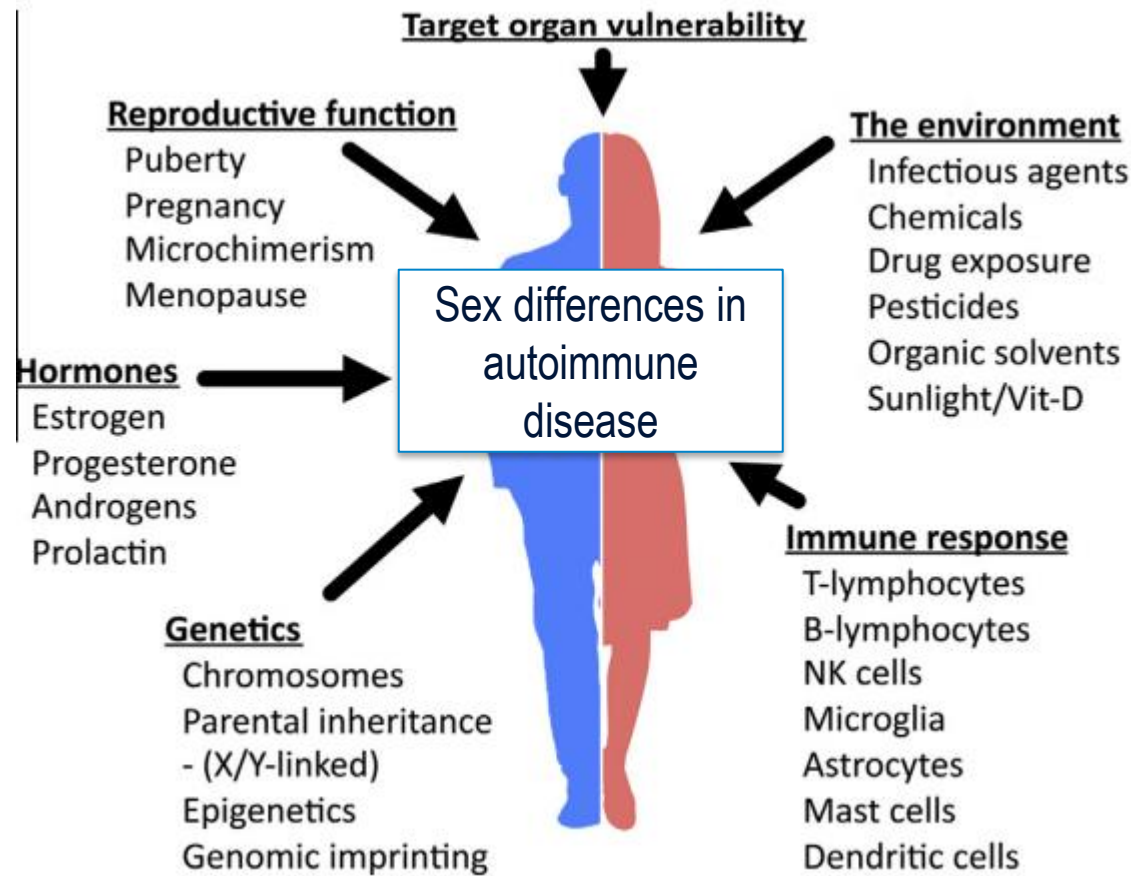
# Sex Differences in Cardiovascular Disease

- Men-to-women mortality rate ratios for coronary heart disease and stroke
- Several studies identified premenopausal hormonal dysfunction and hypertensive disease during pregnancy as being highly associated with cardiovascular disease



(Bots S., 2017)

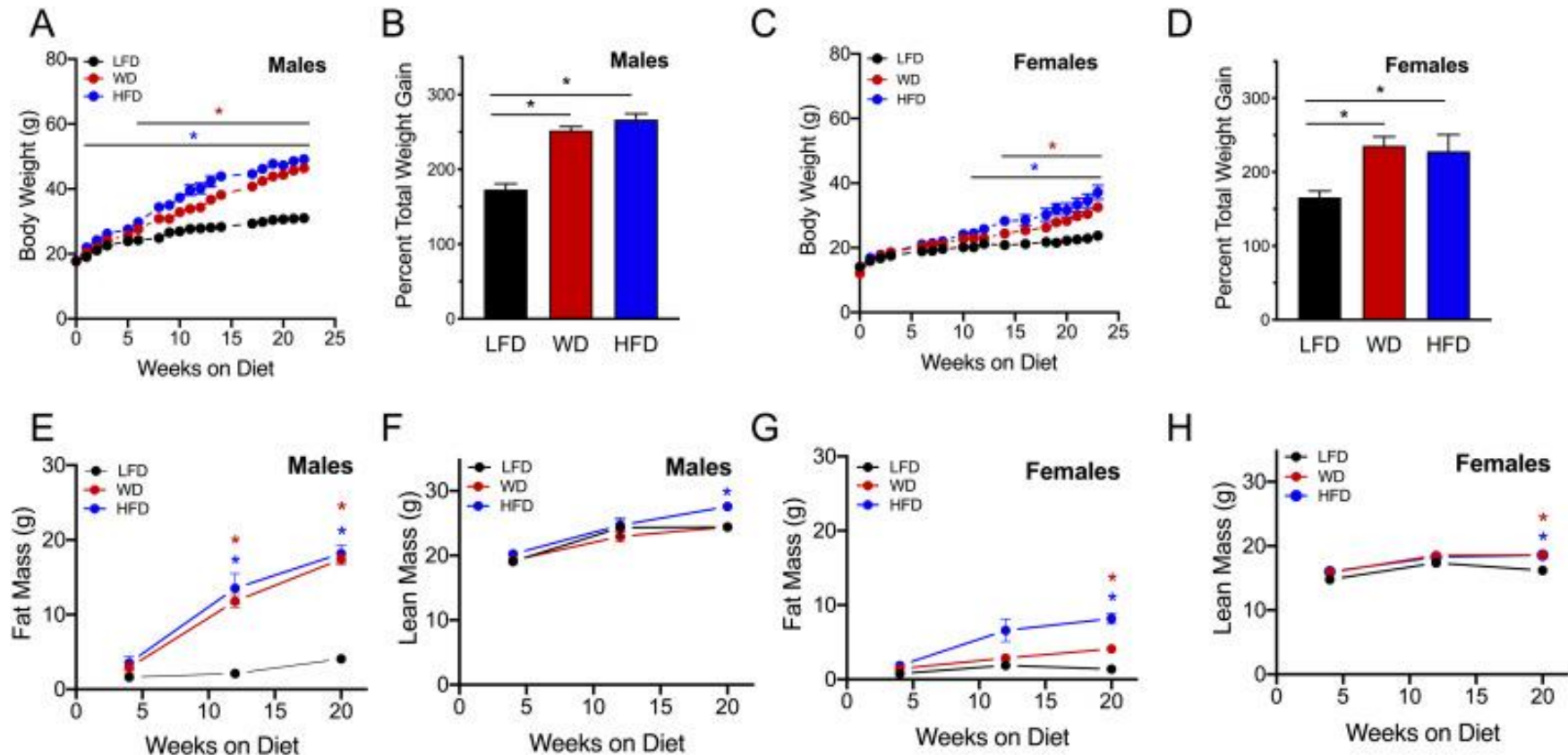
# Sex Differences in Autoimmunity



- Sex has a significant influence on the development of autoimmune diseases
- Potential mechanisms for sex differences include different immune response, pregnancy, sex hormones, genetic predisposition, parental inheritance, epigenetic, and environmental factors

# Sex Differences in Metabolism

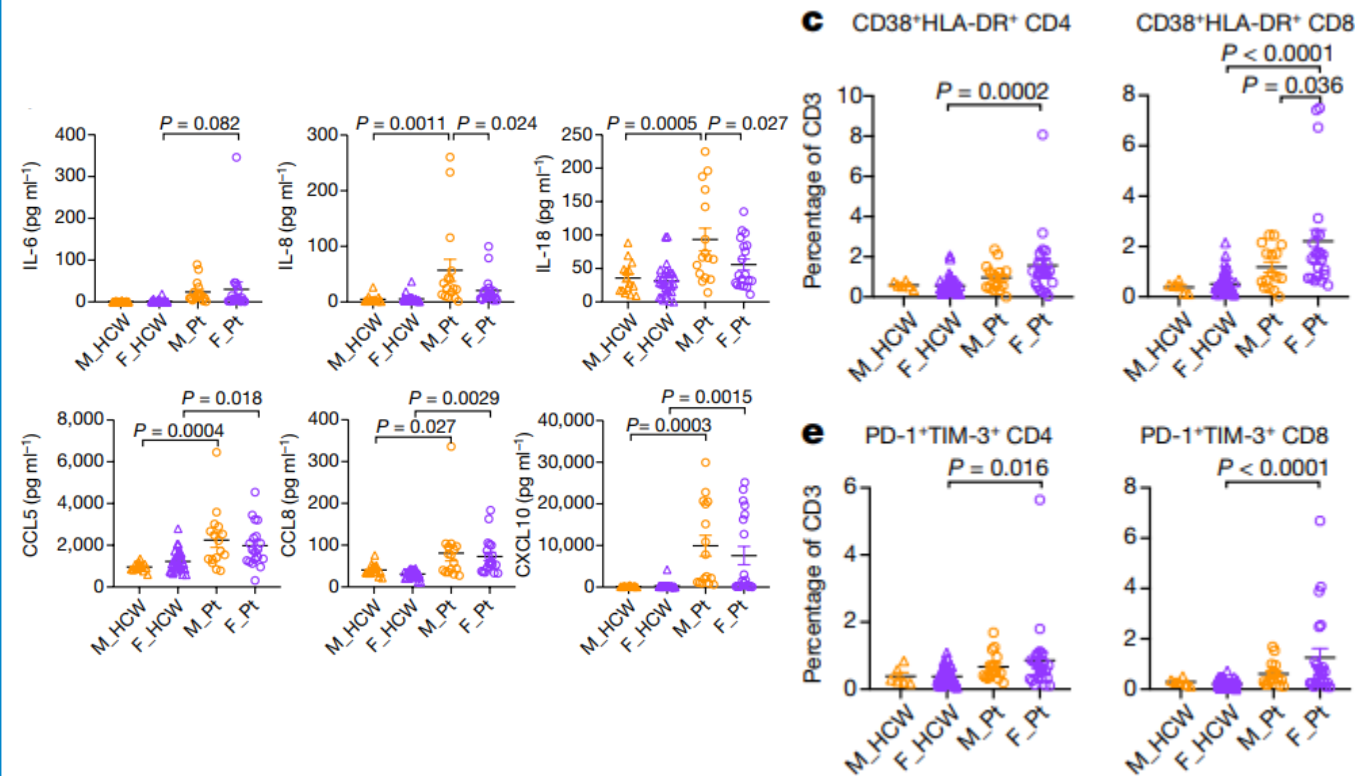
- Considerations for experiments
  - estrous cycle
  - vivarium environment
  - stress



<https://www.sciencedirect.com/science/article/pii/S1056872720305894>

# Sex Differences in Immune Responses to COVID-19 Infection

- There are differences in immune responses during the disease course of SARS-CoV-2 infection in male and female patients.
- Several important pro-inflammatory innate immunity chemokines and cytokines such as IL-8, IL-18 (at baseline) and CCL5 (longitudinal analysis) were higher in male patients
- A more robust T cell response among female patients than male patients at baseline



(Takahashi T et al., 2020)

# Conclusions

- There are sex differences in multiple disease disciplines
- Male and female mice are phenotypically different and responding differently to the therapeutics
- It is recommended that researchers should report and analyze experimental results by sex.

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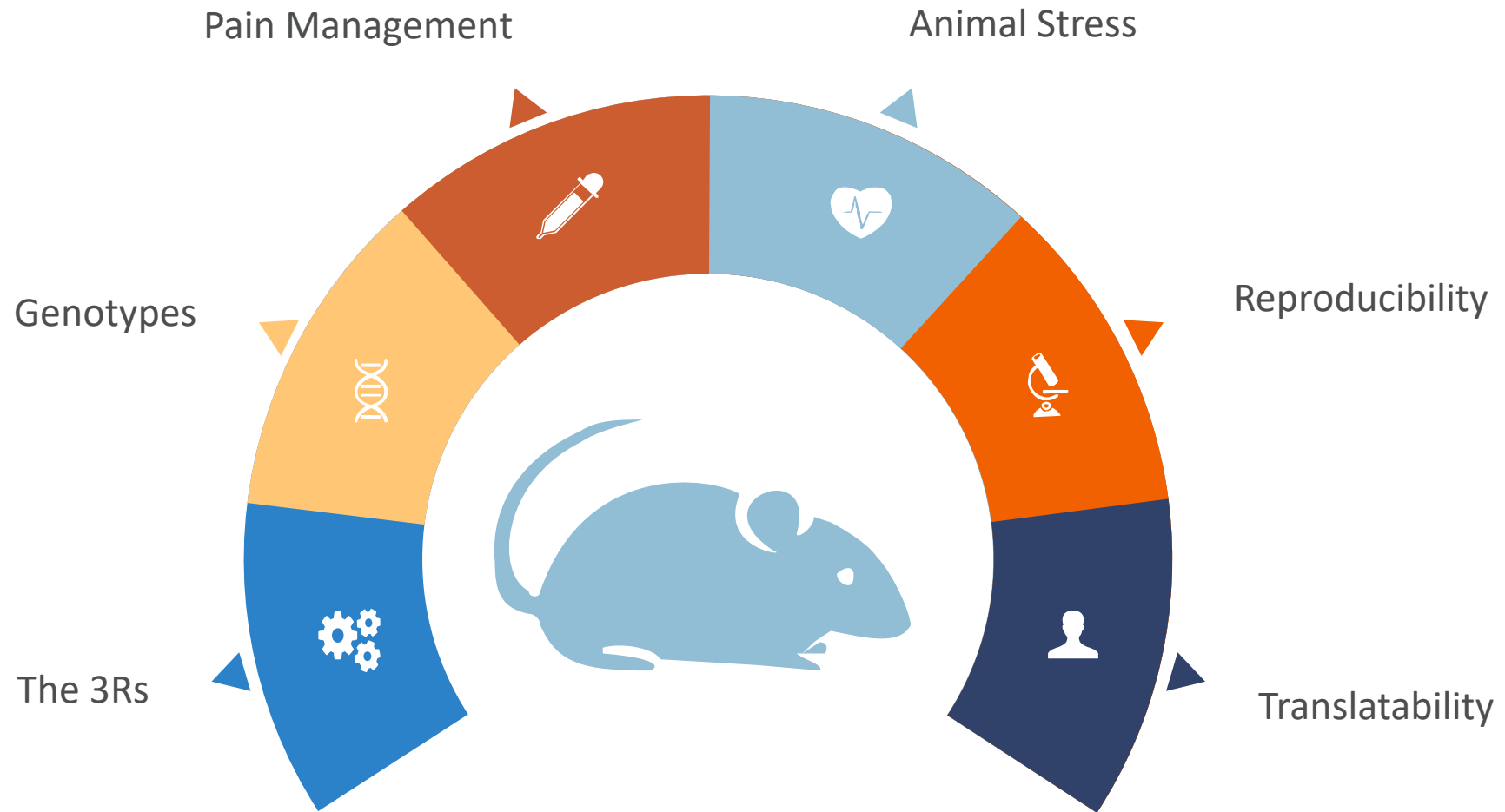
# SEX BIAS IN PRECLINICAL RESEARCH

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The Impact on Animal  
Welfare and the 3Rs

# OVERVIEW

## Animal Welfare Considerations of Sex Bias in Research



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# The 3Rs

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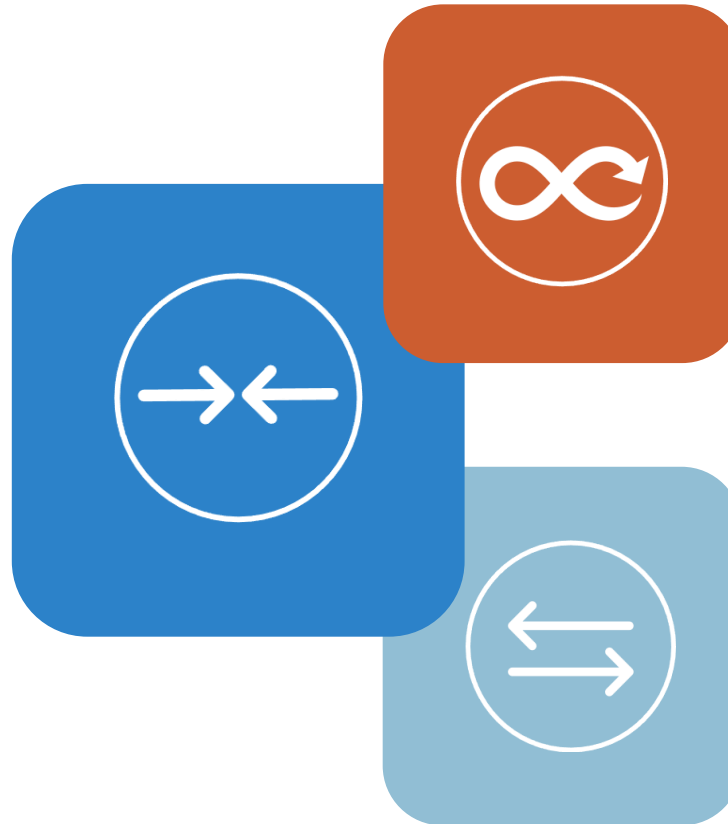
Section subhead (Calibri, size 18)

# WHAT ARE THE 3RS?

Subtitle (Sentence case, Calibri, size 18)

## *Reduction*

Methods for obtaining comparable levels of information from the use of fewer animals, or for obtaining more information from the same number of animals



## *Refinement*

Methods which alleviate or minimize potential pain, suffering or distress, and which enhance animal well-being

## *Replacement*

Methods which permit a given purpose to be achieved without conducting procedures on animals

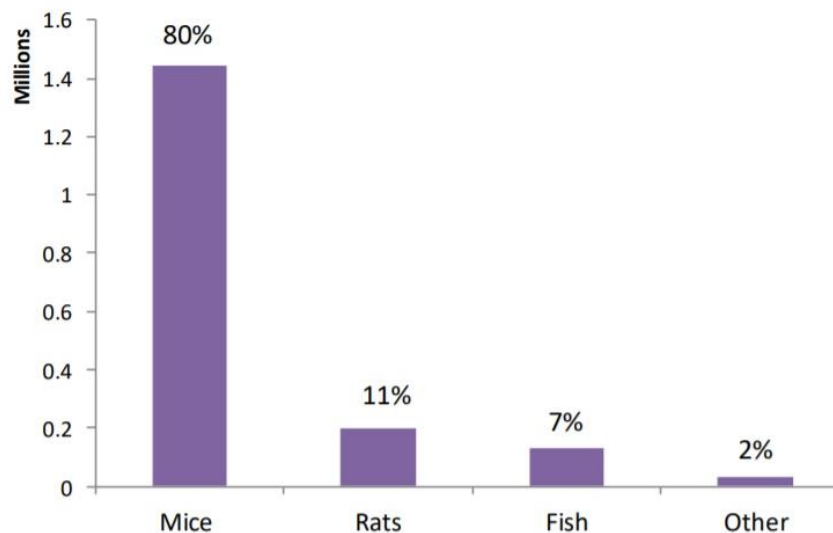
# ANIMALS BRED FOR RESEARCH AND NOT USED

UK Home Office: animals bred for scientific purposes 2017

**1.8 million non-genetically modified animals** were bred for scientific purposes but were euthanized or died prior to use in procedures

- 80% mice, 11% rats, 7% fish, 2% other
- breeding animals (i.e. **wild types**) that underwent no procedures themselves
- **Wrong gender** for a particular purpose
- **Necessary surplus** resulting from the breeding of animals to ensure adequate supply for scientific purposes (eg. Right age and weight) or needed to provide tissue.

Figure 1: Animals that were bred for scientific procedures but were killed or died without being used in procedures, Great Britain, 2017



Source: Home Office, Additional statistics on breeding and genotyping of animals for scientific procedures, [Table 1.1](#)

**2017 The European Commission also announced that 12,597,816 animals were bred but not used in scientific research. Wrong gender was cited as one of the leading reasons.\***

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# Genotypes

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# SINGLE SEX STUDIES CONTRIBUTE TO ANIMAL EXCESS

Subtitle (Sentence case, Calibri, size 18)

Creating animals just to use only one sex leads to excess animal production...



Wrong sex



Wrong genotype



# GENOTYPES



## Reduction

- Application of 'Reduction'
- Single-sex litters



Article | [Open Access](#) | Published: 03 December 2021

### CRISPR-Cas9 effectors facilitate generation of single-sex litters and sex-specific phenotypes

[Charlotte Douglas](#), [Valdome Maciulyte](#), [Jasmin Zohren](#), [Daniel M. Snell](#), [Shantha K. Mahadevaiah](#), [Obah A. Ojarikre](#), [Peter J. I. Ellis](#)  & [James M. A. Turner](#) 

*Nature Communications* **12**, Article number: 6926 (2021) | [Cite this article](#)

**18k** Accesses | **376** Altmetric | [Metrics](#)

#### Abstract

Animals are essential genetic tools in scientific research and global resources in agriculture. In both arenas, a single sex is often required in surplus. The ethical and financial burden of producing and culling animals of the undesired sex is considerable. Using the mouse as a model, we develop a synthetic lethal, bicomponent CRISPR-Cas9 strategy that produces male- or female-only litters with one hundred percent efficiency. Strikingly, we observe a degree of litter size compensation relative to control matings, indicating that our system has the potential to increase the yield of the desired sex in comparison to standard breeding designs. The bicomponent system can also be repurposed to generate postnatal sex-specific phenotypes. Our approach, harnessing the technological applications of CRISPR-Cas9, may be applicable to other vertebrate species, and provides strides towards ethical improvements for laboratory research and agriculture.

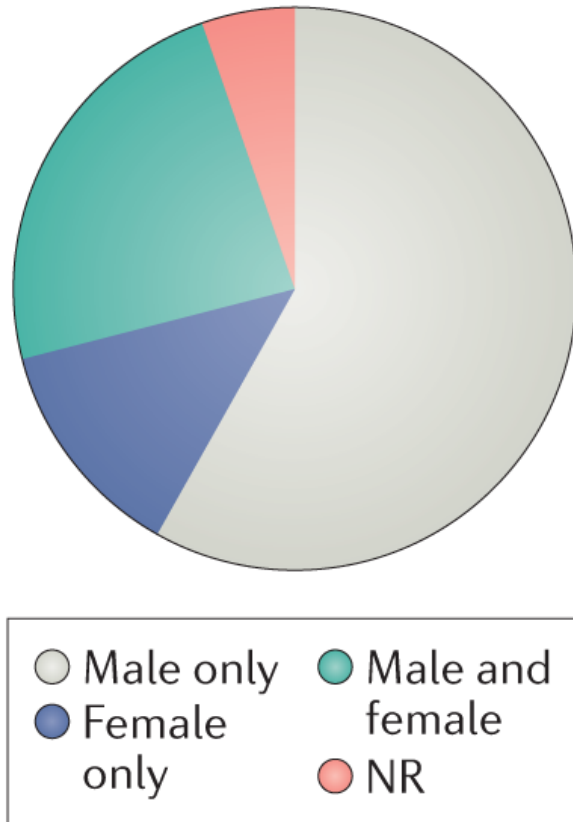
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# Pain Management

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# PAIN MANAGEMENT

## Refinement



Analysis suggests that young, naive, inbred C57BL/6 male mice & young, naive, outbred Sprague Dawley male rats are the current status quo for preclinical pain studies. (Sadler 2022)

Several anatomic/physiologic factors come into play when interpreting sex differences regarding pain and analgesia:

- adult male rodents have greater percentage of body fat than females
- sexual dimorphism can affect the distribution of highly lipophilic drugs and influence analgesia potency, and duration of action.
- metabolism, immune response, activity level, and response to analgesics all have a sex difference.

# SINGLE SEX STUDIES & ANALGESIA UNDERDOSING

Subtitle (Sentence case, Calibri, size 18)

Male mice utilize microglia in the spinal cord to mediate pain, whereas females preferentially use T cells in a similar manner. The difference can be traced to differences in cell populations, differences in suppression by hormones, and disparate cellular responses in males and females....females are more likely to experience chronic pain (Sorge & Totsch 2016 JNR)



## Male vs. Female

Pain studies done in males

Mouse estrus cycle influences:

- pain sensitivity
- pain threshold
- Pain tolerance



## Female pain goes untreated

- Opioids are more potent in males
- Females likely underdosed

<https://doi.org/10.1002/jnr.23841>

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# Animal Stress

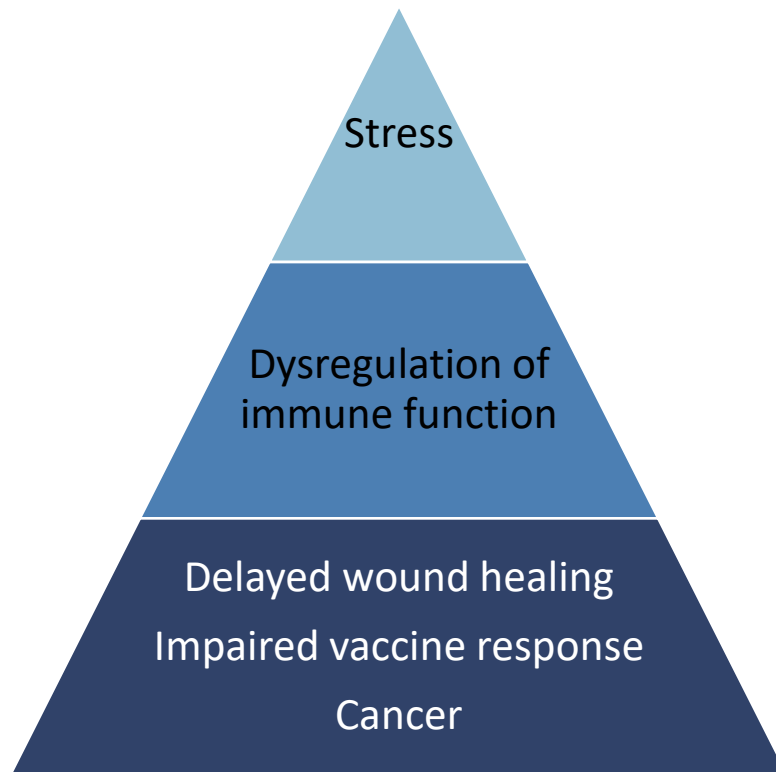
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Section subhead (Calibri, size 18)

# ANIMAL STRESS

Males & Females experience different stress factors in different ways

*Click to edit Master text styles*



Males are often single housed

- Socially housed male mice may show territorial behaviour, aggression and fighting, depending on strain, previous experience and cage enrichment (Van Loo et al., 2001).

Females have lower levels of stress-induced analgesia than males after experiencing the same stressors (Wiesenfeld-Hallin 2005)

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# Reproducibility

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Section subhead (Calibri, size 18)

# RESEARCH QUALITY AND REPRODUCIBILITY: THE CURRENT CRISIS

## EDITORIAL

### Step up for quality research

In response to calls for change from within and outside the scientific community, funding agencies, journals, and professional societies are developing new requirements to promote reproducibility and integrity in research. Amid this activity, the voices of academic institutions—both their leadership and rank-and-file faculty—have largely been quiet. Yet journals, societies, and funding agencies are not in the laboratory, clinic, or field. They do not analyze data, write manuscripts, or prepare figures. Institutions that comprise the global academic community can do more to help their researchers produce the highest-quality results. The three areas of actions, described below, echo points highlighted in the recent U.S. National Academies of Sciences, Engineering, and Medicine report, *Fostering Integrity in Research*, as well as at the 2017 World Conference on Research Integrity in May.

University leaders should better promote the critical importance of research quality. It is easy to focus on funding successes and high-profile publications as



***“The university community must ask itself tough questions...”***

issues. The symposium inspired other institutions to consider hosting similar events.

Universities must also strengthen their research integrity offices, review and improve research integrity training programs, and develop proactive programs to prevent research misconduct. Focusing on two areas—mentoring and data management—could go a long way toward preventing falsification and fabrication. Programs like the University of Wisconsin–Madison’s research mentor training program, which was tested and found effective in 16 academic medical centers, should be adopted. Another model is Columbia University’s Research and Data Integrity (ReaDI) Program, which provides researchers with hands-on support and resources for data management and reproducibility.

The university community must ask itself tough questions: What incentives may influence or impede research integrity and reduce research quality and reproducibility of results? How can the value of quality control activities in research be promoted? How should young scientists be taught to avoid pitfalls? What is the

## THE CONVERSATION

Academic rigor, journalistic flair

Search analysis, research, academics...

Arts + Culture Economy + Business Education Environment + Energy Ethics + Religion Health + Medicine Politics + Society **Science + Technology**

### The science ‘reproducibility crisis’ – and what can be done about it

March 15, 2017 5:49am EDT



Science and integrity is under the microscope. Shutterstock

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#### Disclosure statement

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Email

Reproducibility is the idea that an experiment can be repeated by

# REPRODUCIBILITY

How to manage conflicts with regard to the 3Rs?



- 3R principles in animal care and use suggest reduction, replacement and refinement in laboratory research for the sake of animal welfare.
- Performing experiments now on both sexes requires more subjects to be tested, which is against the main principles of 3R because they suggest using as few animals as possible to obtain valid and statistically powerful data.....
- But we cannot ignore substantial evidence pointing towards sex as an important variable.
- Results can be reproduced successfully only if they are robust against the variation that exists between independent replicate studies.

**Therefore, careful planning of experimental design and validating results by replication  
is a prerequisite of excellent science**

# HOW DO WE FIX THIS?

## Scientific concerns & welfare concerns

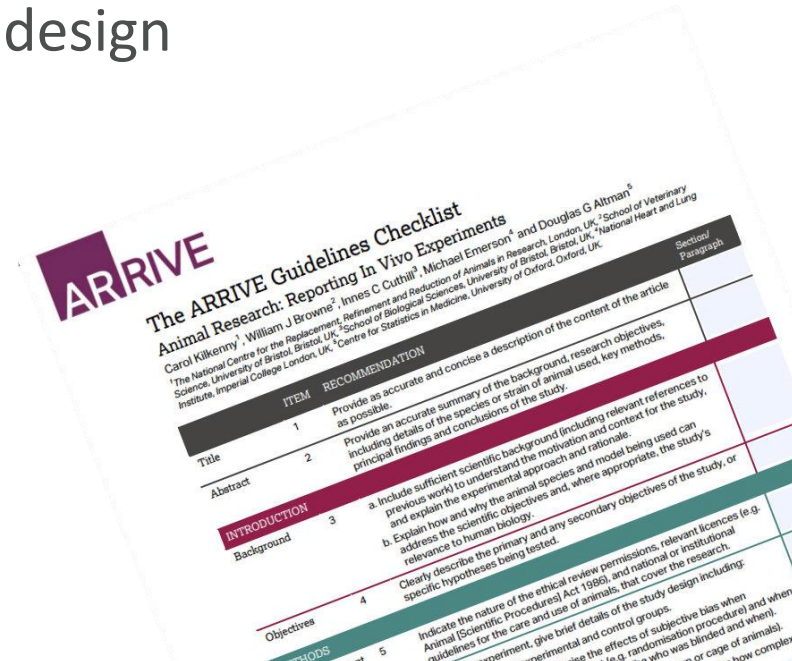
Researchers are required to use both sexes of laboratory animals, or clearly state and defend by reasonable arguments, why they are using male-only or female-only animals in their study.

- National Institutes of Health (NIH) – <https://orwh.od.nih.gov/sex-gender/nih-policy-sex-biological-variable> 2014, Sex as a biological variable (SABV)
- Canadian Institutes of Health Research (CIHR) - <https://cihr-irsc.gc.ca/e/49346.html> applicants must report sex integration in their proposal
- European Commission (EC) - systematically questions whether and in what sense, sex and gender are relevant in the objectives and in the methodology of projects



## Scientific concerns & welfare concerns

# NC3Rs – Experimental design



41 | EVERY STEP OF THE WAY

# SUMMARY

- Consider sex as biological variable, exploring the prevalence of sex having an impact on experiments
- Keep in mind why sex as bias is existing in studies
- If a disease is sex-linked, use the appropriate sex animal model
- The significant effect of sex bias on animal welfare
- 3Rs guidelines and recommendations to eliminate sex bias in preclinical studies
- Translational value. We don't hit the target in single sex studies



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