

# Pseudoreplication: How to avoid it in experimental design and analysis

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Data Science Talk  
Oct 15, 2018

# The P-value



**The probability of getting your experimental result (or something more extreme), given that the null hypothesis is true.**



**NOT:** The probability that the null hypothesis is wrong

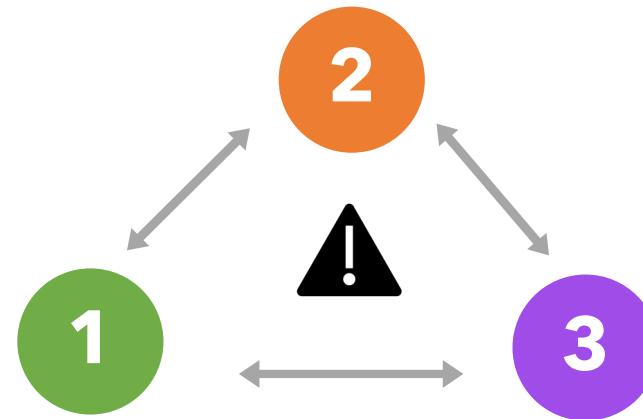
**NOT:** The probability that the your hypothesis is wrong



$P = 0.65$  means a 65% chance that you would get the data you did (or something more extreme), if there is no true difference between groups.

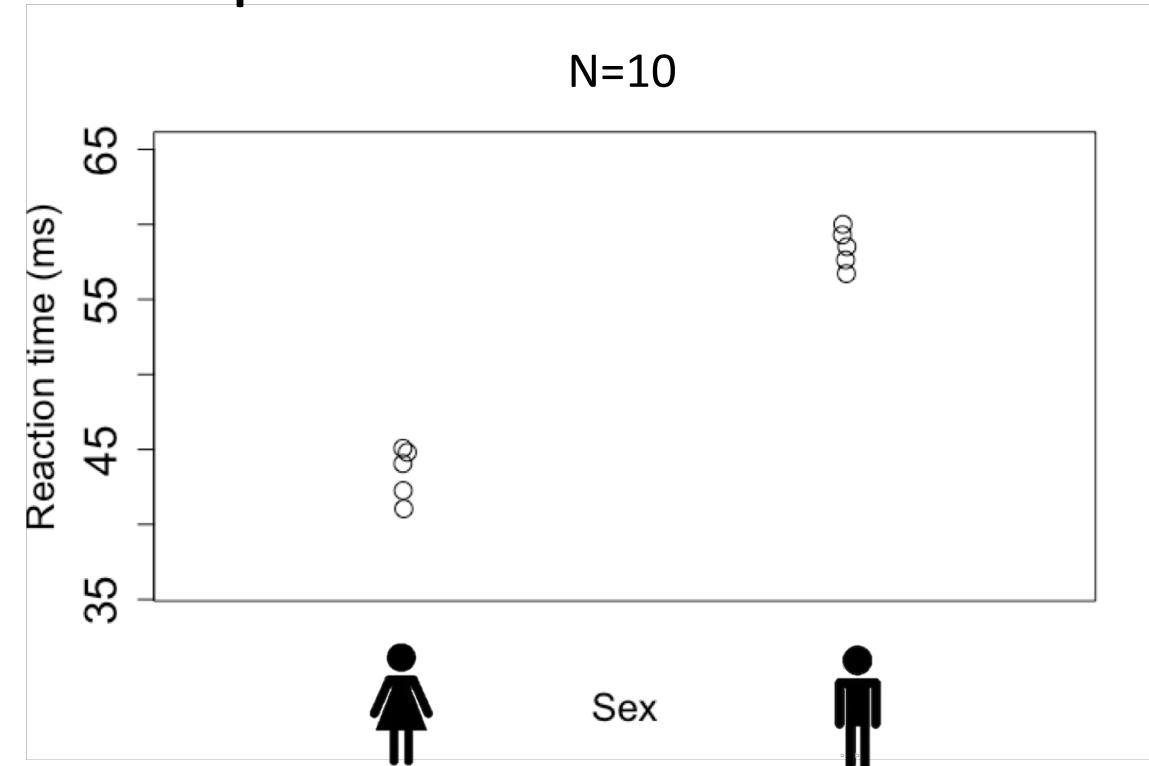
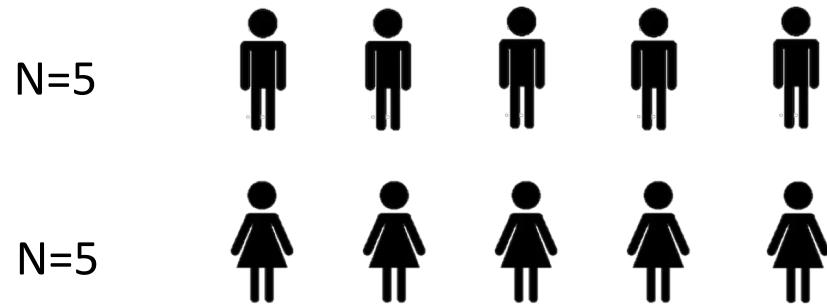
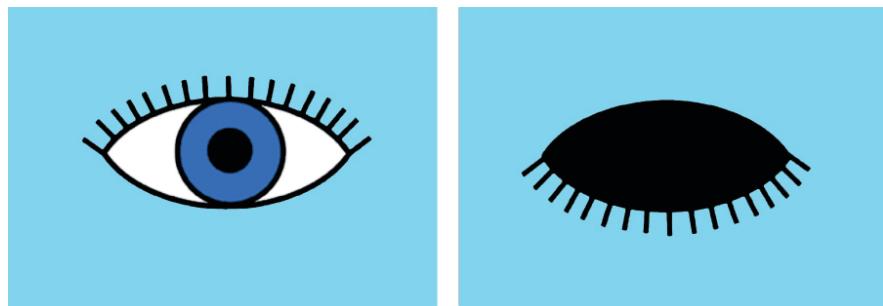
# Can you trust your p-value?

**Assumption:** Observations cannot be correlated (they must be independent) for our inferences to be correct



**Pseudoreplication** occurs when data points are not independent but treated as if they are

# Extreme example of pseudoreplication



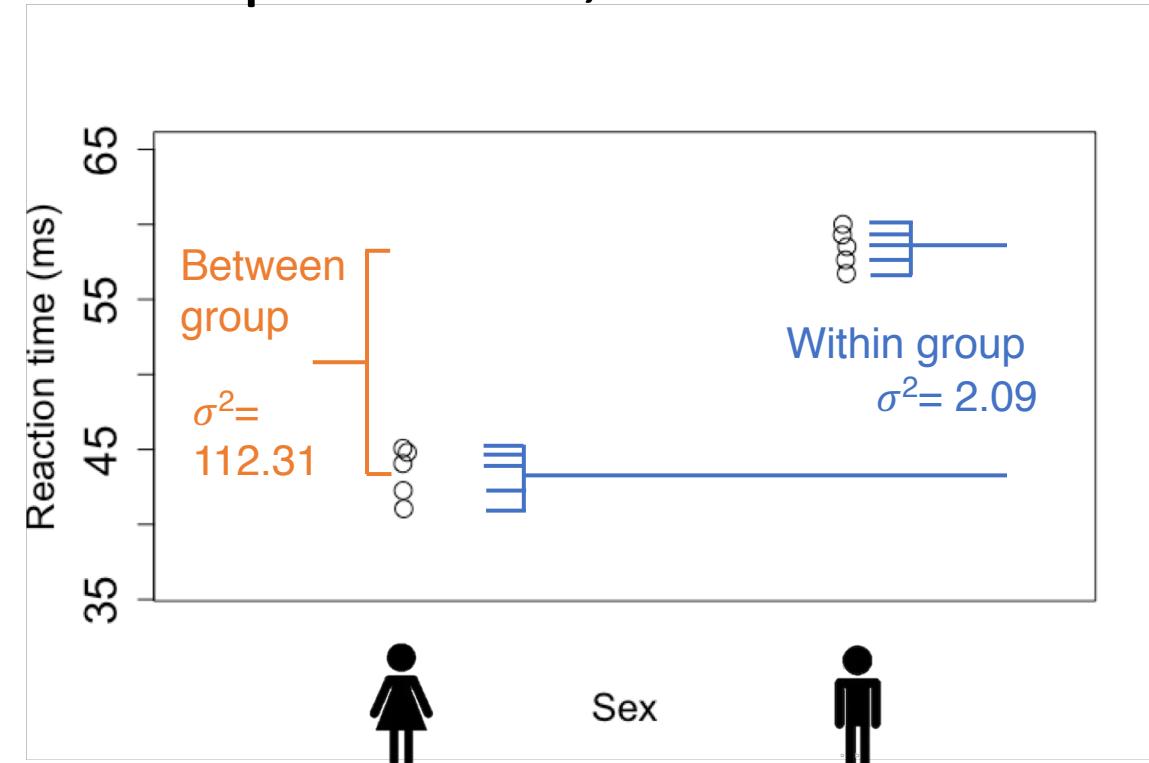
# Determining the effective sample size, N

## Intraclass correlation (ICC)

$$\text{ICC} = \frac{\sigma^2_{\text{between.group}}}{\sigma^2_{\text{between.group}} + \sigma^2_{\text{within.group}}} = \frac{112.31}{112.31 + 2.09} = 0.98$$

$$\text{design effect} = 1 + (n - 1) \times \text{ICC} \\ = 1 + (5 - 1) \times 0.97 = 4.93$$

$$N_{\text{effective}} = \frac{N \times n}{\text{design effect}} = \frac{2 \times 5}{4.89} = 2.03$$

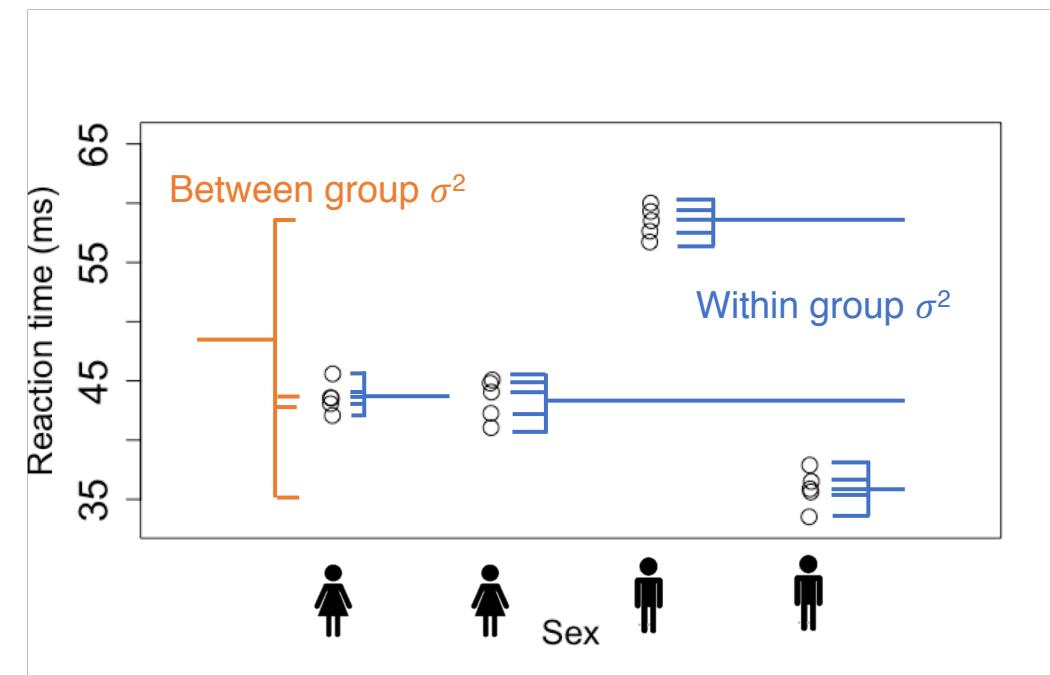
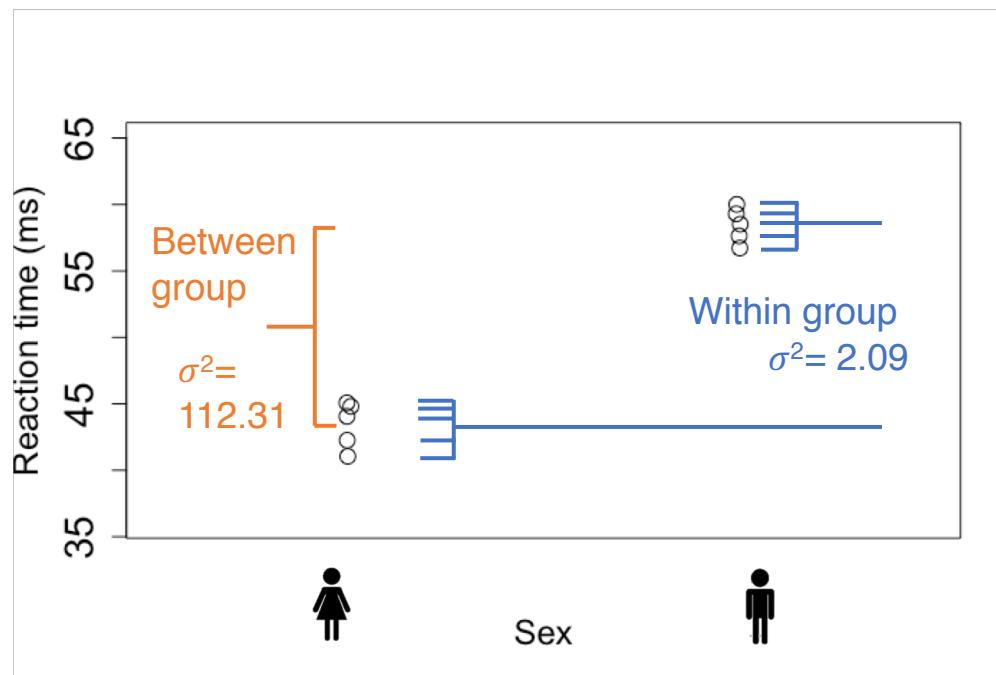


# Markdown for calculating the ICC

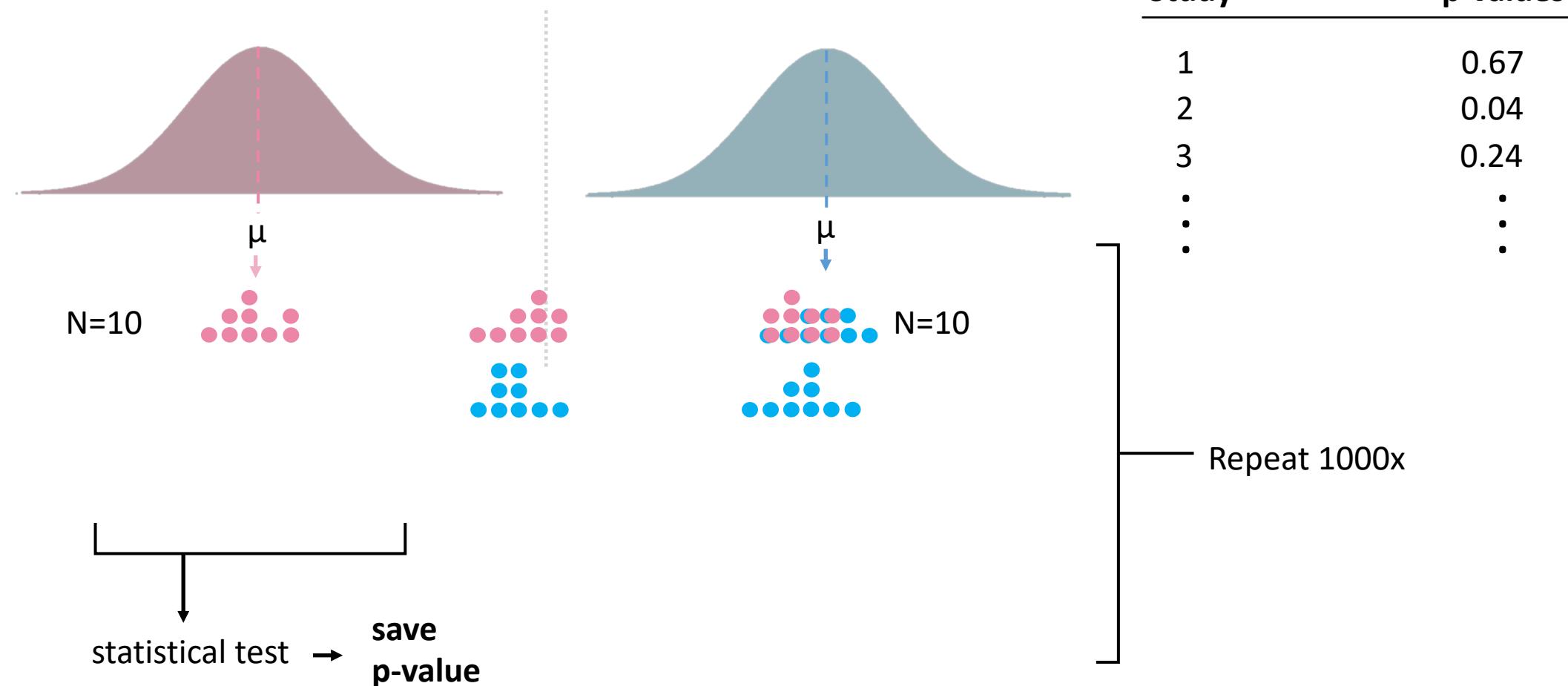
# Determining the effective sample size, N

## Intraclass correlation (ICC)

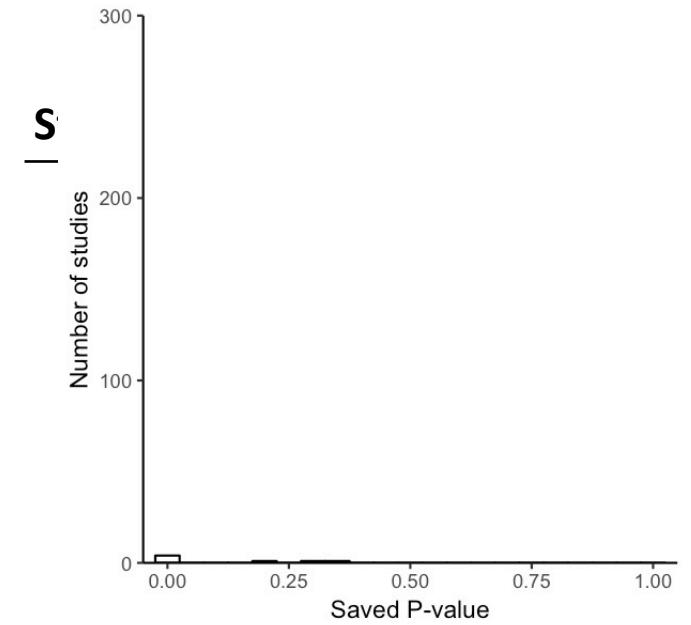
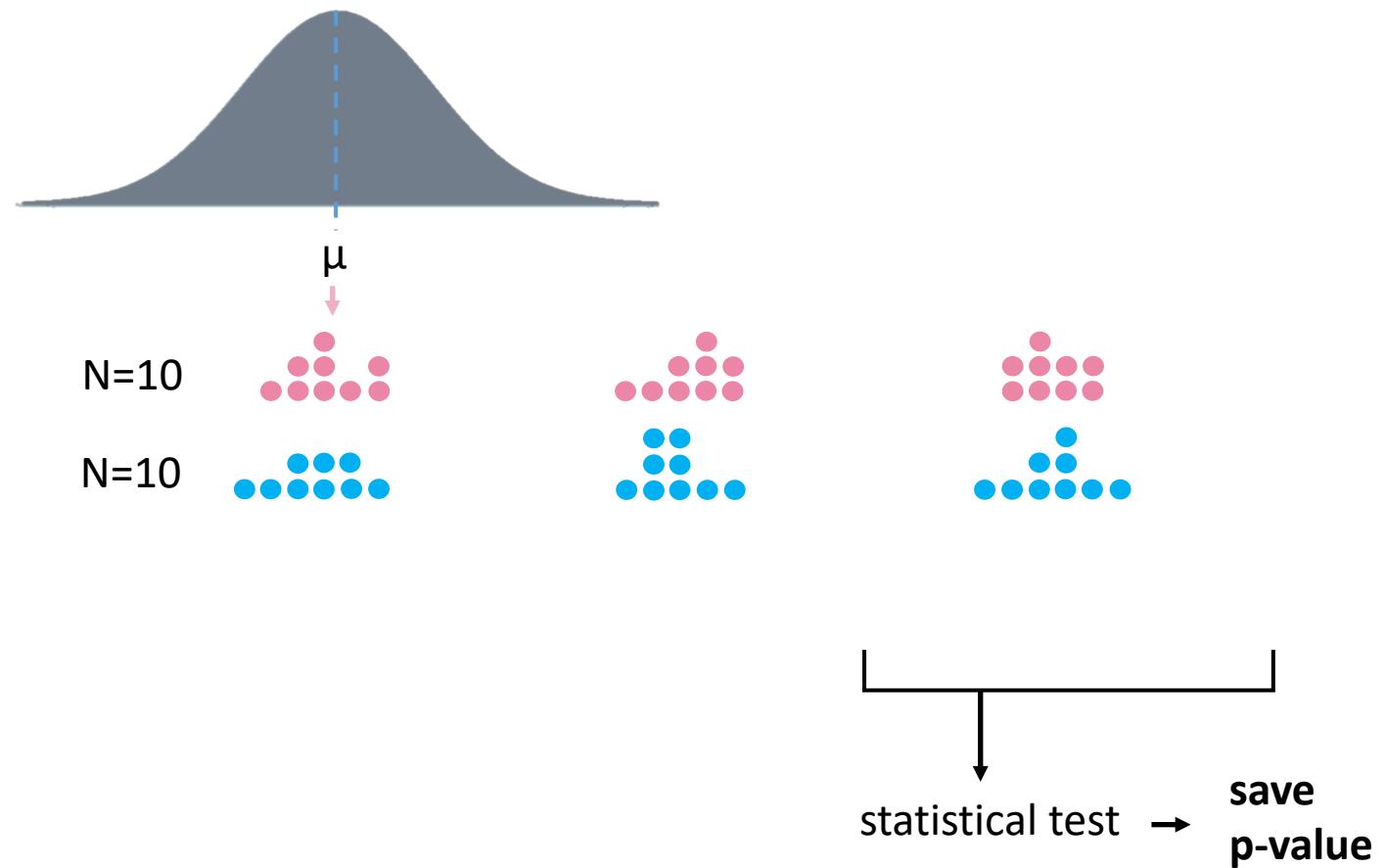
$$\text{ICC} = \frac{\sigma^2_{\text{between.group}}}{\sigma^2_{\text{between.group}} + \sigma^2_{\text{within.group}}}$$



# Why is pseudoreplication a problem?

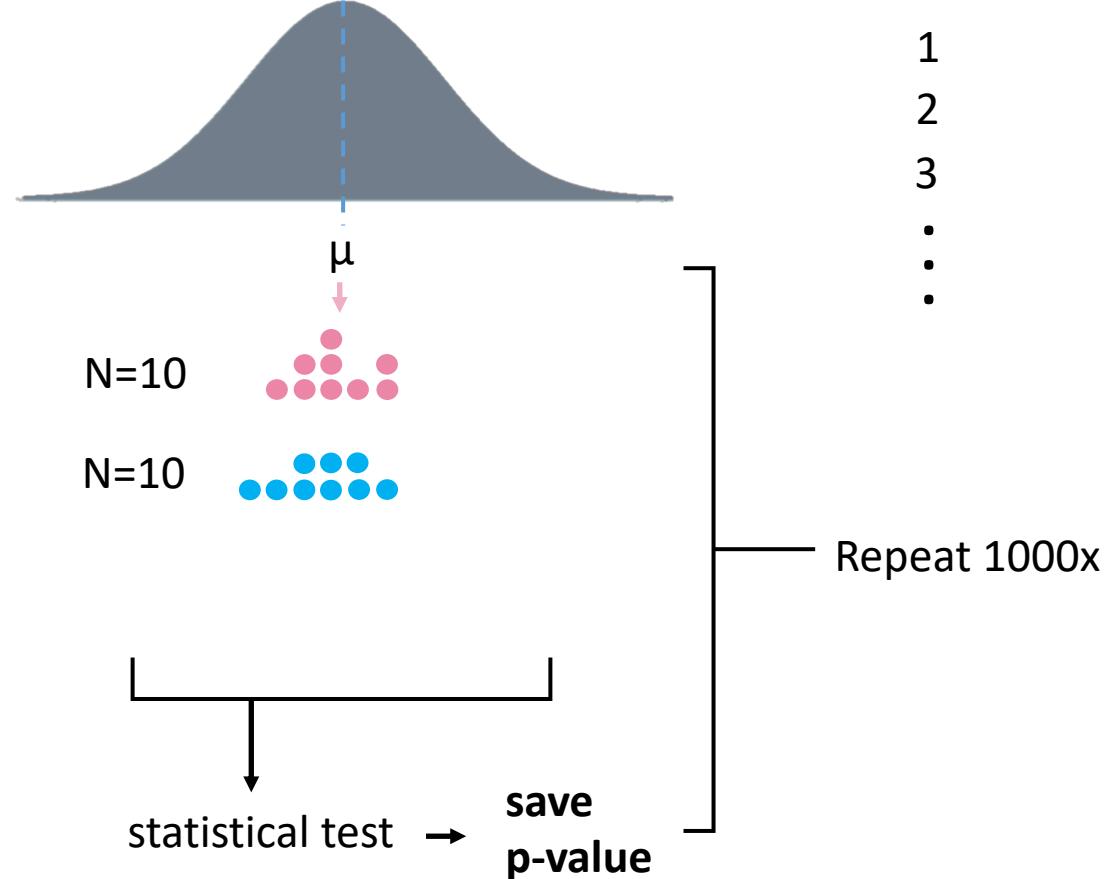


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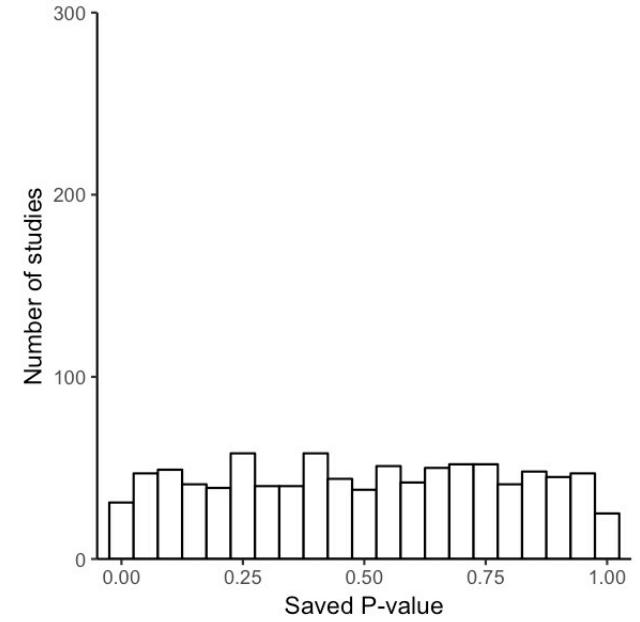


Repeat 1000x

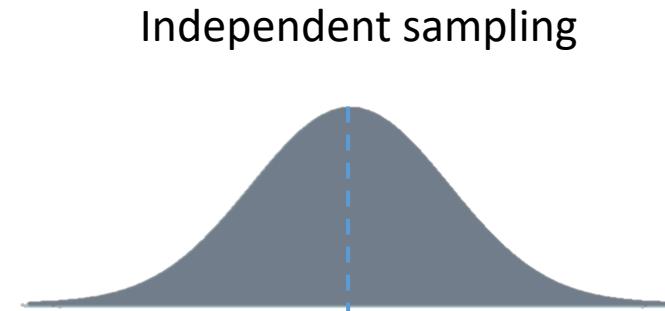
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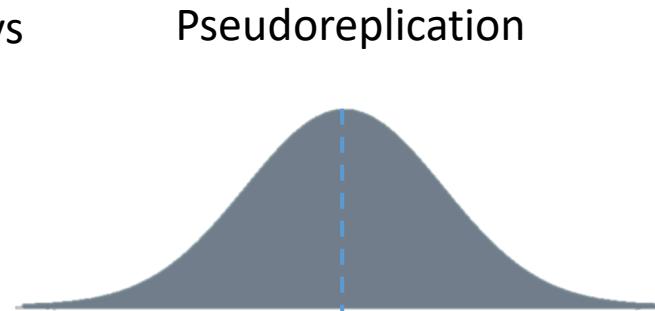
Study	p-values
1	0.67
2	0.04
3	0.24
•	•
•	•
•	•



# Why is pseudoreplication a problem?



vs



N=10

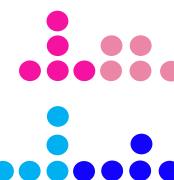


N=10

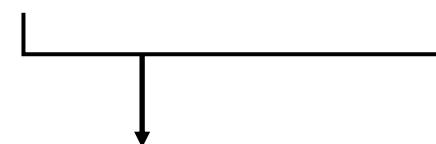


statistical test → save  
p-value

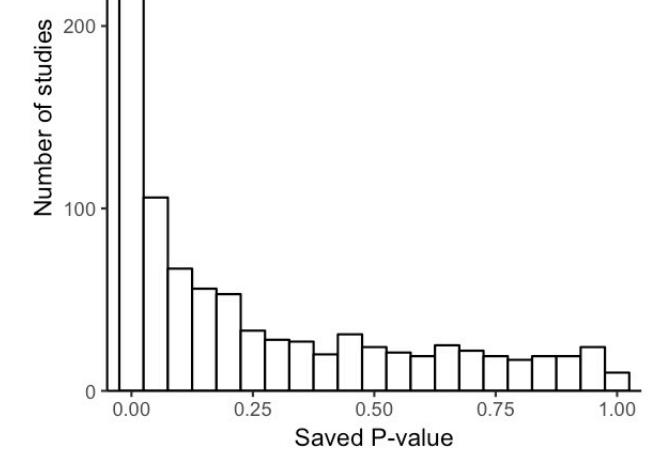
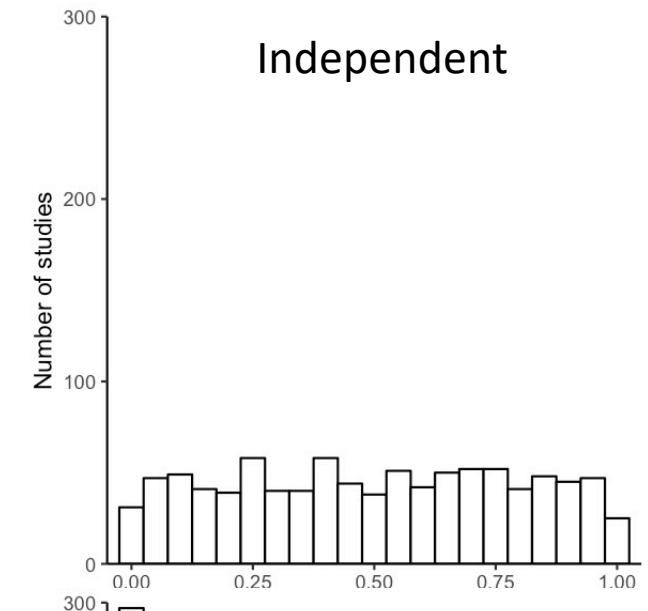
N=10



N=10



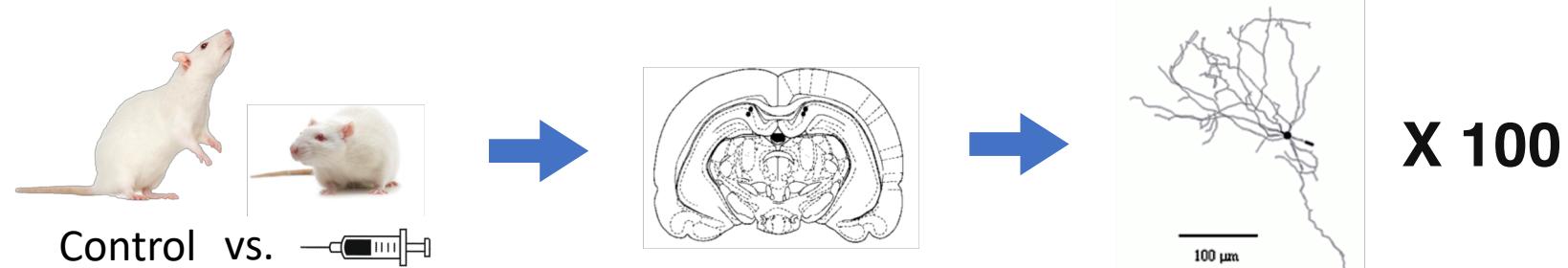
statistical test → save  
p-value



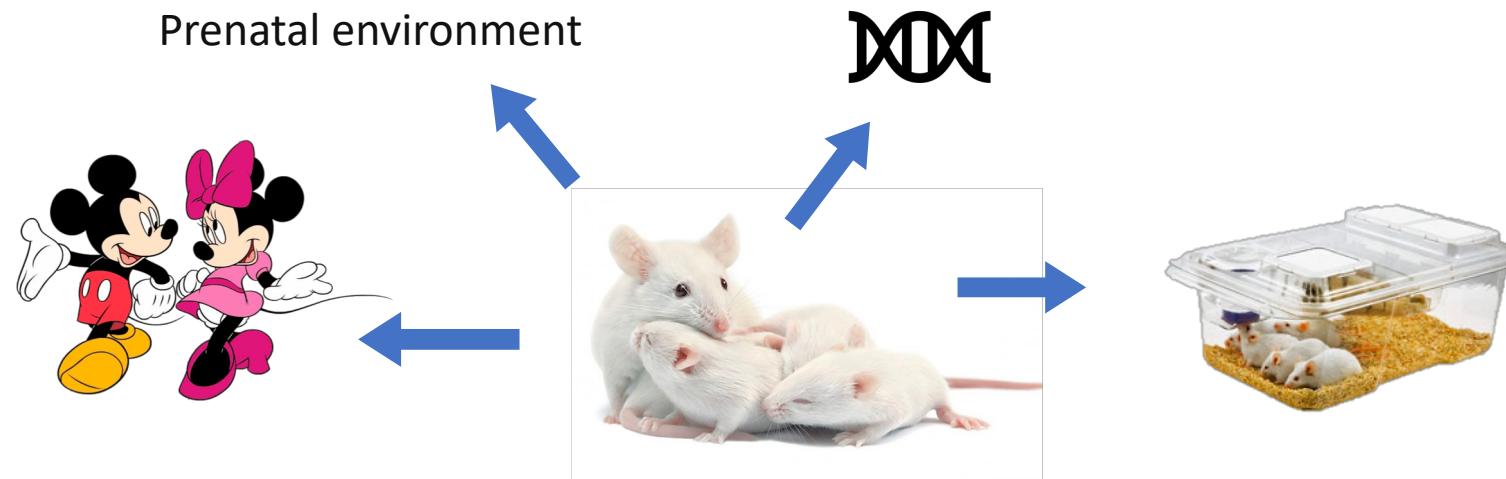
# Markdown for false-positive simulation

# Common designs with pseudoreplication

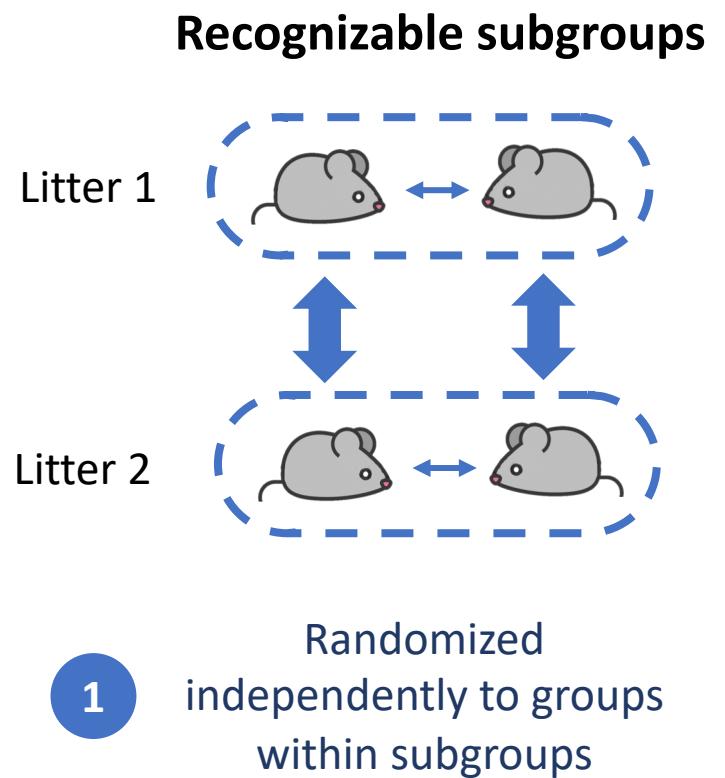
Samples from different levels of biological organization



Animals within a litter or other groupings are not independent

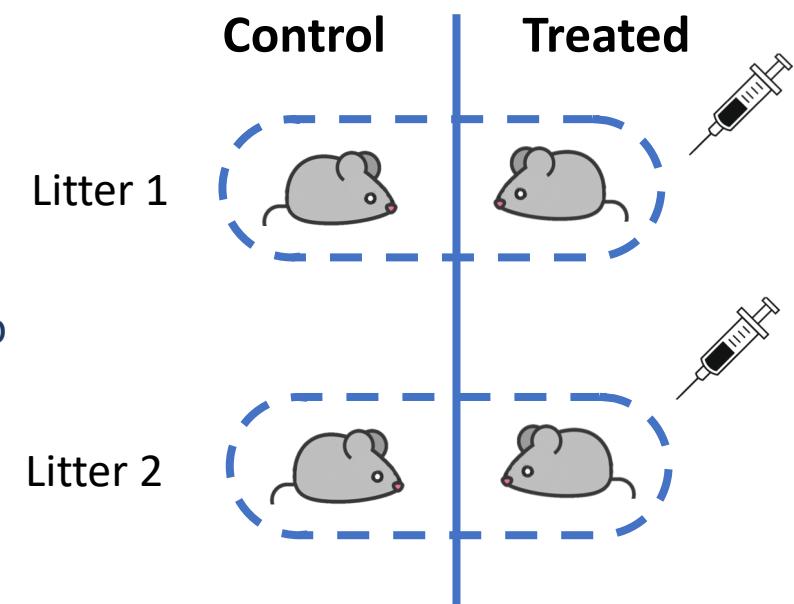


# What can you do?



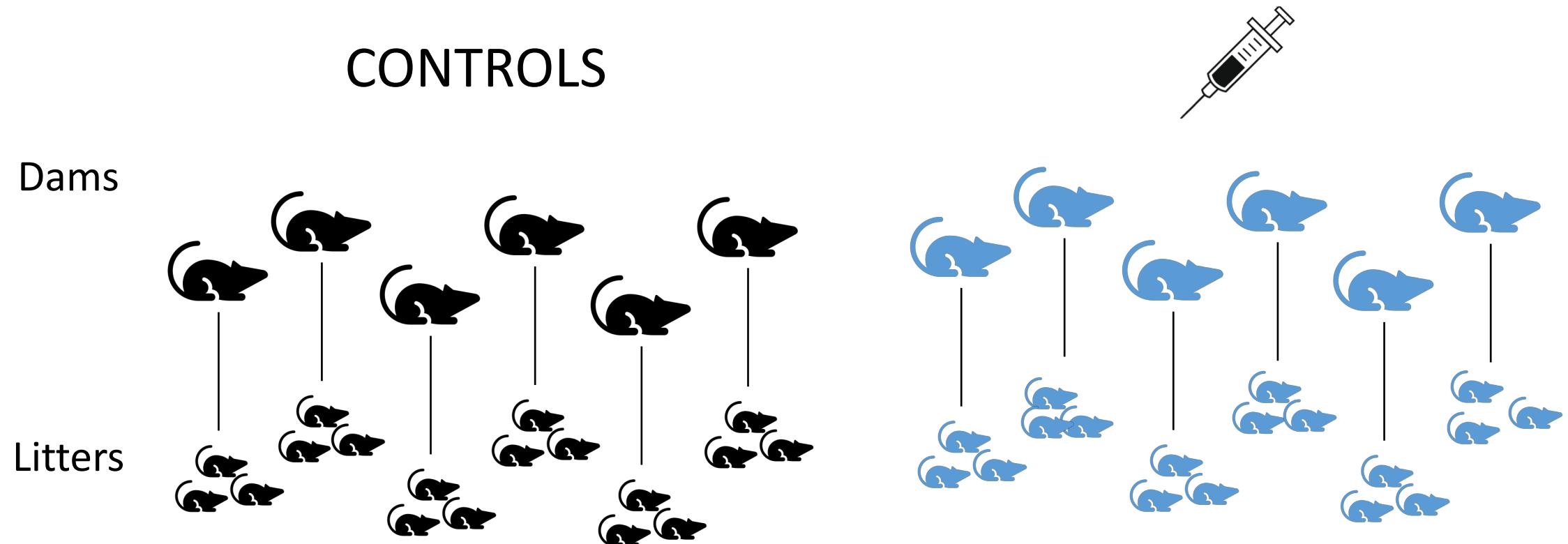
2

Treatment applied independently and no spillover



Adapted from Lazic et al. 2018

# Example: Valproic acid (VPA) model of autism



# VPA Example: Wrong vs Better way

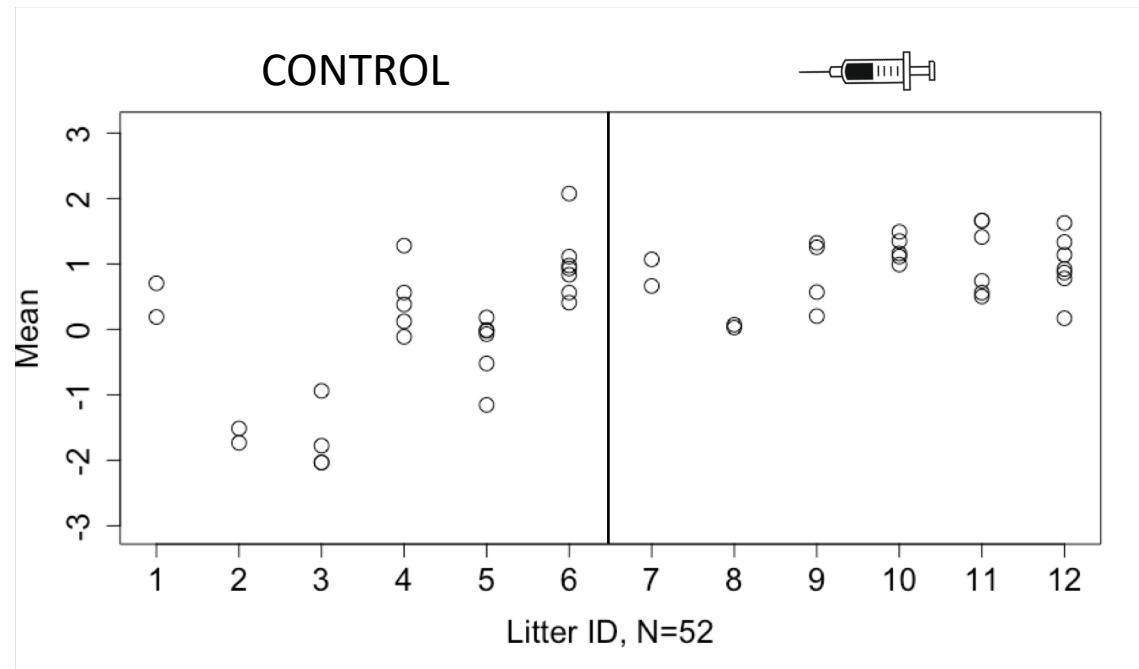


## The Wrong Way

N=52 animals total (26 vs 26)

6 litters of N= 2,2,4,5,6,7 per group

P=  $8.79 \times 10^{-5}$

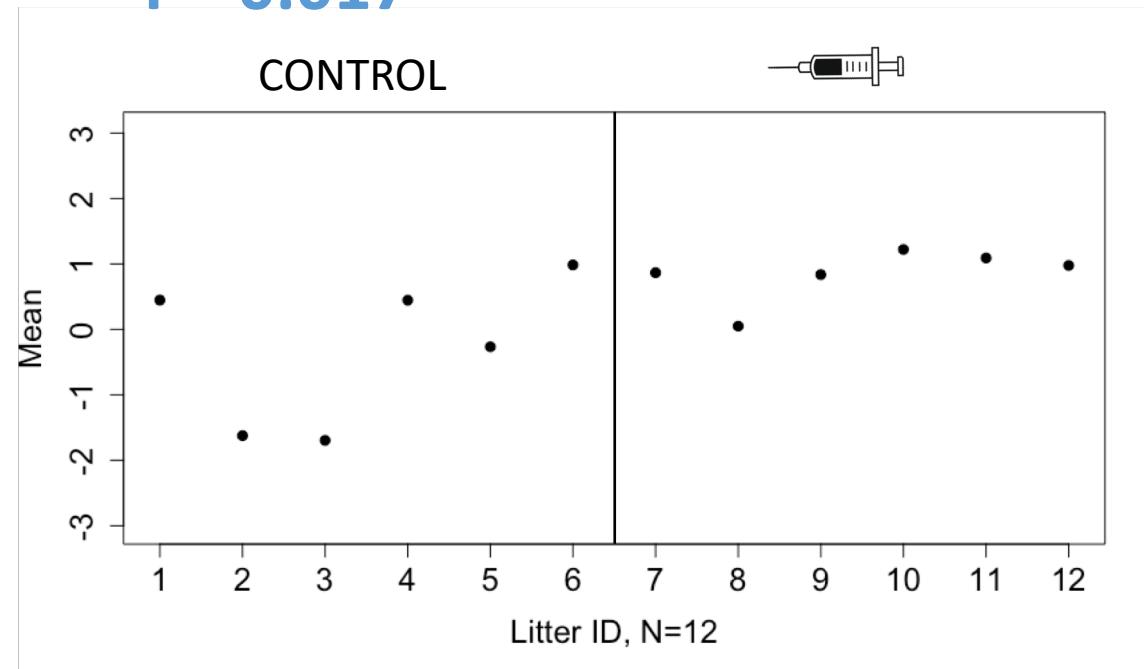


## The Better Way

N=12 animals total (6 vs 6)

Unit of analysis is the litter

P= 0.017

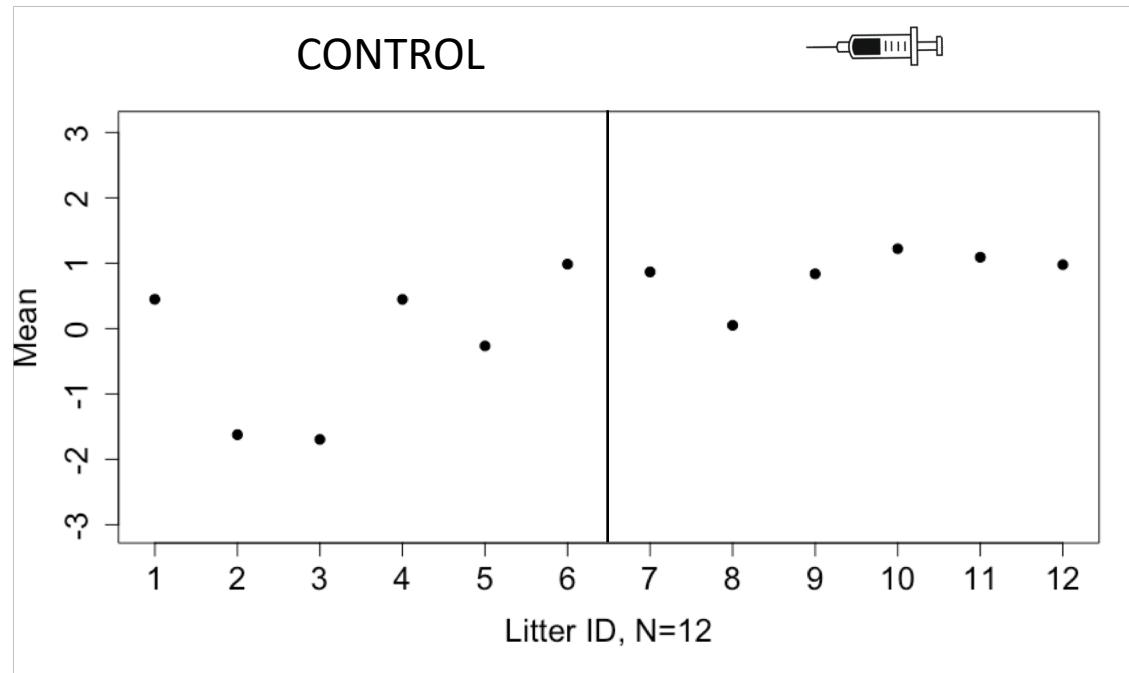


# Markdown for VPA example

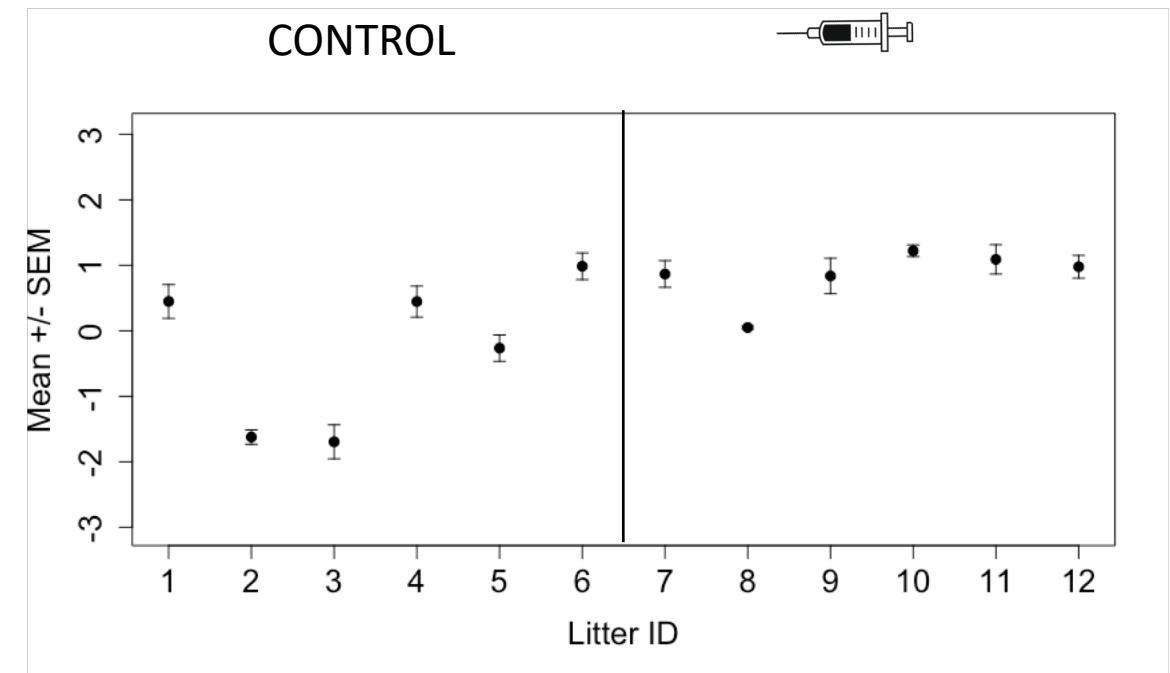
# The downside to discarding the information about litter size



Using Average per Litter



Taking into account variability in litters



# Code for plotting average values with SEM

Generating the SEM

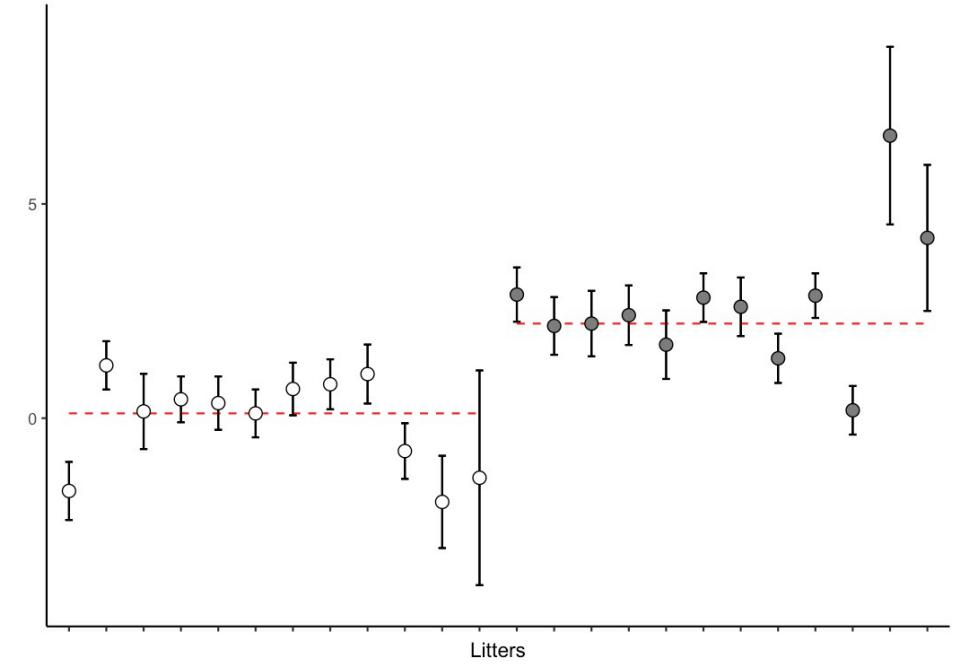
Plot with SEM as “arrows”

# Correcting with a mixed model

Flexible modeling framework

Compromise between individual  
and group level analysis

Uses group level info to shrink  
estimates toward grand mean

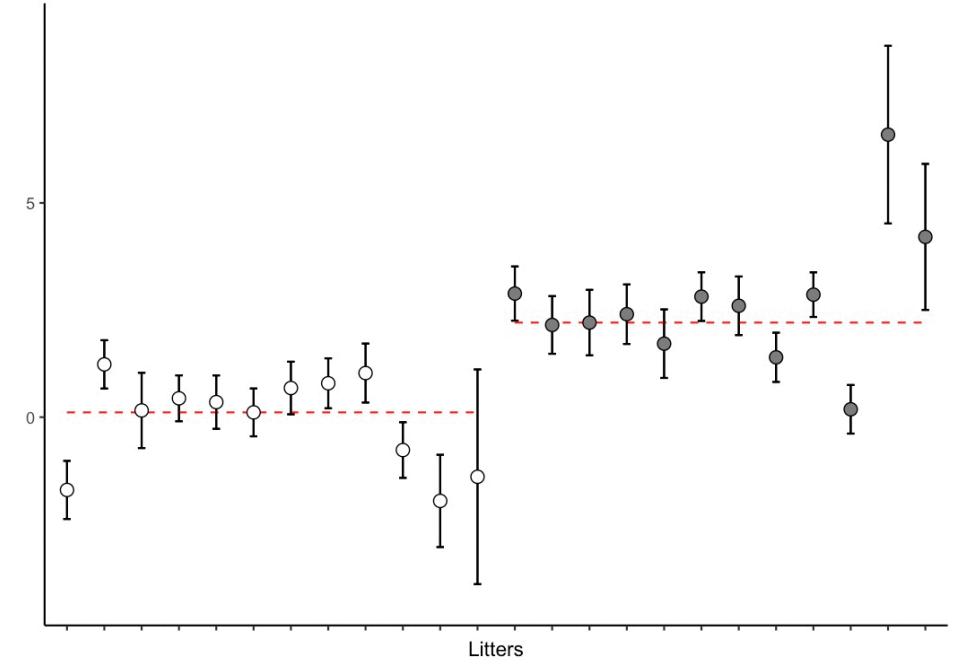


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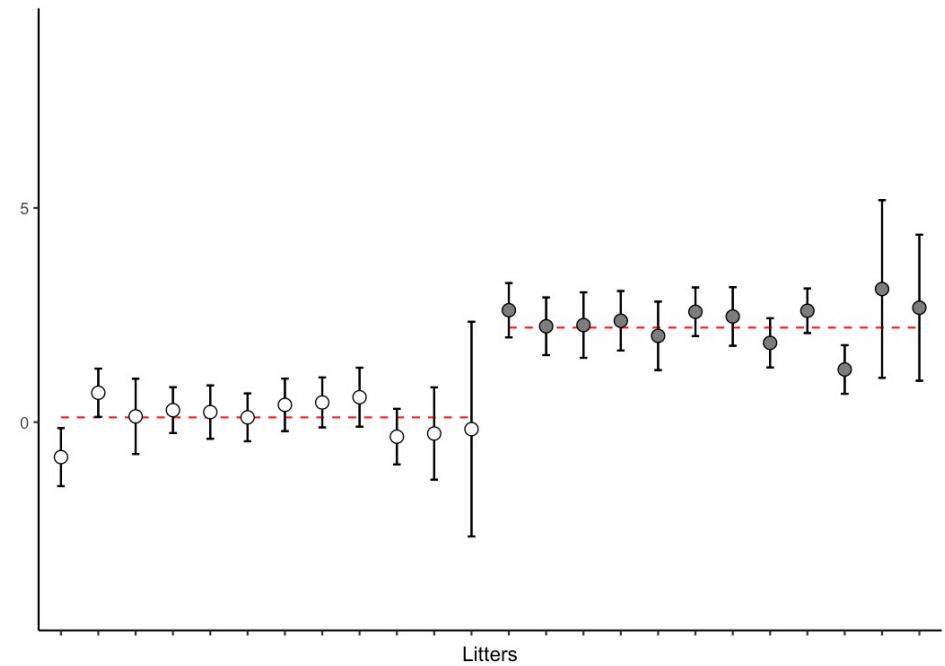


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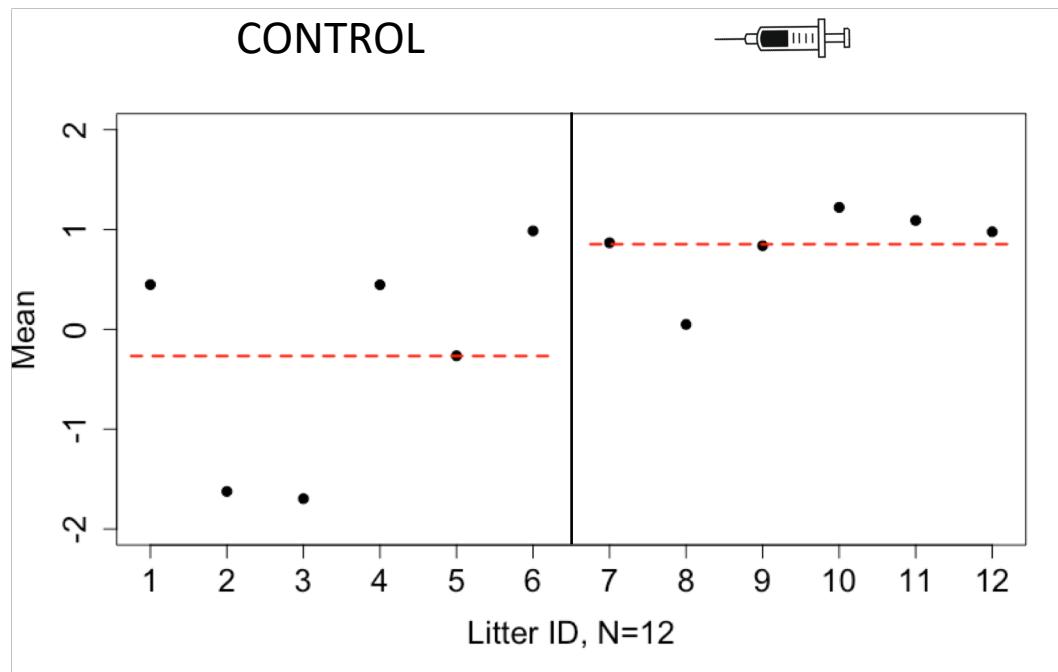
# Mixed Model: R function lme()

# Revisiting the VPA example



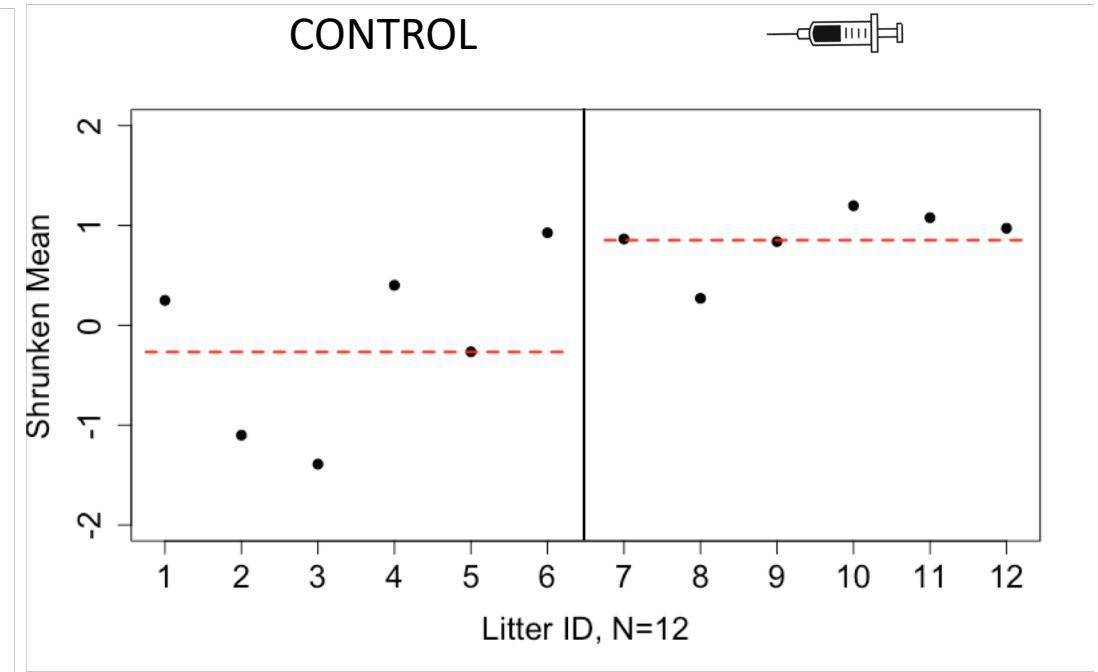
Using Average per Litter

P= 0.017

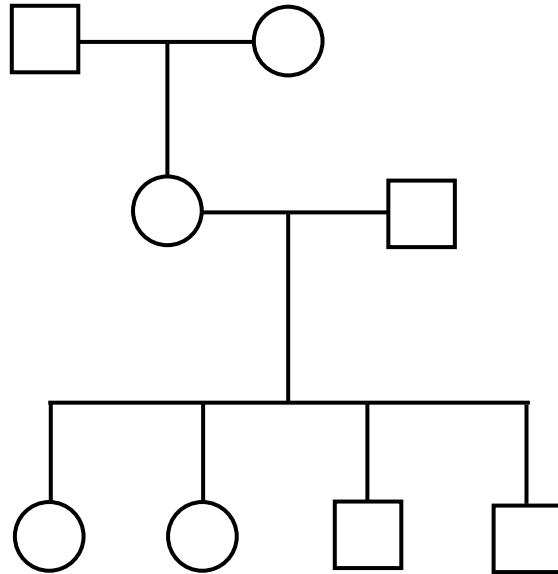


After shrinkage

P= 0.045



# Pseudoreplication and Relatedness

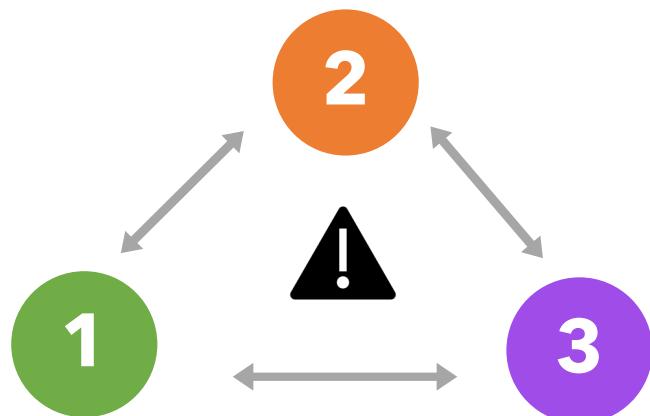


Subject	ID	Father	Mother
RYv8	RNw5	REa6	
RUy5	RQA4	RSd4	
RVd10	RUg4	RBc6	
RKu9	RZc6	RHc6	
RVw9	RZc6	RPh7	
RCq9	RZc6	RRu5	



- Can use Pedigree information as a random effect in mixed model

# CONCLUSIONS



- Ignoring pseudoreplication leads to increased likelihood of false positives
- Correlated observations must be accounted for
  - Randomize
  - Look at your data, calculate the ICC
  - Take the average
  - Use a mixed model

$$s^2 = \frac{\sum (X - \bar{X})^2}{N-1}$$

Group's mean

Grand mean

$$s^2 = \frac{\sum (X - \bar{X})^2}{N-1}$$

Individual Observation

Group mean (M or F)