Improving basic and translational science by accounting for litter-to-litter variation in animal models

(What is 'N')

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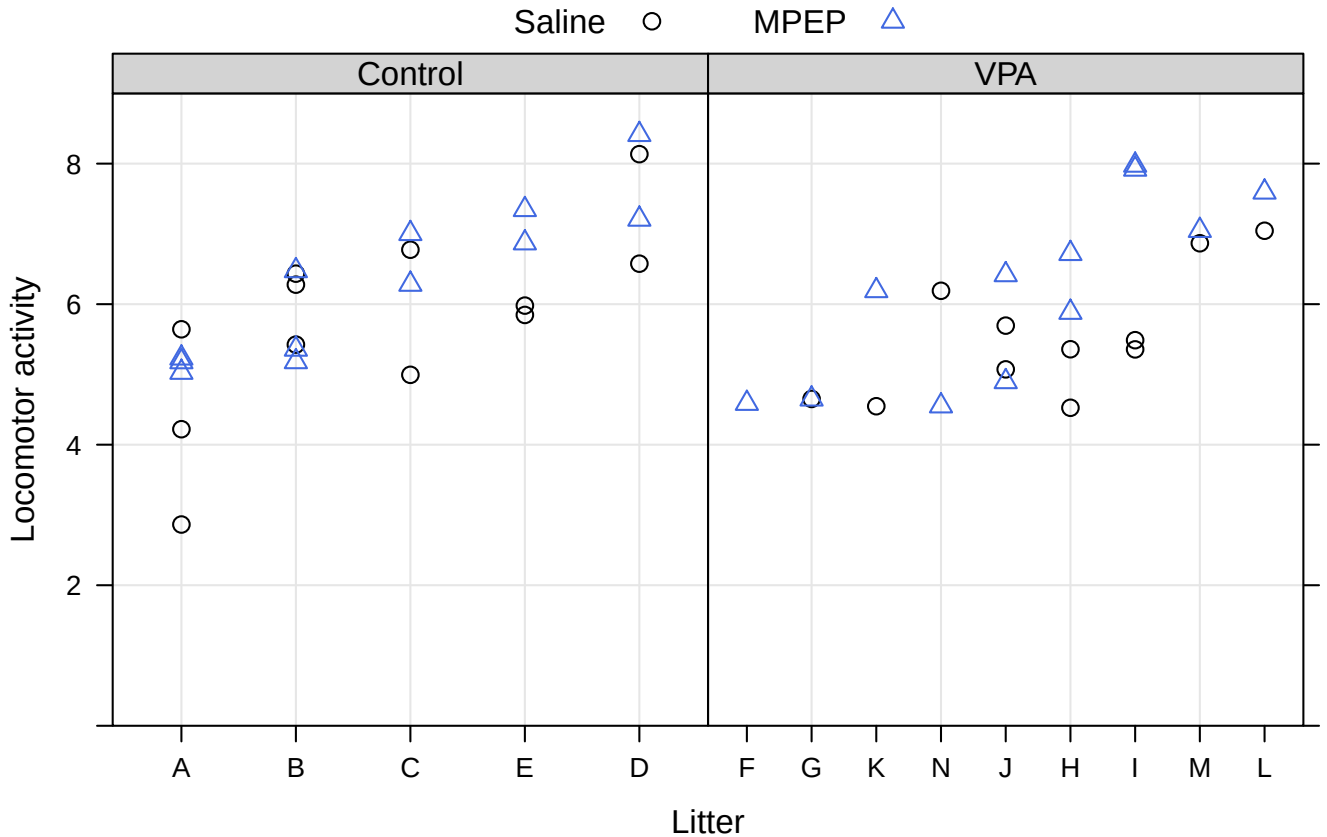
Litter effects

Definition: Variation between litters such that animals within a litter are more alike than animals between litters on an outcome variable.



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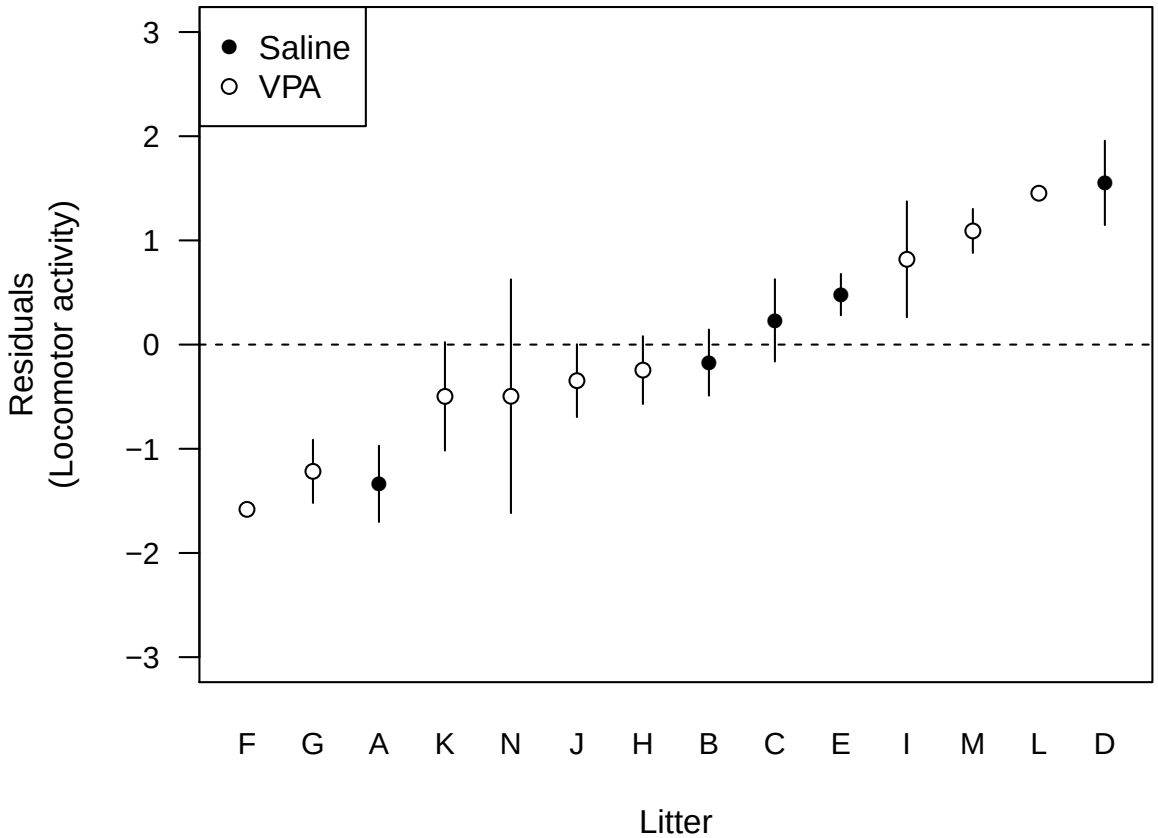

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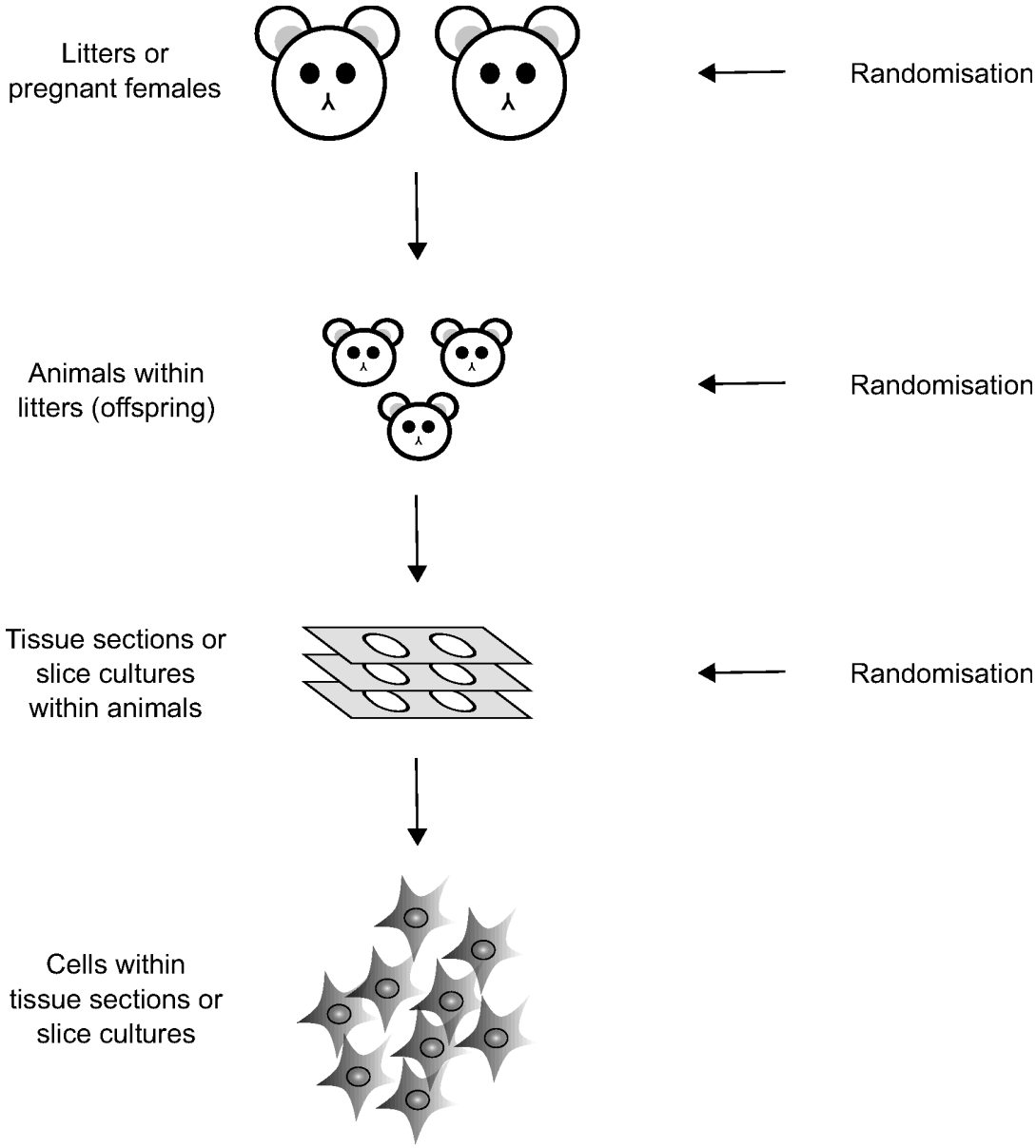
Biological and technical hierarchies



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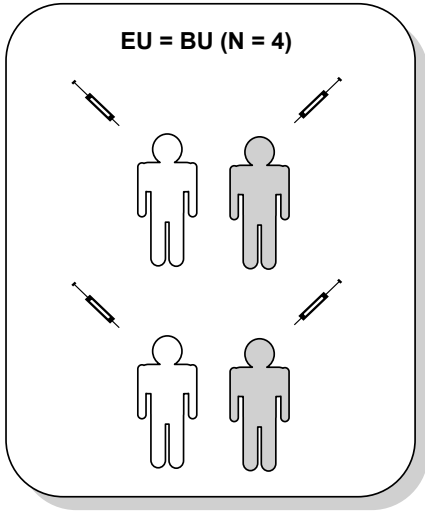
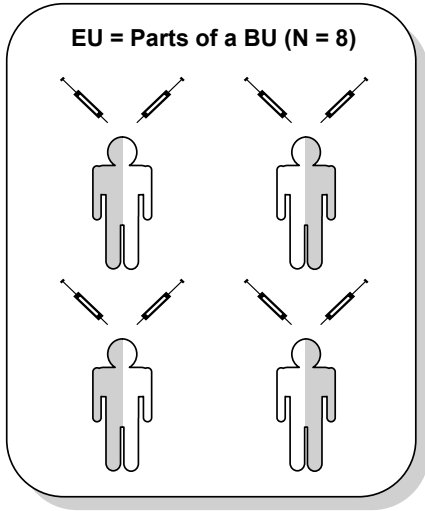
3) **Observational unit (OU):** the entity on which measurements are taken; they may differ from the experimental and biological units of interest. Increasing the number of OUs does not increase the sample size.

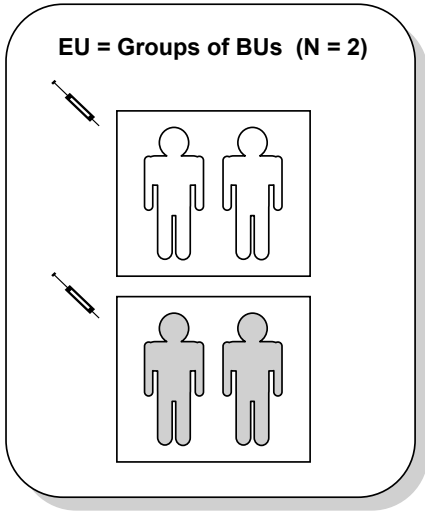
Experimental unit

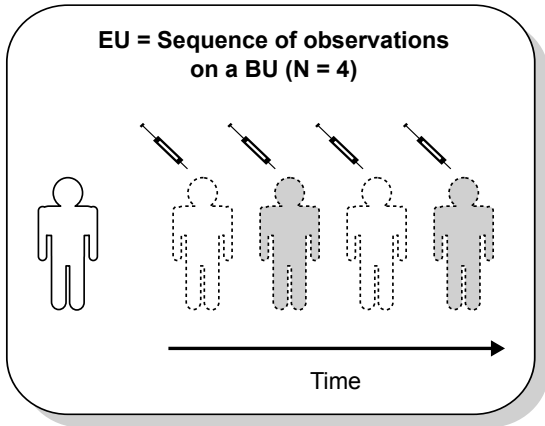
An EU may correspond to:

- a biological unit of interest
- groups of biological units
- parts of a biological unit
- a sequence of observations on a biological unit

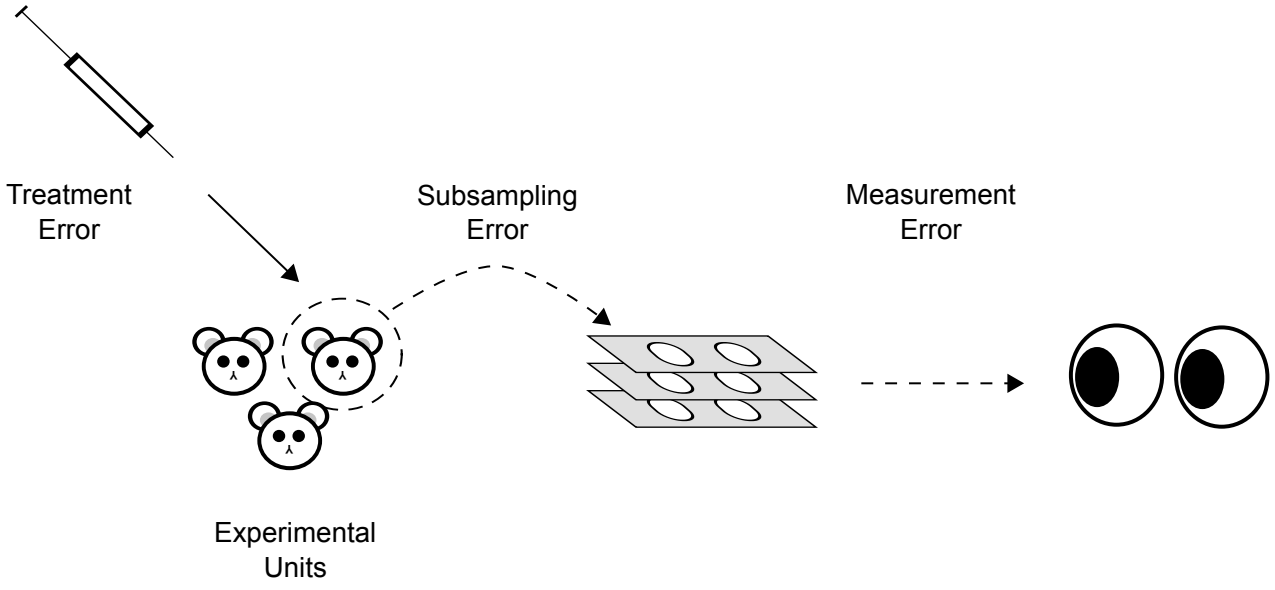








Sources of error (variation, not mistakes!)



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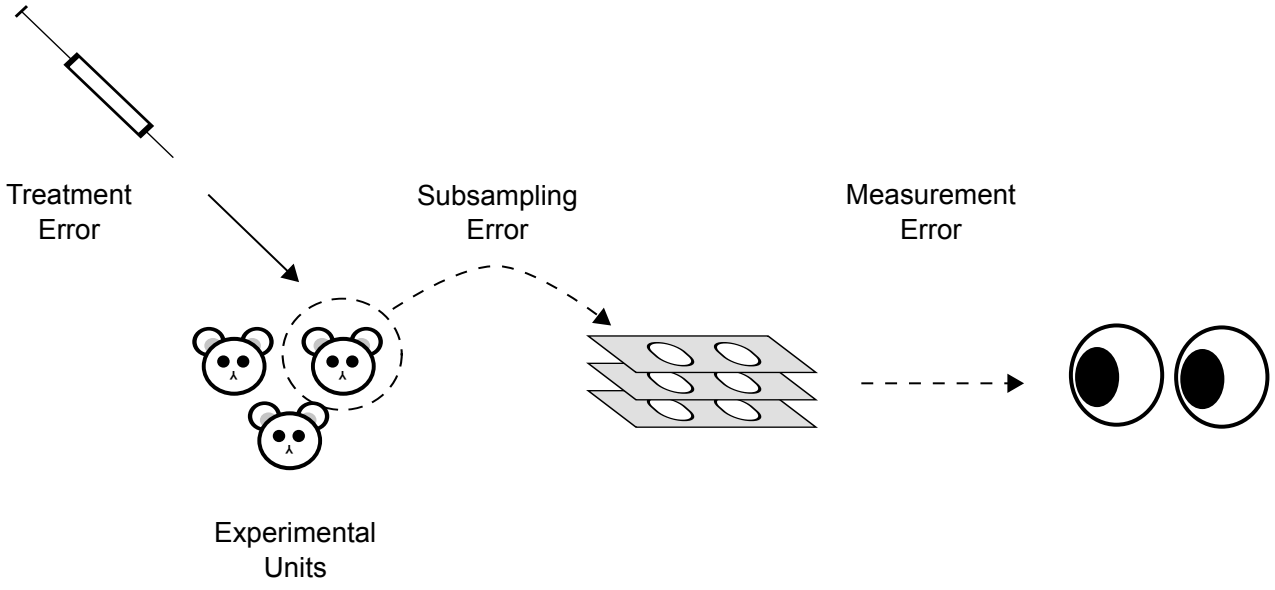

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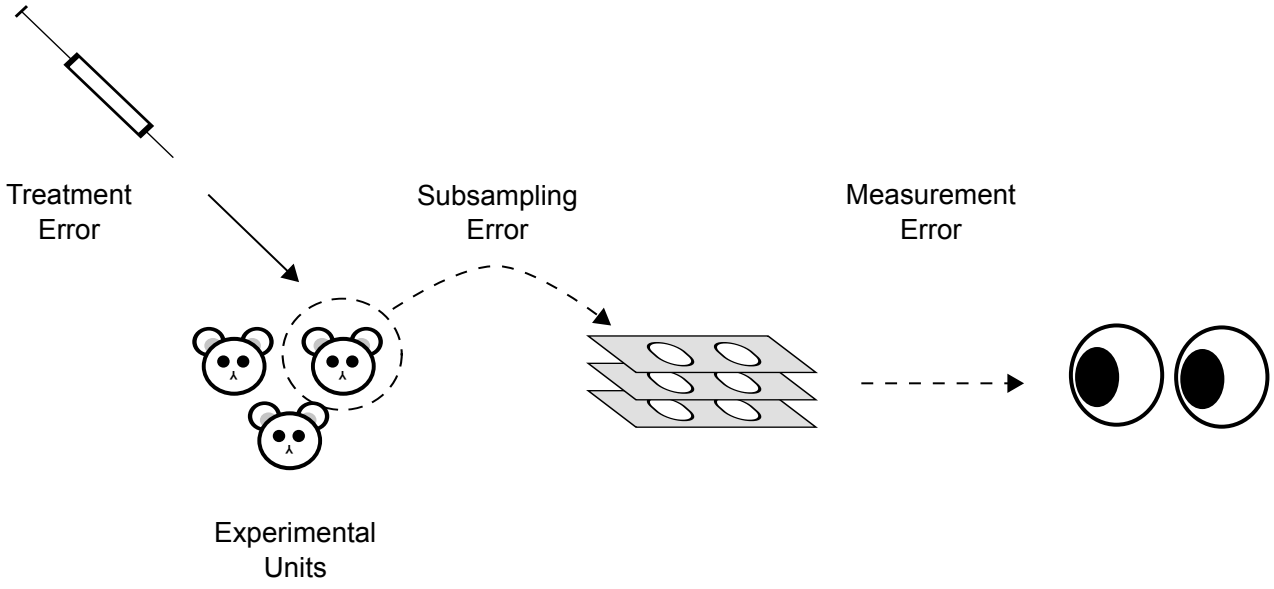

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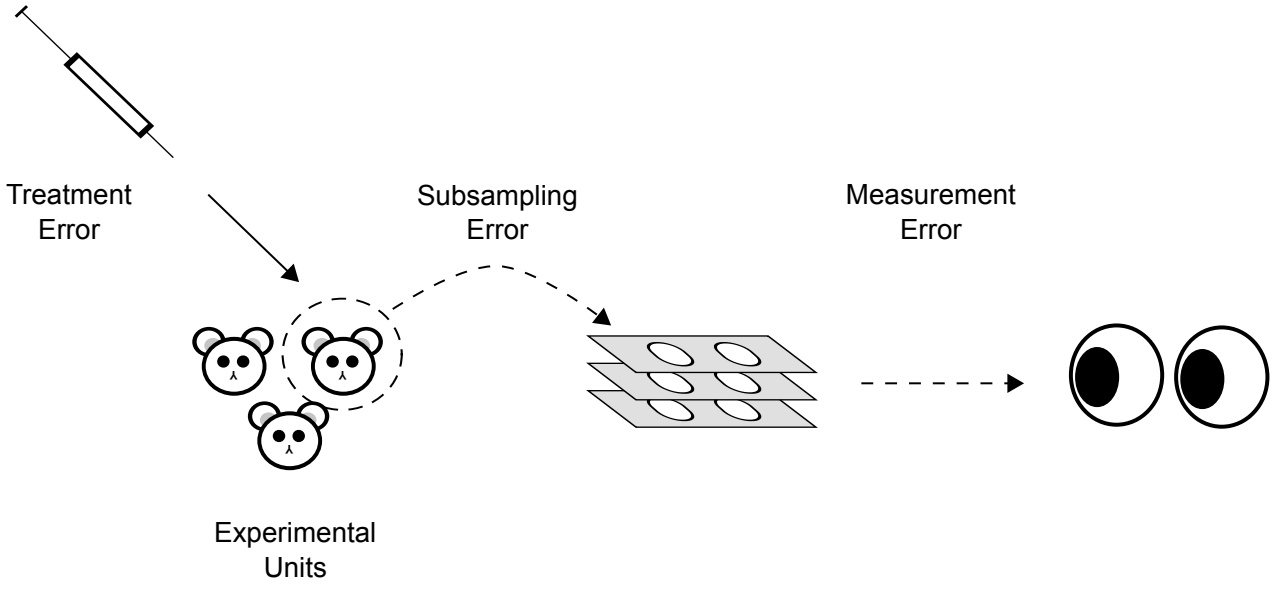

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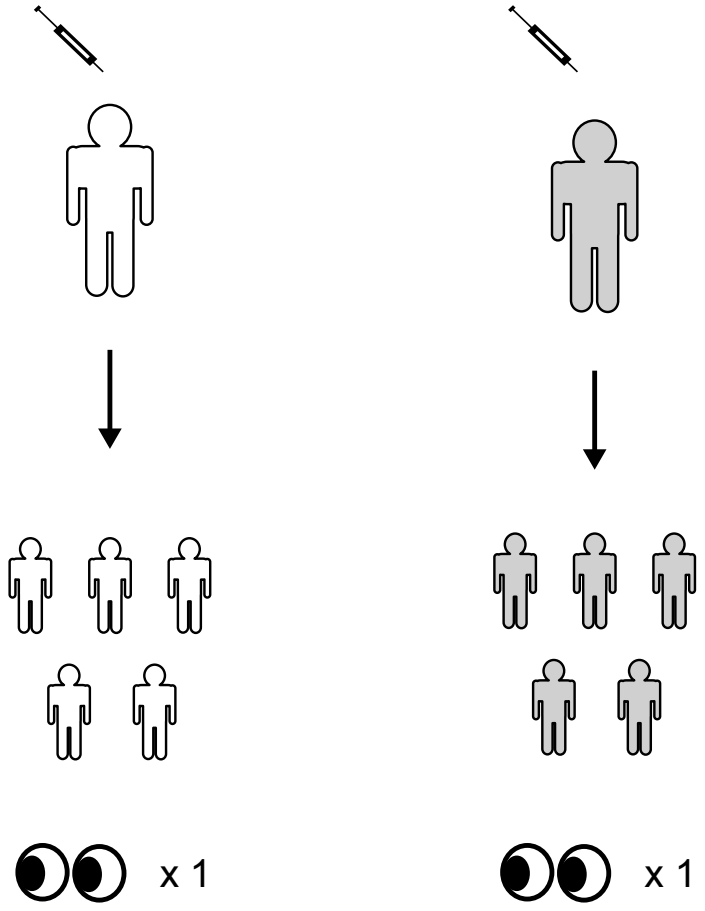
- **(Sub)sampling error:** Samples differ from the whole population.
- **Measurement error:** Measurements are never perfect.
- **Experimental error:** Reflects the natural biological variation from EU to EU (plus treatment error). This is the appropriate error for testing hypotheses.

Design 1: No replication!

- Treatment applied to 2 pregnant females (EUs).
- Observations taken on 10 offspring (BU and OU).



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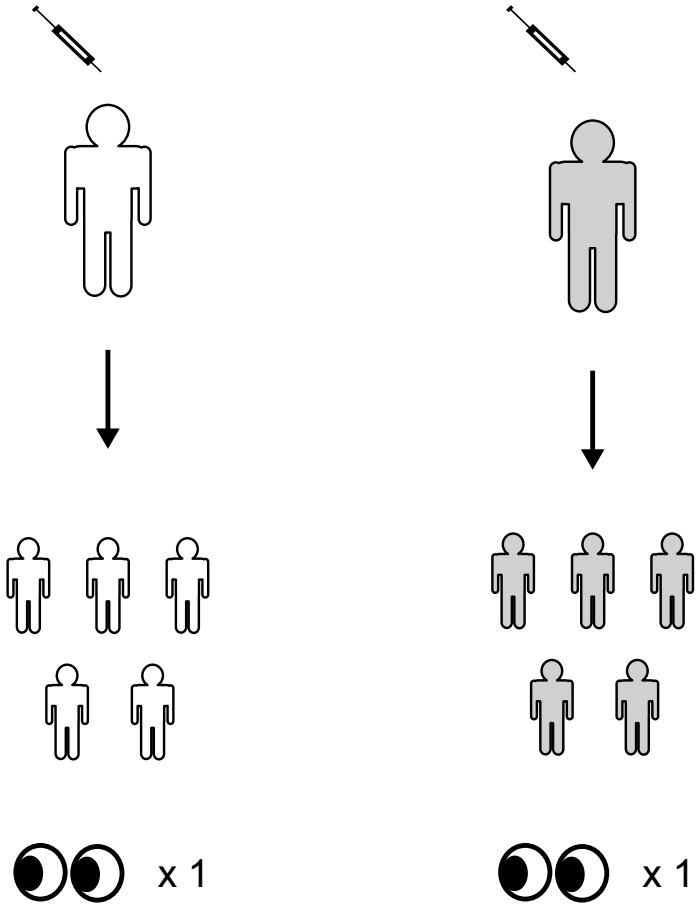
- No valid inference can be made $\rightarrow$ cannot separate treatment effects from differences between litters.
- Need to increase number of females, not number of offspring.



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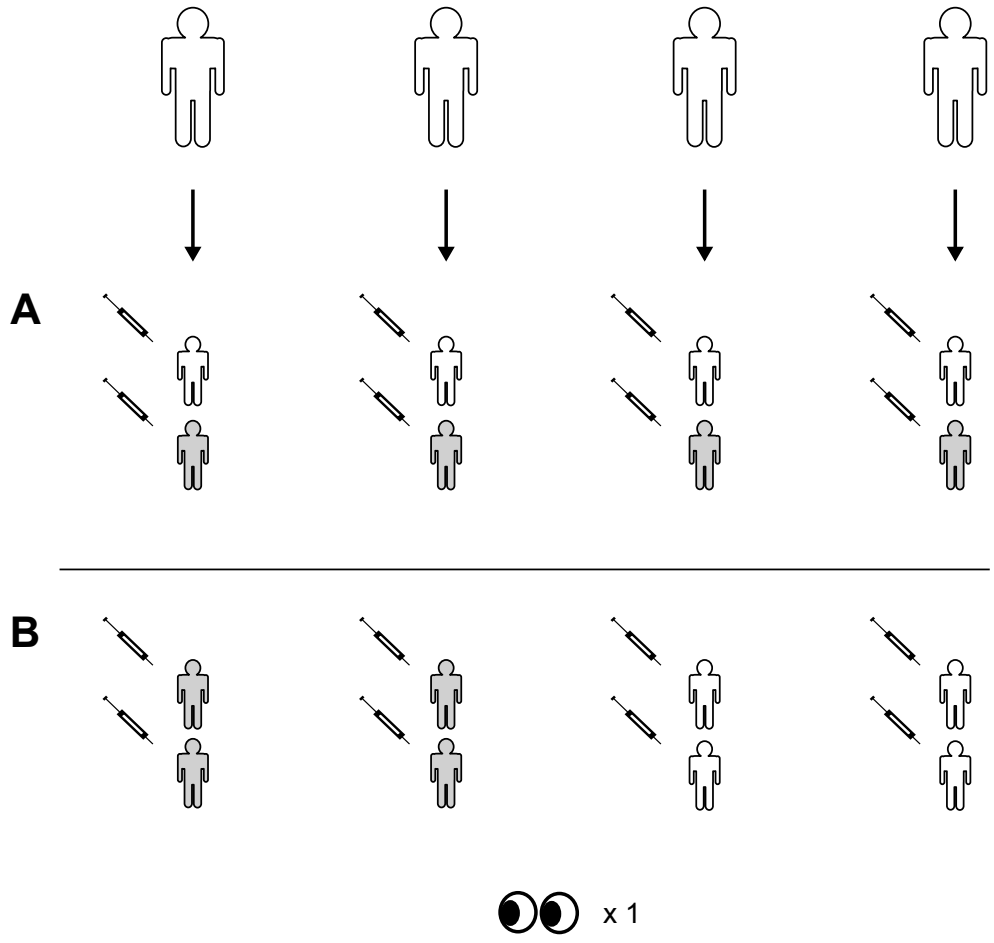

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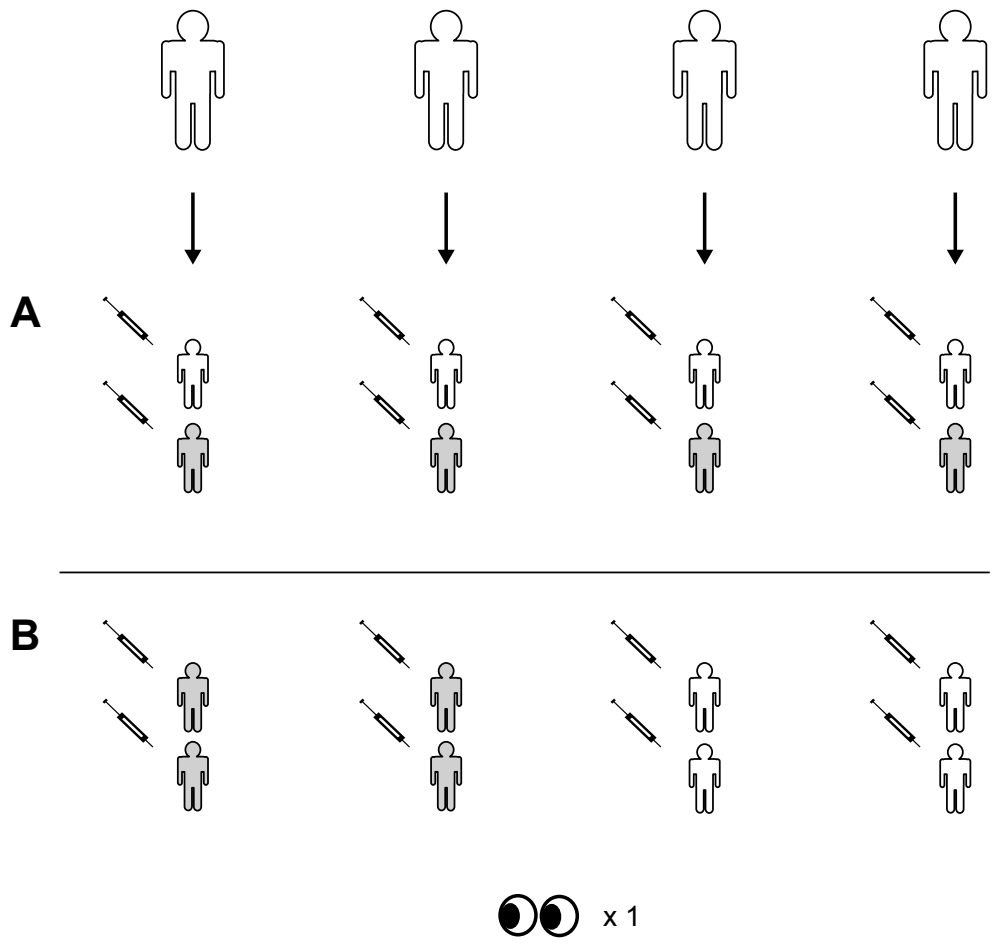

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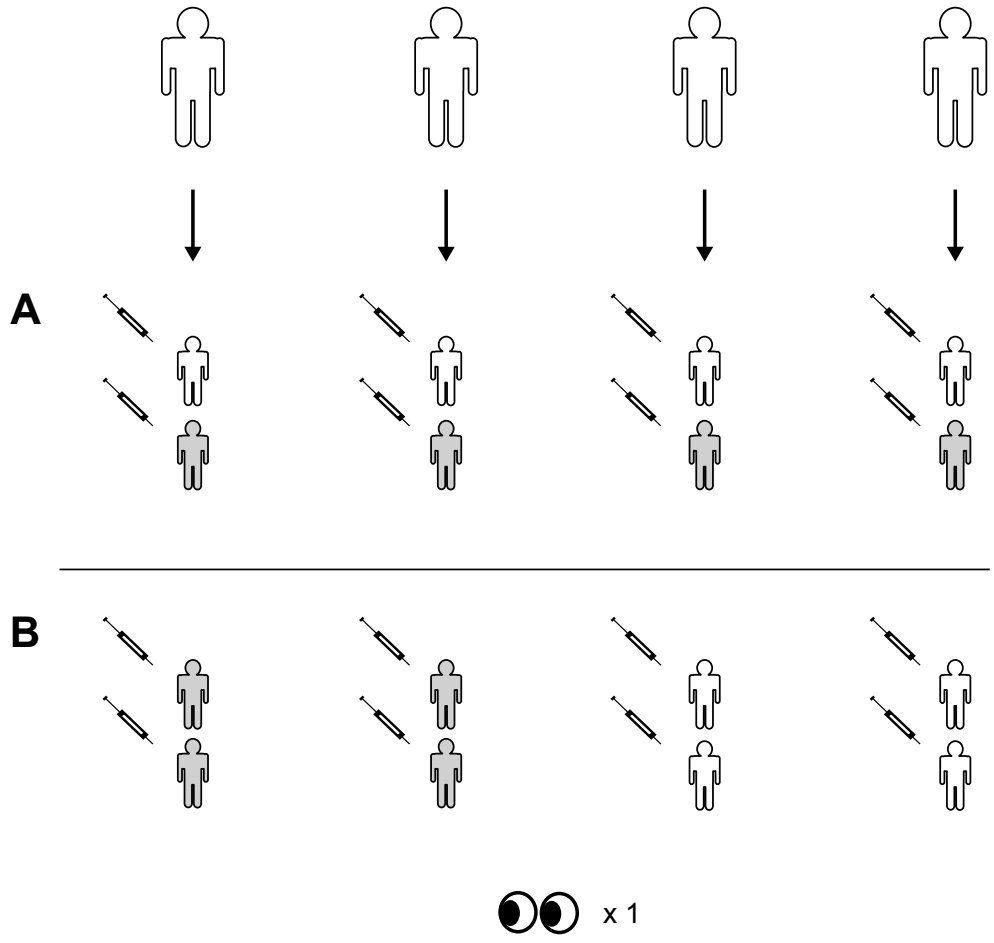

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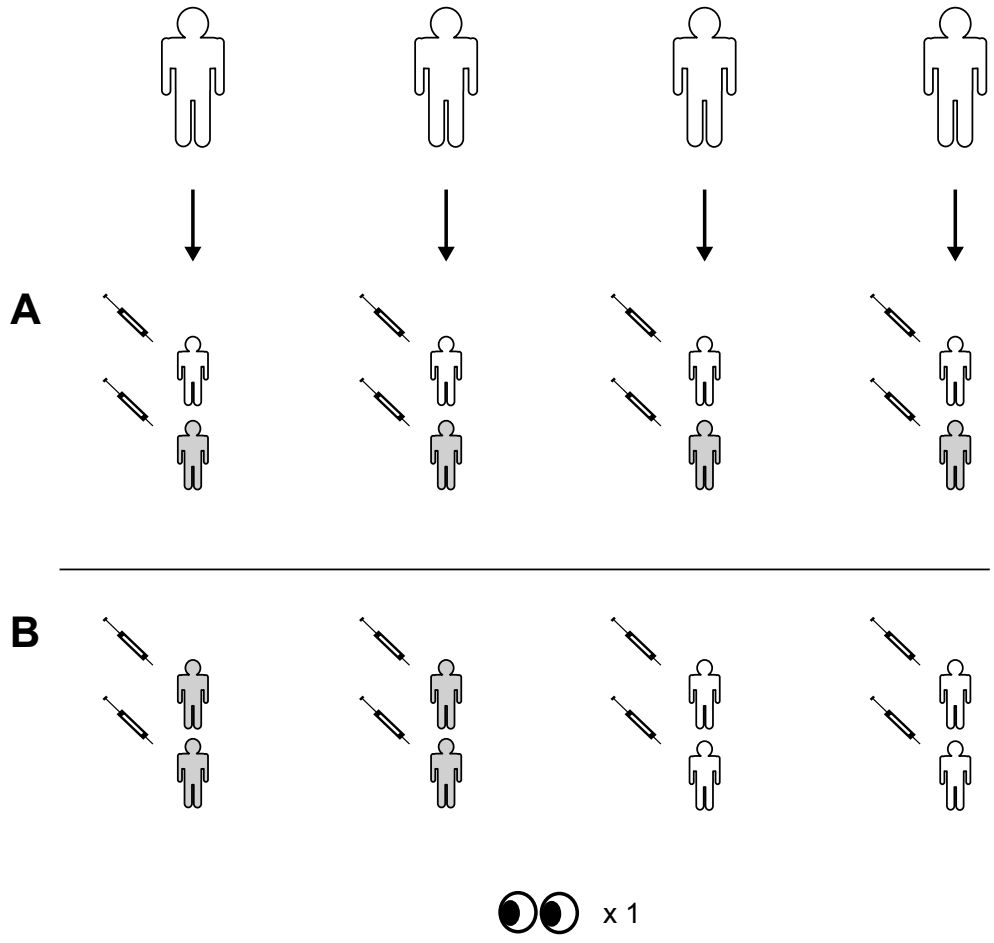

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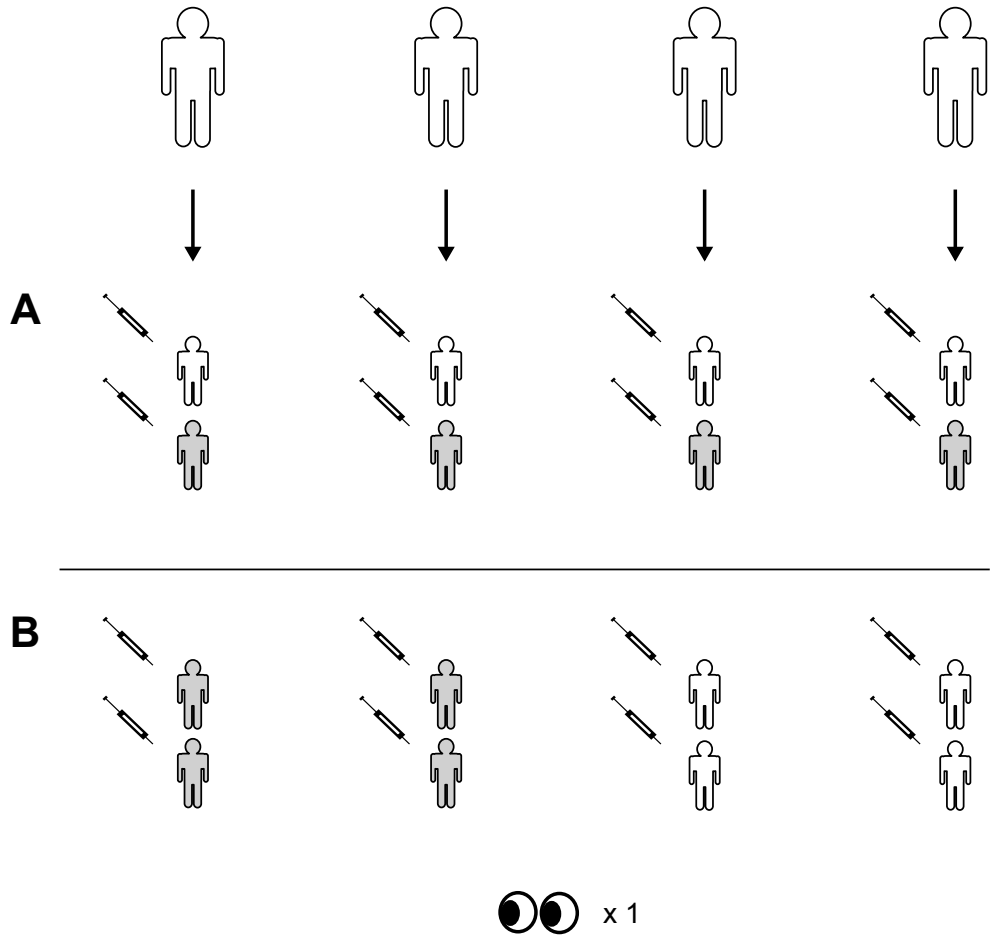
- Completely randomised design is worst (not shown).



Design 3: Split unit design (more than one type of EU)

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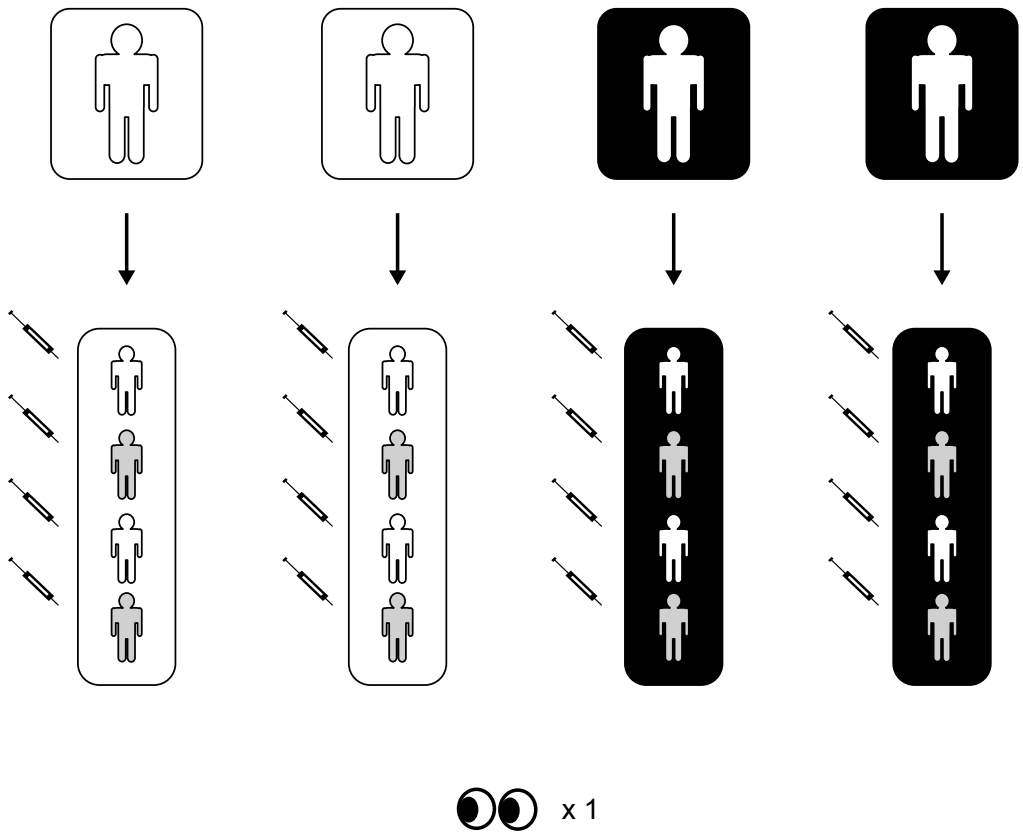

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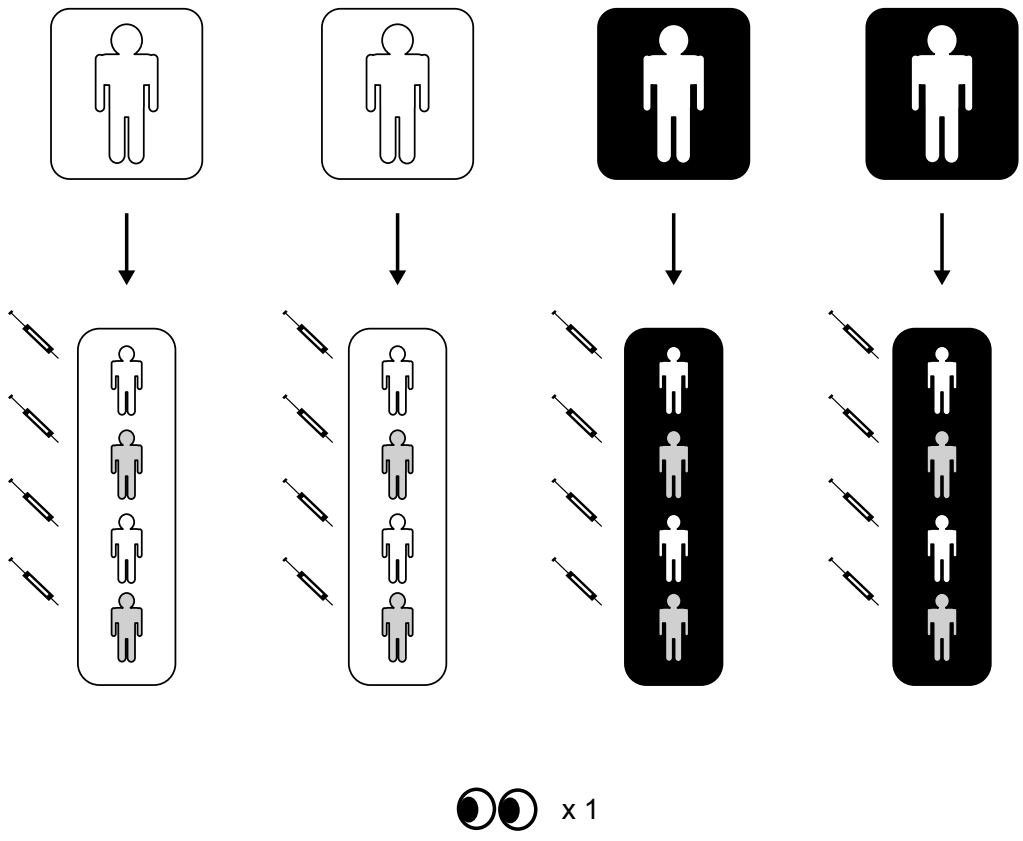

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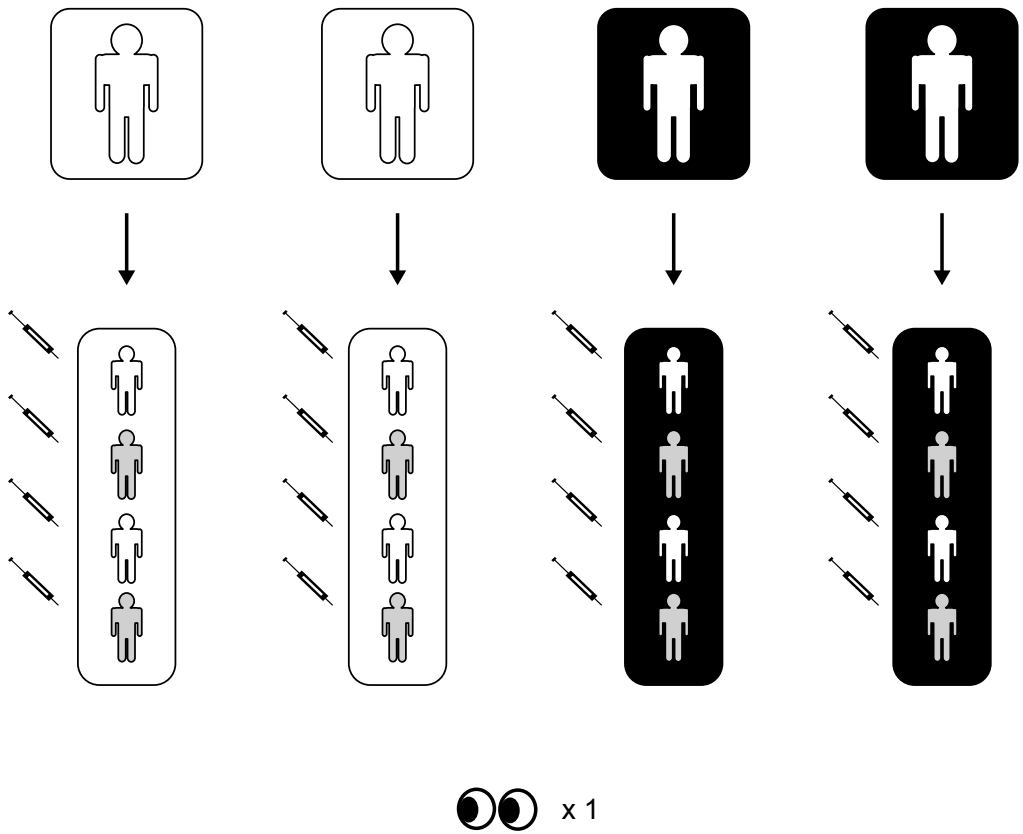
- 16 Offspring randomised to groups within litters.
- The offspring are the BUs and OUs, but
- 4 EUs for environment effect and 16 EUs for treatment effect.



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- Multiple OUs per EU must be accounted for in the analysis.
- When reporting results, make it clear what the EU is, the number of EUs, if the OU was different, and how multiple measurements or subsamples were handled.
- Cross treatment groups and litters (avoid nesting).

Further information

1) **Lazic SE** (2016). *Experimental Design for Laboratory Biologists: Maximising Information and Improving Reproducibility*. Cambridge University Press.
2) **Lazic SE** (2013). Technical comment on "Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice." *Science* 340(6134):811.
3) **Lazic SE**, Essioux L (2013). Improving basic and translational science by accounting for litter-to-litter variation in animal models. *BMC Neuroscience* 14(1):37.
4) **Lazic SE** (2010). The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis? *BMC Neuroscience* 11:5.