# Applying the Scientific Method in Animal Research

Pat Lonergan

UCD School of Agriculture and Food Science

UCD DUBLIN

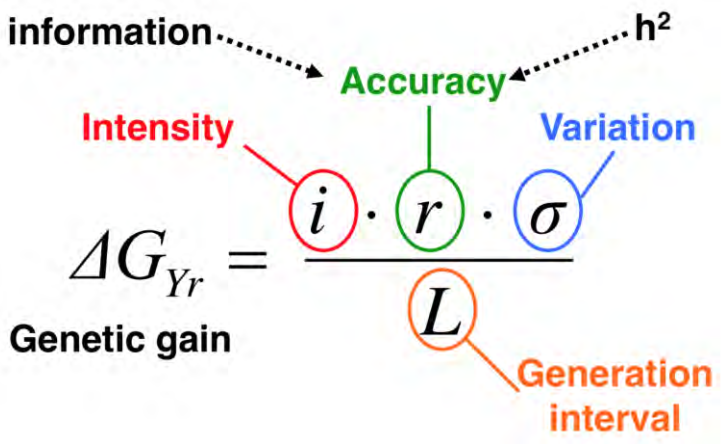
PDST Professional Development Service for Teachers | An tSeirbhís um Fhorbairt Ghairmiúil do Mhúinteoirí

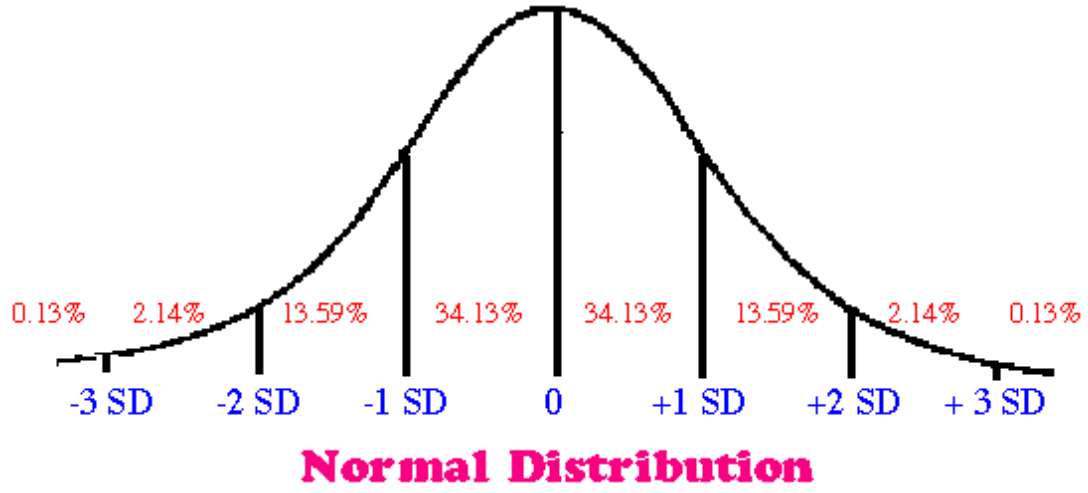

NATIONAL GEOGRAPHIC
The New Europe
China's Unknown Gobi
World of Islam
EVOLUTION OF DOGS
WOLF TO WOOF


Science
AAAS

# Genetic improvement

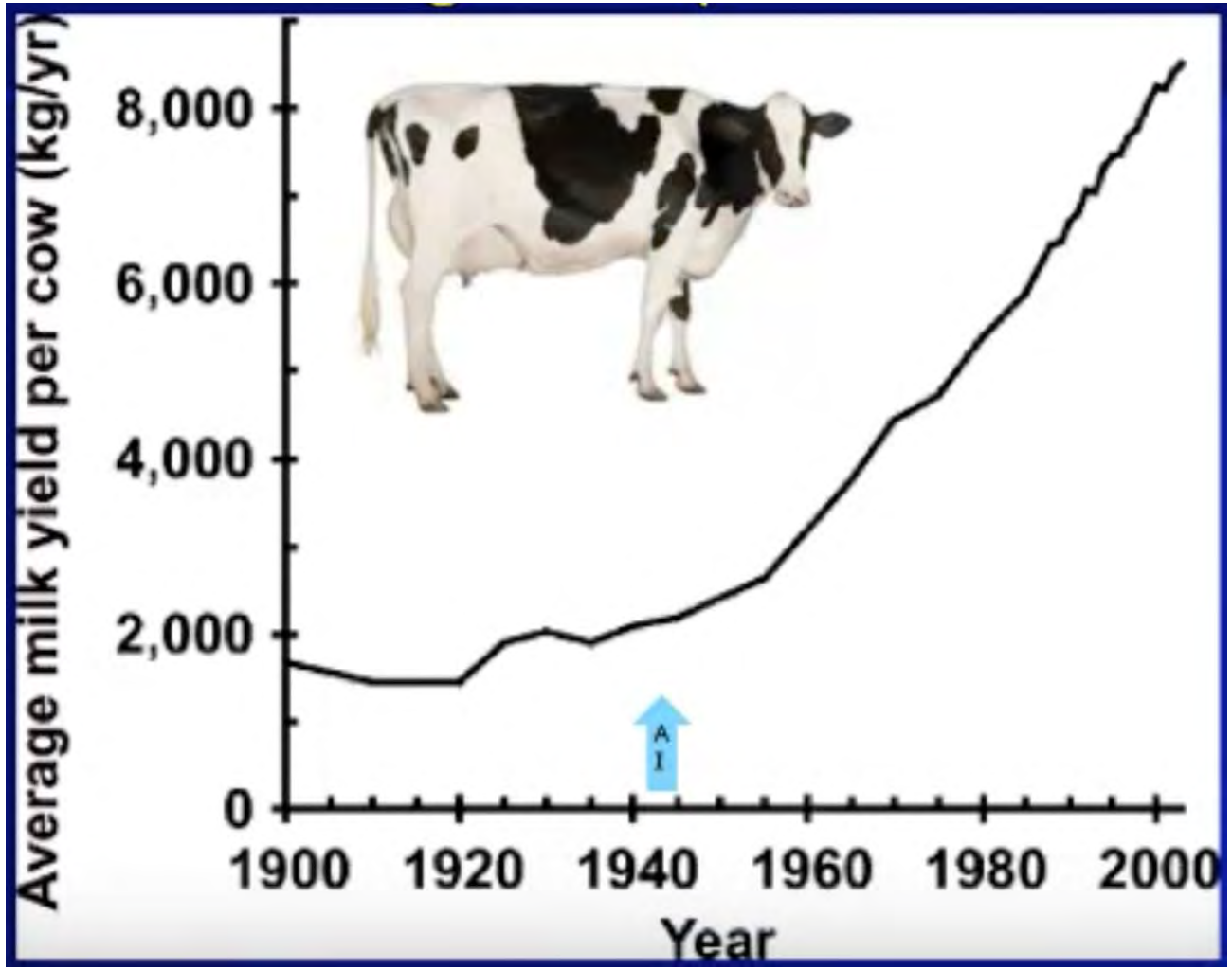
- Permanent and cumulative, cost effective and sustainable
- Rate of genetic improvement depends on
  - Selection intensity
  - Accuracy
  - Amount of genetic variation
  - Generation interval



1944: 25.6 million animals. Total annual milk production 53.1 billion kg
1997: 9.2 million animals. Total annual milk production 84.2 billion kg

1.6 times the amount of milk from one-third the number of cows



# Outline

- The ‘Scientific Method’
- What is an experiment?
- Understanding variation
- Data presentation
- Regulations governing animal research
- Some examples

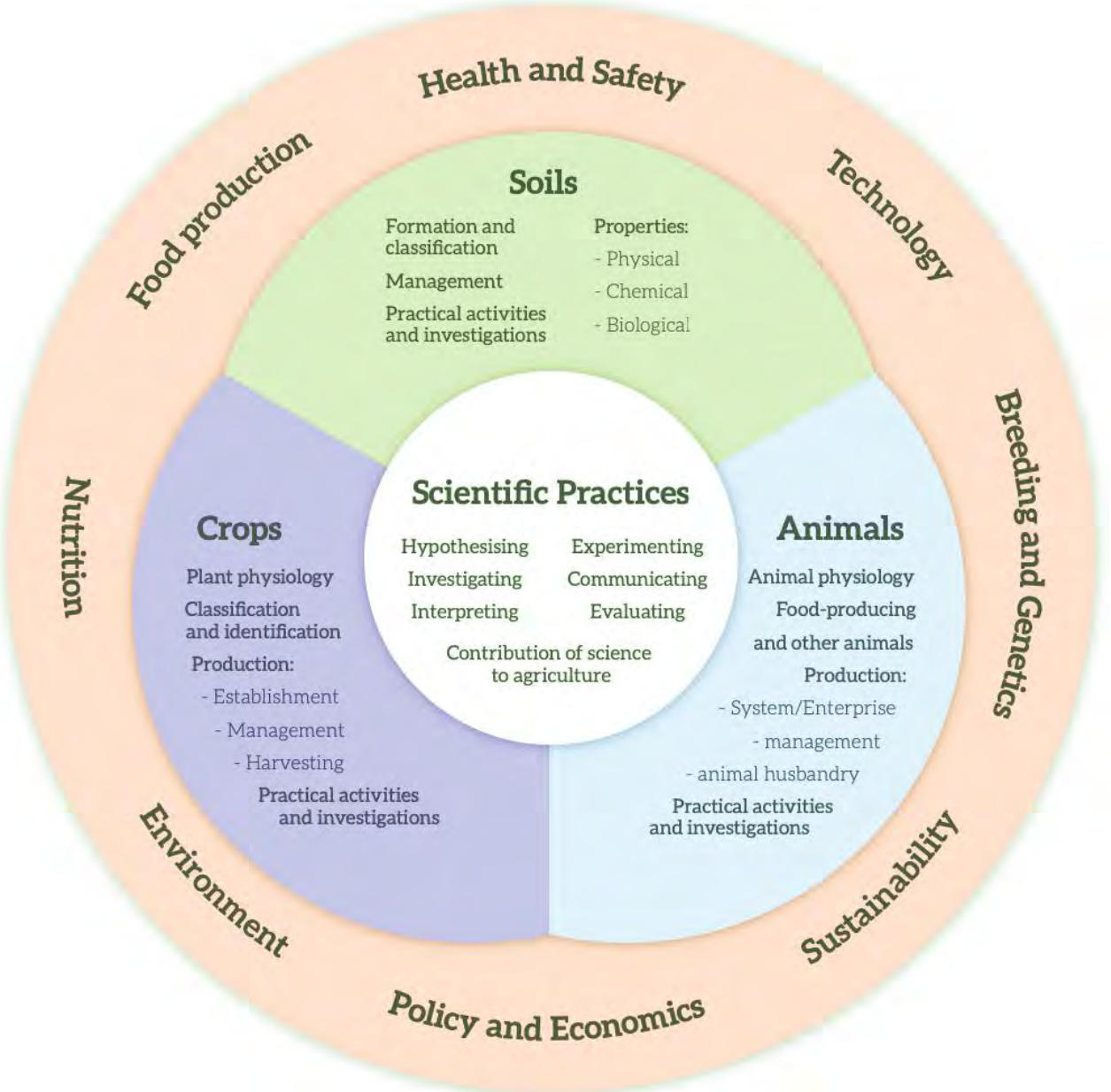
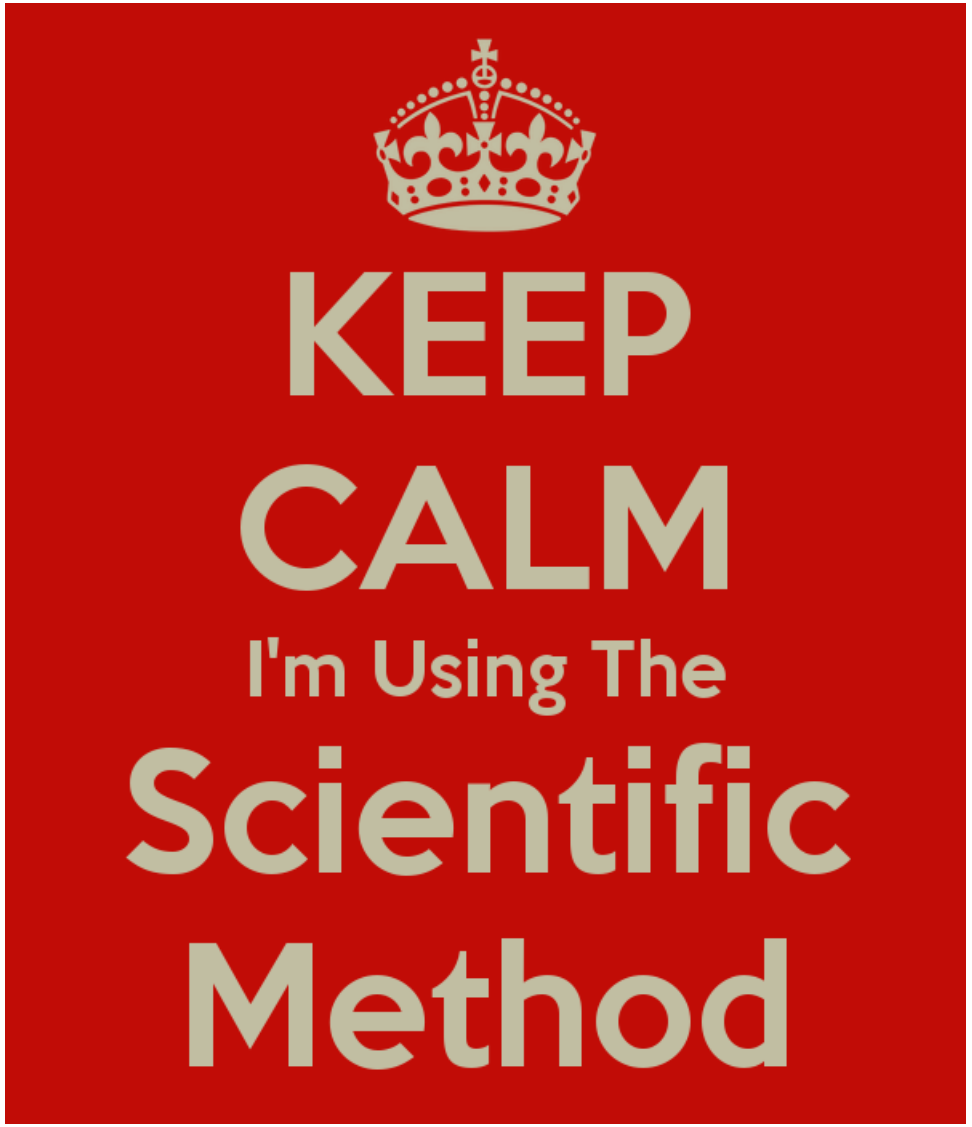



*Figure 4: Structure of the specification*




# The Scientific Method

## Purpose
What question are we trying to answer or discover?

## Hypothesis
What is an educated guess of the answer to our question?

## Materials
What supplies or equipment will we need for our experiment?

## Procedure
How are we going to conduct our experiment, step-by-step?

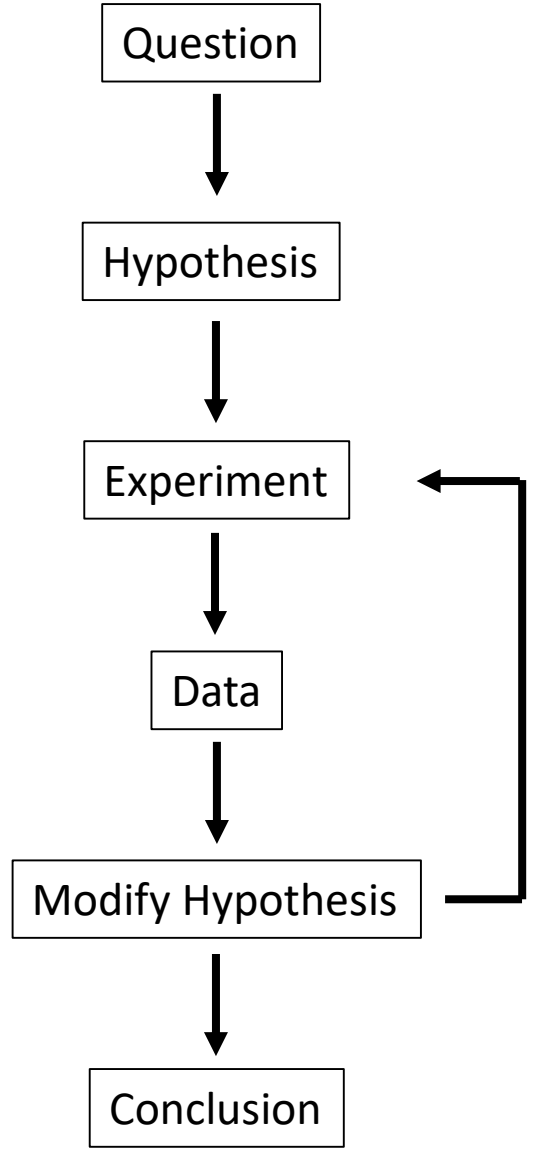
## Results
What happened in our experiment?

## Conclusion
Was our hypothesis correct?

# 5 phases of research

- Conceptualisation
- Planning
- Execution
- Analysis
- Reporting

- Each vital to the whole process



# Planning

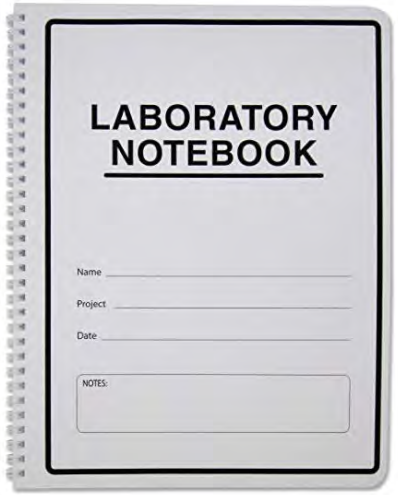
- Defining clear objectives for proposed work
- Identify context for work
  - Is the problem of national/global importance?
  - Is it relevant to the research organization, the industry, the end-user etc?
  - Are the results translatable e.g., potential application to human or animal health, improved understanding of biological processes?

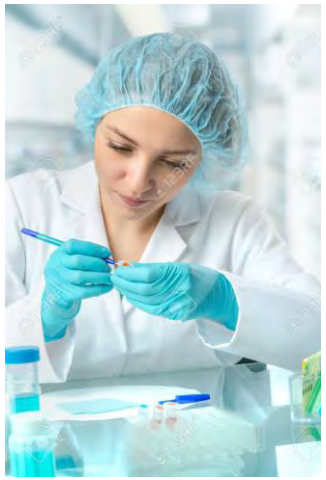
# Planning

- Justification
- Literature review
- Experimental design
- Recording system
- Physical layout/selection of experimental material
- Evaluation of facilities required
- Task timetable
- Personnel plan
- Financial budget

# Execution

- Easier if planning done well
- Adherence to experimental protocol
- Clear and accurate recording
- Experimental diary
- Clear system of labelling (bus rule)



# Analysis

- Ease of analysis directly related to planning and execution
- Consult a statistician before the experiment!


"Data don't make any sense,
we will have to resort to statistics."

# Reporting

- Possibly most important step
- Identify target audience
- Method of dissemination
  - Scientific peer-reviewed articles
  - Local press, radio, TV
  - Open days (e.g., Teagasc, UCD, farm walks etc)
  - Seminars

# Where to Publish?

- Depends on the discipline and sub-discipline
  - Find a society and belong to it








Always aim high


BIOLOGY of REPRODUCTION
Official Journal of the Society for the Study of Reproduction
Journal of Dairy Science
PHYSIOLOGICAL GENOMICS
human reproduction
BJN
BRITISH JOURNAL OF NUTRITION
Journal of Animal Science
animal reproduction SCIENCE
animal
Journal of Animal Ecology
Molecular Reproduction & Development
PNAS
Proceedings of the National Academy of Sciences of the United States of America
www.pnas.org
27 February 1997
International weekly journal of science
nature
A flock of clones
Extrasolar planets Fading from view
Climate cycles Eccentricity finds a role
Archaeology Hunting 400,000 years ago
New on the market
Genetics
Science
Livestock Decoded
AAAS

# Statistics and experimental design can help in relation to:

- Validity
  - Is the study relevant to the question?
- Analysis
  - How do I analyse, interpret and report this study?
- Efficiency
  - Are resources used efficiently; how big should the experiment be?

- Selecting appropriate **design** increases information obtained
- Selecting appropriate study **size** ensures interesting treatment effects will be detected

# Statistical and systematic uncertainty

- Random error (noise)
  - always present
  - Inconsistent measurement values when repeated measures of a constant attribute are taken
- Systematic error
  - Not determined by chance – introduced by inaccuracy
  - Imperfect machine calibration (scales)
  - Imperfect method of observation (heat detection)

# Accuracy v Precision

- Accuracy
  - How close a measurement is to the true value
- Precision
  - How close measures of the same item are to each other
  - How reproducible measurements are

# Causation v correlation

- Causation: Action A causes Action B
- Correlation: a relationship – Action A relates to B but neither causes the other (both may be caused by C)
- Correlation is a statistical measure (expressed as a number) that describes the size and direction of a relationship between two or more variables. A correlation between variables, however, does not automatically mean that the change in one variable is the cause of the change in the values of the other variable.

# Correlation v Regression

- Correlation determines co-relationship or association of two variables.
- Regression describes how an independent variable is numerically related to the dependent variable.
- Regression indicates the impact of a unit change in the known variable (x) on the estimated variable (y).

- Difficult to design the perfect experiment
  - Too small – may not detect important biological effects
  - Too big – wasteful of resources and animals
  - Choice of animals (species, strain, sex, pregnancy status, parity etc)
  - Diet, housing and other environmental factors
  - Choice of treatments (dose levels, route of administration etc)
- How to reduce variability
  - Heterogeneity will reduce power of experiment to demonstrate a statistical difference should one exist
- Use proper methods for statistical analysis

- On ethical grounds, difficult to justify poorly designed experiments
- When treatment effects are subtle, risk of being obscured by ‘noise’ (false negative = Type II error) or generating false positives (Type I error)
  - Both minimized through proper experimental design
- Blocking improves precision
- Randomization to avoid potential bias
- Conclusions should be supported by the data (avoid over-reaching)

- The method of assigning **treatments** to **units** is called **experimental design**.
- The **experimental unit** is the smallest division of the experimental material such that any 2 units may receive different treatments in the actual experiment
- *the smallest entity that is assigned <u>independently</u> of all other units to a particular treatment*
- Animals or groups of animals are the experimental units.
- Procedures, drugs etc. under comparison are the **treatments**

# Experimental Unit

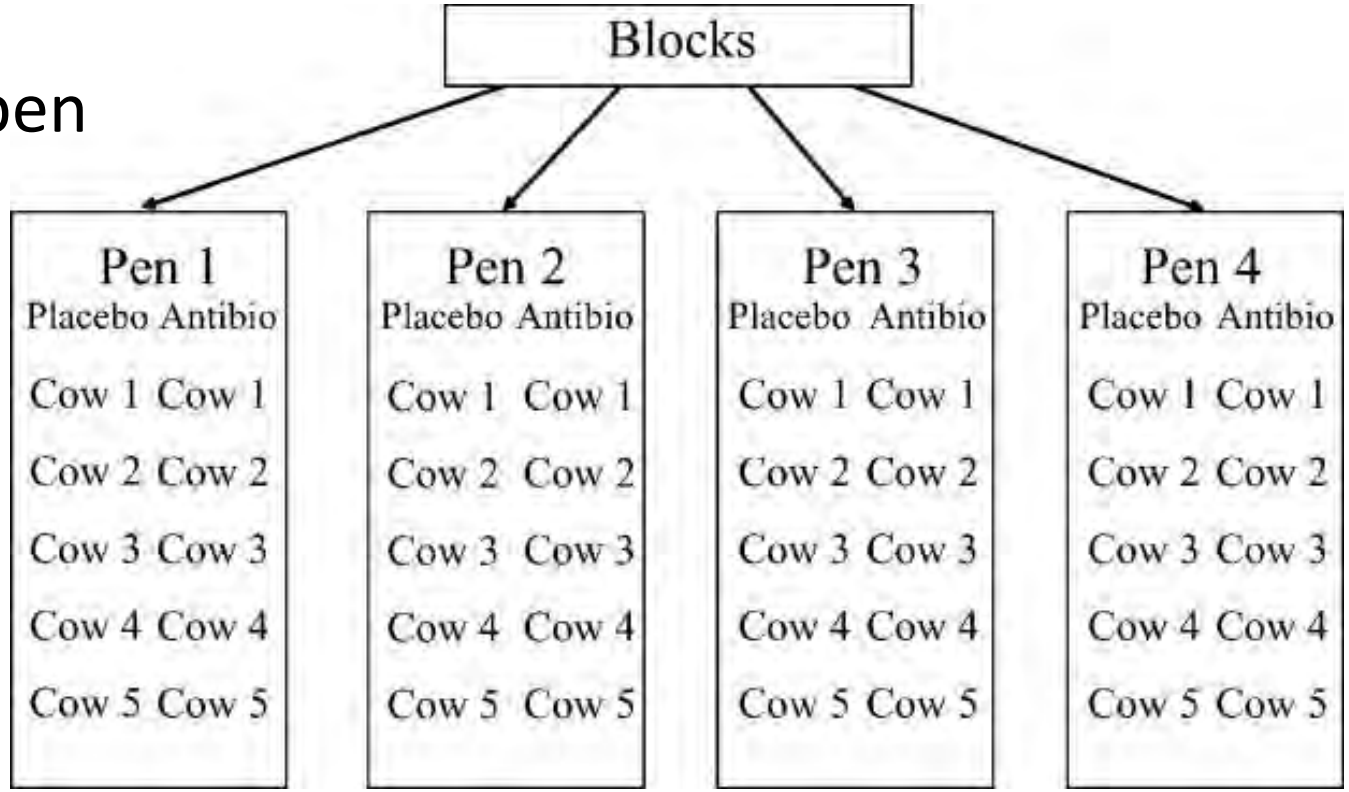
- The entity that can be assigned at random to a treatment, independently of other experimental units
- Often the individual animal, but
  - in a study looking at maternal nutrition during pregnancy on lamb birth weight, the pregnant female is the experimental unit, not the in utero lambs
  - With several mice in a cage, the cage is assigned to treatment group (e.g., diet) and the group of mice is the experimental unit
  - But if mice in the same cage can be assigned to different treatments (e.g., compound delivered by injection), then individual mouse is unit

# Experimental Unit

- Individual animal can sometimes serve as experimental units
  - E.g. if treatments were different types or doses of antibiotics individually injected to treat mastitis.
    - cows still be housed together in pens
    - individual cows within a pen are randomly assigned to different treatments
- May be of interest to compare diets that, for logistical reasons, are commonly fed (i.e., randomly assigned) to pens
  - all cows in the same pen are offered the same diet
  - randomly assign diets A and B each to a different random set of pens, with each pen holding several cows
  - Pen is experimental unit
  - To separate diet effects from pen effects would require more pens - diets need to be replicated to multiple pens

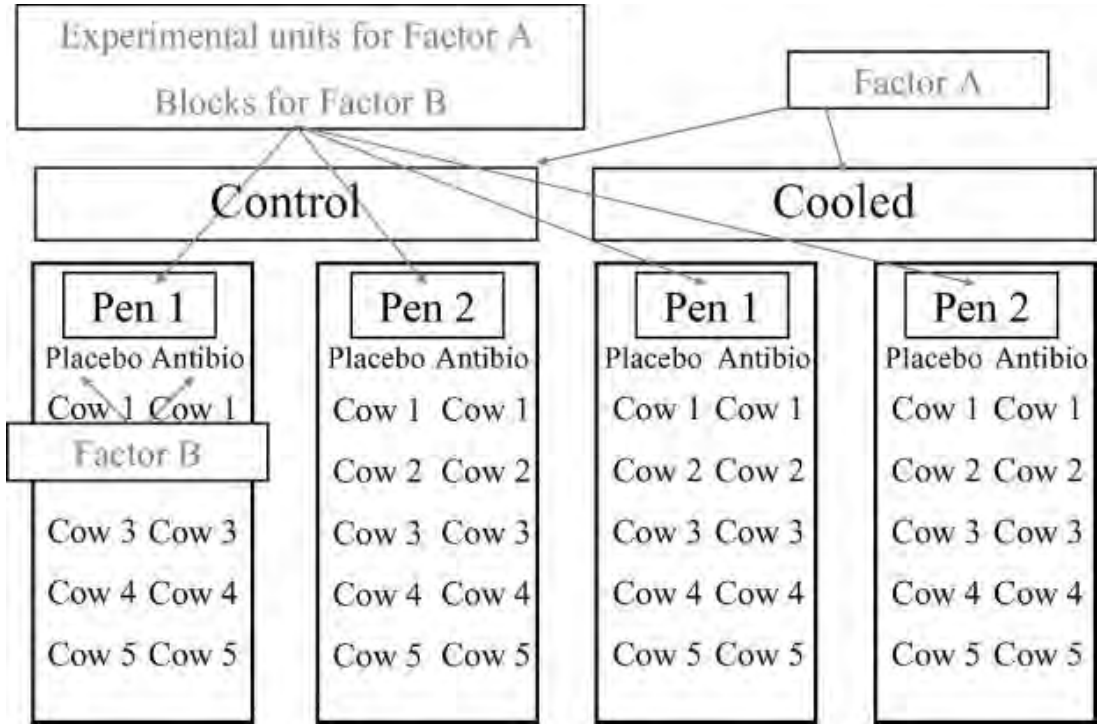
# Test new antibiotic in cows

- Placebo v antibiotic
- 40 cows in 4 pens of 10
- Cows assigned at random to pen
- Within pen (randomly)
  - 5 get placebo
  - 5 get antibiotic
- Randomized complete block



# Test new antibiotic and cooling in cows

- As Exp 1, but
- 2 of 4 pens randomly selected to be cooled with fans and sprinklers (cooled), whereas the 2 other pens were selected to be uncooled (control).
- Aims, to determine
  - efficacy of a new antibiotic
  - the effect of cooling
  - interaction



# All experimental designs consist of 3 structures

- Treatment structure
  - the sets of treatments, treatment combinations, or populations that the experimenter has selected to study
- Design structure
  - the grouping of the experimental units into homogeneous groups or blocks
- Error structure
  - The interactions between elements of the design structure and the treatment structure

# All experimental designs consist of 3 structures

- In Exp 2, the treatment design is a 2 × 2 factorial
  - main factors are the cooling treatments (control vs. cooled), and the injection treatments (placebo vs. antibiotic).
- the design structure is associated with the 4 pens. Although the cows within each pen are randomly assigned to the injection treatments, it is the pens that were randomly assigned to the cooling treatments. Thus, the experimental units are different for the 2 factors. The experiment design is a classic **split plot**

# 3 elements to a designed experiment to ensure validity

- **Comparative** – more than 1 treatment
- **Replication** – each treatment tested on more than 1 experimental unit
- **Randomization** – allocation of treatments to experimental units is random

# 3 principles of experimental design

- Replication
- Randomization
- Local control

# Replication

- Application of each treatment to more than one experimental unit
- Farmer treats one cow with mastitis with new drug – full recovery
  - proof of effectiveness?
  - Is it an experiment?
- 2 cows with mastitis – one treated with new drug, one untreated
  - Is this an experiment?
- 6 cows with mastitis – 3 treated, 3 untreated
  - Replicated experiment
  - Amount of replication depends on variation between animals

# Randomization

- Randomize allocation of treatments to units such that the probability of a particular treatment being allocated to a particular unit is the same for all treatments and units
- Assigning cows to be treated at random
- Removes bias

# Local control - Blocking

- Involves imposing some restriction on the random allocation of treatments to animals to take account if known initial differences
- Done by ***blocking***
  - Cows 1 and 2 least affected
  - Cows 3 and 4 intermediate
  - Cows 5 and 6 most affected
  - Allocate treatments at random to cows within a block
- No treatment ends up with monopoly of extreme units

# Local control - Blocking

- 2 treatments, identical twins
  - Each treatment assigned to one of each pair
  - Expect twins to be basically alike – even a small trt effect will be obvious
- Milk yield in cows increases with increasing lactation number (for low lactation numbers)
  - If not taken into account – background variation will be large – more expensive resources required to establish differences exist
  - Grouping on lactation number first and then assigning to trt at random within groups – lower noise – more precise

# Completely randomized design

- When animals (units) are assigned to treatments completely at random
  - E.g. effect of different diets on ewe body weight
- Useful when experimental units are homogenous
  - E.g., all first parity, same age, same breed
  - E.g., all singleton male lambs

# Randomized Complete Block

- Treatments allocated randomly to positions within each block
- Complete – each block contains every treatment
- Blocking increases precision by reducing error variance

# What is an experiment? A hypothesis?

- **Experiment**. A test under controlled conditions to demonstrate a known truth, examine the validity of a hypothesis, or determine the efficacy of something new
- **Hypothesis**
  - A tentative explanation for an observation that can be tested
  - Validity determined using data analysis
  - A statement to be tested
  - Only sure way of fining the truth of a hypothesis – test entire population
  - Instead we examine a sample and draw conclusions
    - Leads to probability/chance

# Test of hypothesis

- Null hypothesis
  - The hypothesis of no effect
- Nutritionist examining a new compound feed for cattle
  - $H_O$: new feed does not increase growth rate
  - $H_A$: new feed does increase growth rate
- Test leads to 4 situations
  - $H_O$ is true and is accepted – a correct decision
  - $H_O$ is true and is rejected – incorrect
  - $H_O$ is false and is accepted – incorrect
  - $H_O$ is false and is rejected – correct

# Type I errors

- Probability of rejecting $H_O$ when it is true
  - Concluding there is a real difference when there is not
  - = alpha = level of significance
  - e.g. if alpha=0.05, there is a 1 in 20 chance of rejecting $H_O$ when it is true
- Easy to control size of alpha because we choose level
- Smaller levels chosen when consequences of a type I error are serious

# Type II errors

- Probability of <u>not</u> rejecting $H_O$ when it is false
  - Concluding there is no real difference when there is
  - = beta = probability of making a type II error
  - Controlled when designing the experiment
  - Central in deciding amount of replication for an experiment
- 1-beta = power of a test
  - = probability of rejecting $H_O$ when it is false
  - Chance of detecting a treatment effect if it exists
  - Generally choose >80%
  - Incidence depends on
    - size of real effect
    - variation in experimental material
    - No of exp units/trt (replication)

# Basic Principles

- At end of experiment, need to decide if sufficient evidence to conclude that treatment had an effect
- Means of two groups treated identically will differ due to chance (and biological variation)
  - How large does a difference have to be to conclude it was caused by treatment?
  - Statistics will estimate probability that a difference at least as large as that observed could have arisen by chance, as a result of variation between subjects
- We assume sample set of subjects is representative of population

- *Null hypothesis*: the two samples are derived from the same population
  - Rejected if very unlikely observed difference arose by chance
  - Accepted if no evidence for a difference due to treatment
- *Alternative hypothesis*: they are from different populations

- ‘Very unlikely’ – 5% probability or less
  - - in about 5% of comparisons the null hypothesis will be rejected when in fact it is true (Type I error)

- Failure to detect a true treatment effect – false negative (Type II error)
  - If experimental material is extremely variable
  - If sample size is too small (large differences between group responses can frequently occur by chance)
- *Power* of an experiment = 1-(type II error rate) – usually expressed as a percentage
- Important to design powerful experiments capable of detecting treatment effects sufficiently large to be of biological importance

# Variation in experiments

- Feeding trial measuring effect of protein level on ewe body weight
- Two main sources of variation
  - Differences between levels of protein in diet
  - Differences between ewes fed the same diet
- Aim of analysis is to divide the total variation to obtain estimate of ‘true’ difference between diets
- How well we measure the true difference given by estimates of
  - Standard error of difference
  - Reflected in size of 95% confidence interval

# Variation in experiments

Observed difference

$$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{{S_1}^2}{N_1} + \frac{{S_2}^2}{N_2}}}$$

Standard error of difference (SED)

- Difference we would expect if treatment had no effect

$\bar{x}_1$ = mean of trt 1

$\bar{x}_2$ = mean of trt 2

$S_1^2$ = variance of trt 1

$S_2^2$ = variance of trt 2

$N_1$ = number of samples in trt 1

$N_2$ = number of samples in trt 2

# Variation in experiments

- Biological variation minimized by selecting according to certain characteristics
  - Age, gender, species, initial body weight, breed, production system

# Measures of variation

- Mean – sum of values divided by number in sample
- Median - value at middle position if values listed in order
- Mode – most commonly occurring value

- For a normal distributed data – they are the same

# Measures of variation

- Range
  - largest value to smallest value
  - Increases as sample size increases
  - Gives no information on how other values vary from each other
    - e.g. 1, 2, 3, 4, 5, 6 vs 1, 1, 1, 6, 6, 6 vs 1, 3, 3, 4, 4, 6
  - Tells us nothing about how data are spread around mean

# Measures of variation

- Interquartile range (IQR)
  - Difference between 1st quartile (which has 25% of values below it) and 3rd quartile (25% above it)
  - describes the middle 50% of values when ordered from lowest to highest
  - Not affected by outliers
  - Used to construct boxplots

# Measures of variation

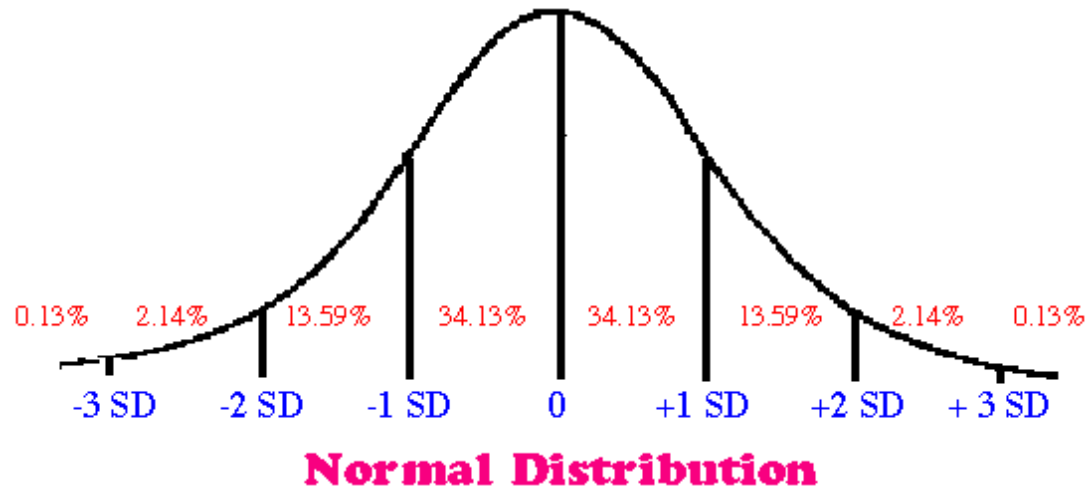
- Sample variance ($S^2$)
  - Measures difference between each observation and the mean
  - Average of squared deviations of each observation from sample mean
  - Units$^2$

$$s^2 = \frac{\sum (x - \bar{x})^2}{n - 1}$$

# Measures of variation

- Standard deviation (S or SD)
  - Square root of the variance
  - Variation of observed values around the sample mean
  - In same units as original observation

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$



# Measures of variation

- Coefficient of variation (CV)
  - Used to compare standard deviations between populations with different means
  - Provides a measure of variation that is independent of measurement units
  - Universal across datasets
  - Expressed as standard deviation as a percentage of the mean

$$CV = \frac{s}{\bar{x}} * 100\%$$

# Measures of variation

- Standard Error of the sample mean (SEM)
  - A measure of how precise our estimate is
  - Standard deviation of the sample is the degree to which individuals within the sample differ from the sample mean
  - SEM is an estimate of how far the sample mean is likely to be from the population mean

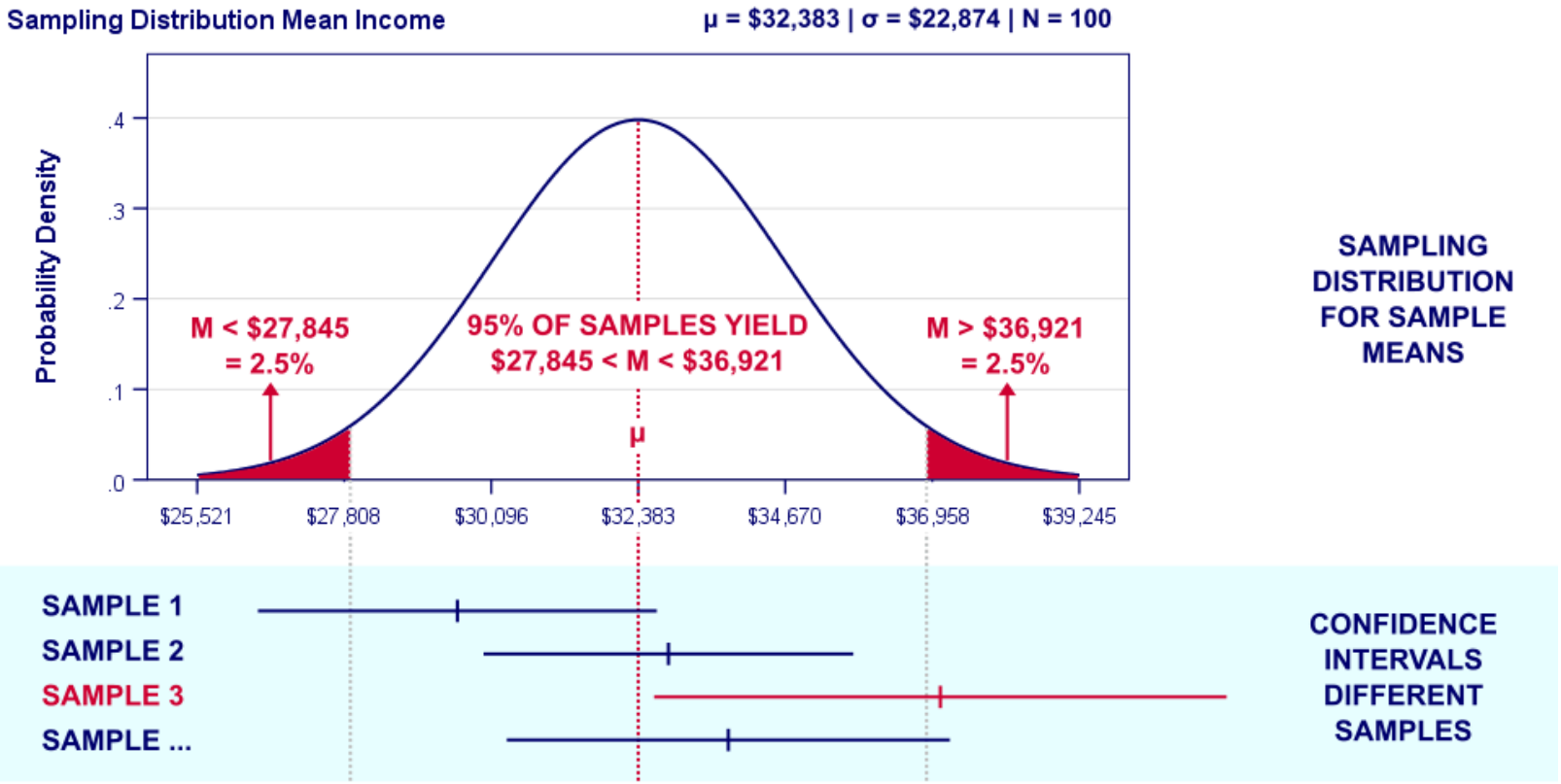
$$SE_{\bar{x}} = \frac{SD}{\sqrt{N}}$$

# Measures of variation

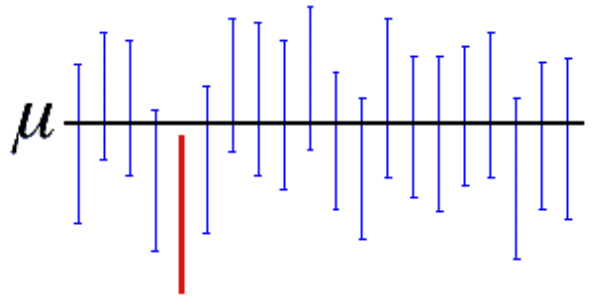
- Confidence Intervals (CI)
  - Determines values in distribution of sample means between which a given percentage (e.g., 95%) of means occur
  - Different random samples from the same population will give different Cis
    - Take 100 samples of size n and calculate the 95% CI for each sample. 95% of samples would contain true population mean. 5% would not

# Measures of variation

- Confidence Intervals (CI)



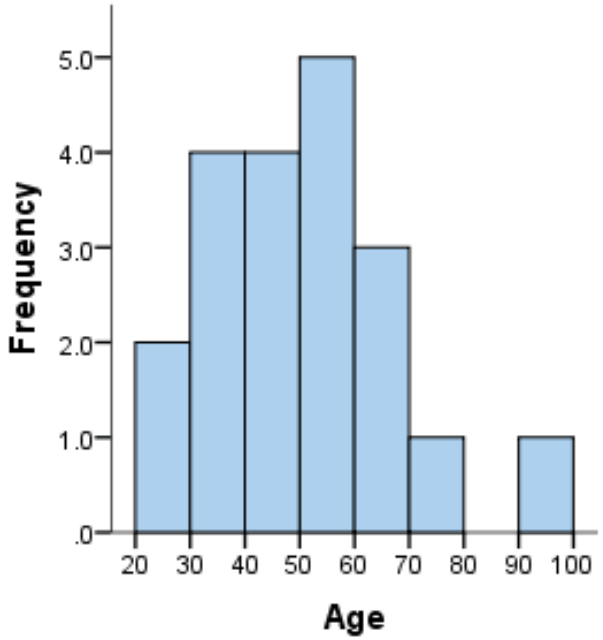

SAMPLING DISTRIBUTION FOR SAMPLE MEANS




A 95% confidence interval indicates that 19 out of 20 samples (95%) from the same population will produce confidence intervals that contain the population parameter.

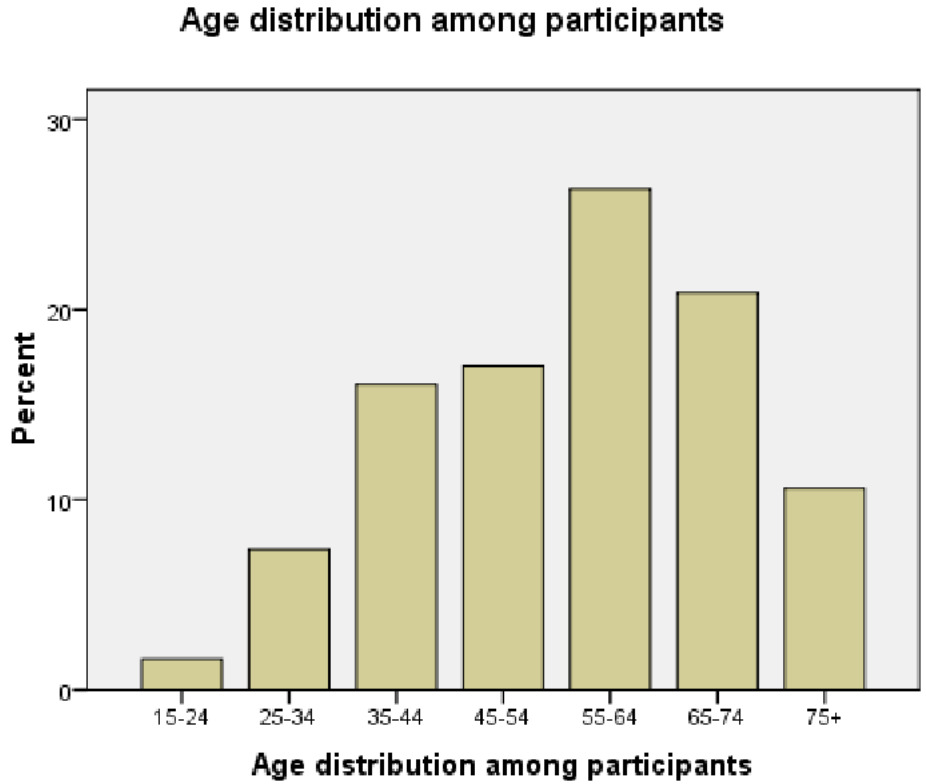
# Graphical summaries

- Histograms
  - Graphical representation of frequency distribution
  - Divide data range into 8-12 intervals
  - Area of column proportional to number of occurrences
  - Used for continuous data



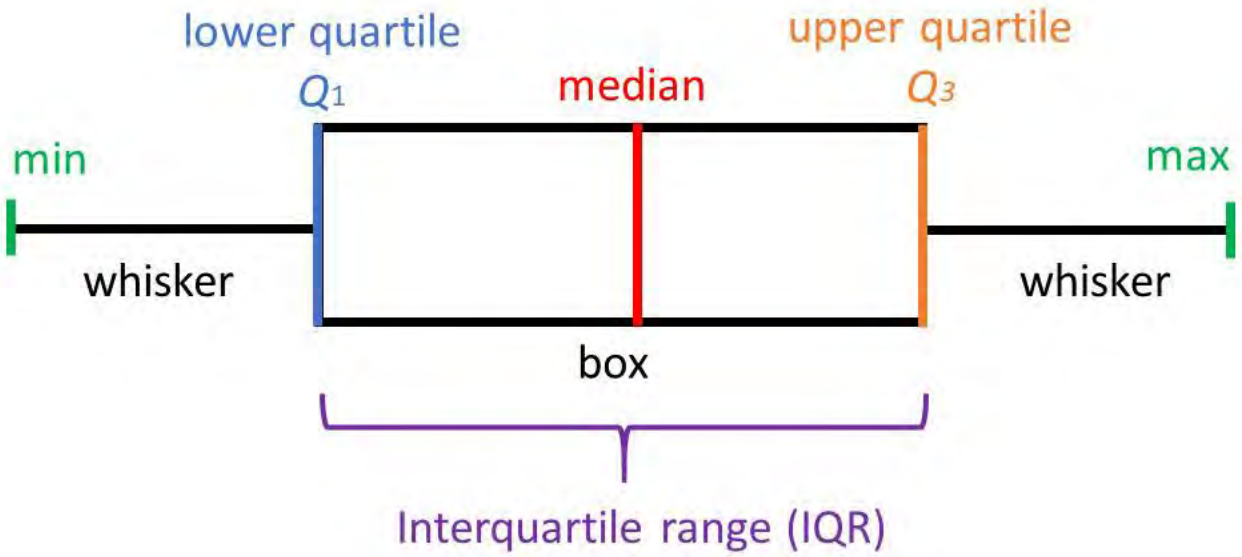
# Graphical summaries

- Bar Charts
  - As histograms, but for ordinal or discrete data
  - Taken numerical data and made it categorical

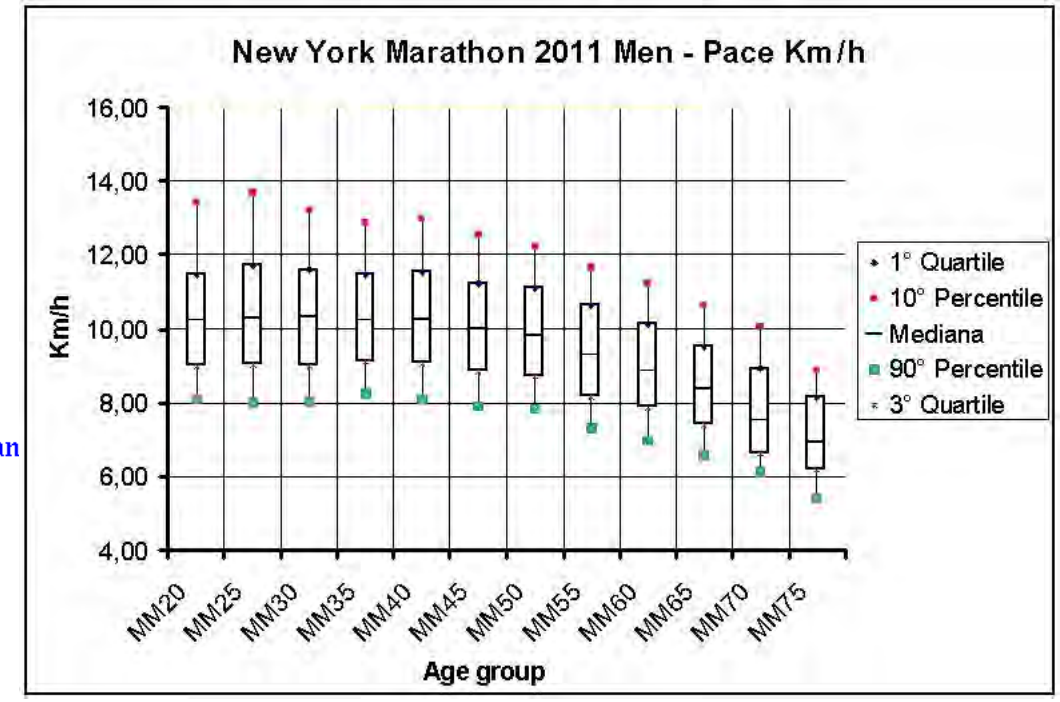


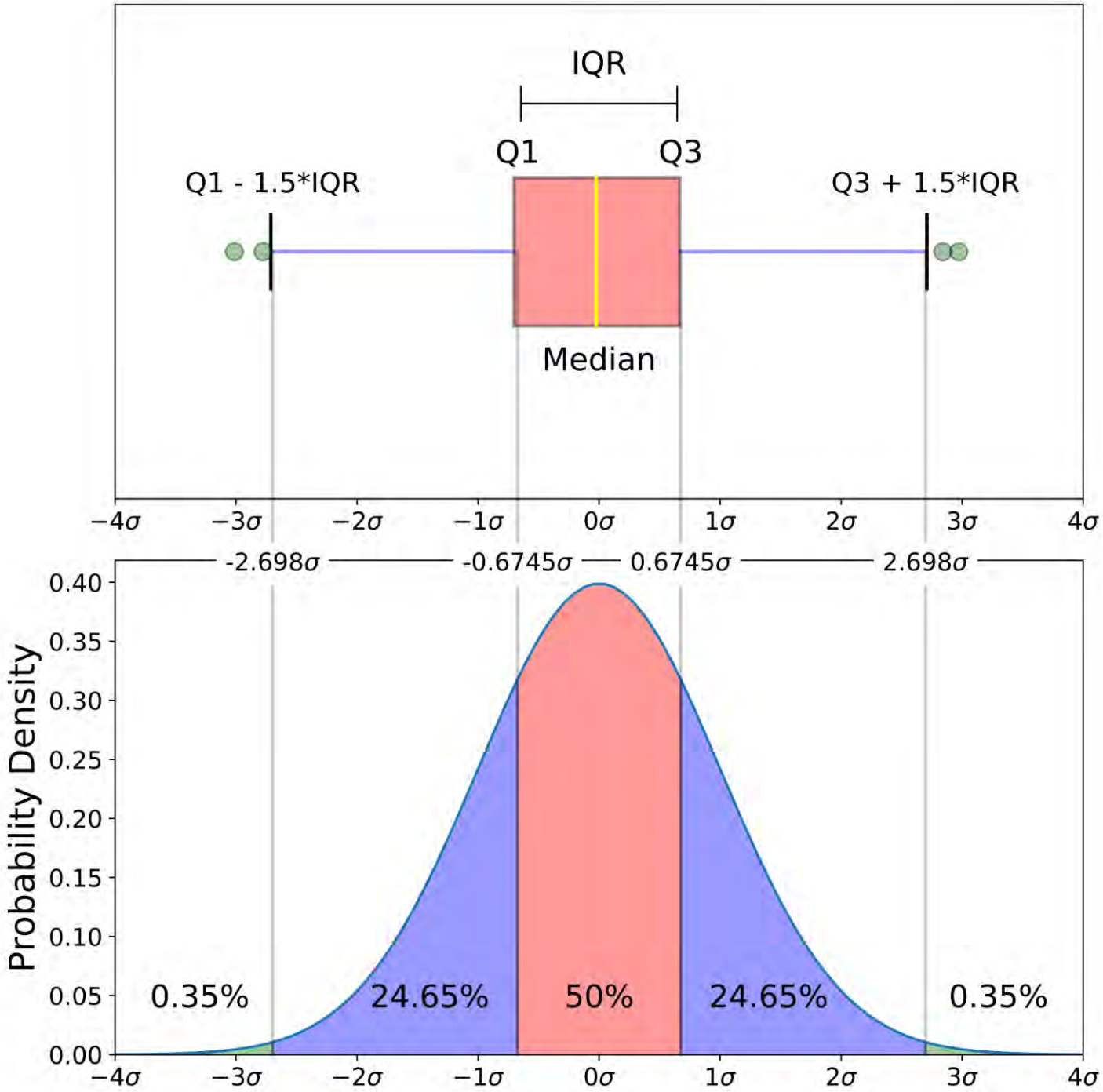
# Graphical summaries

- Box Plots
  - Show median value, 1st and 3rd quartiles and max and min values



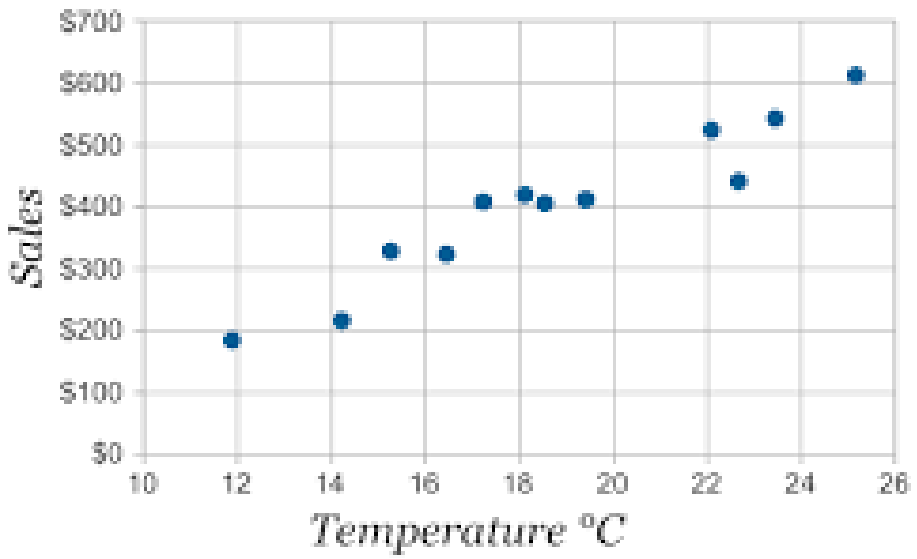

median

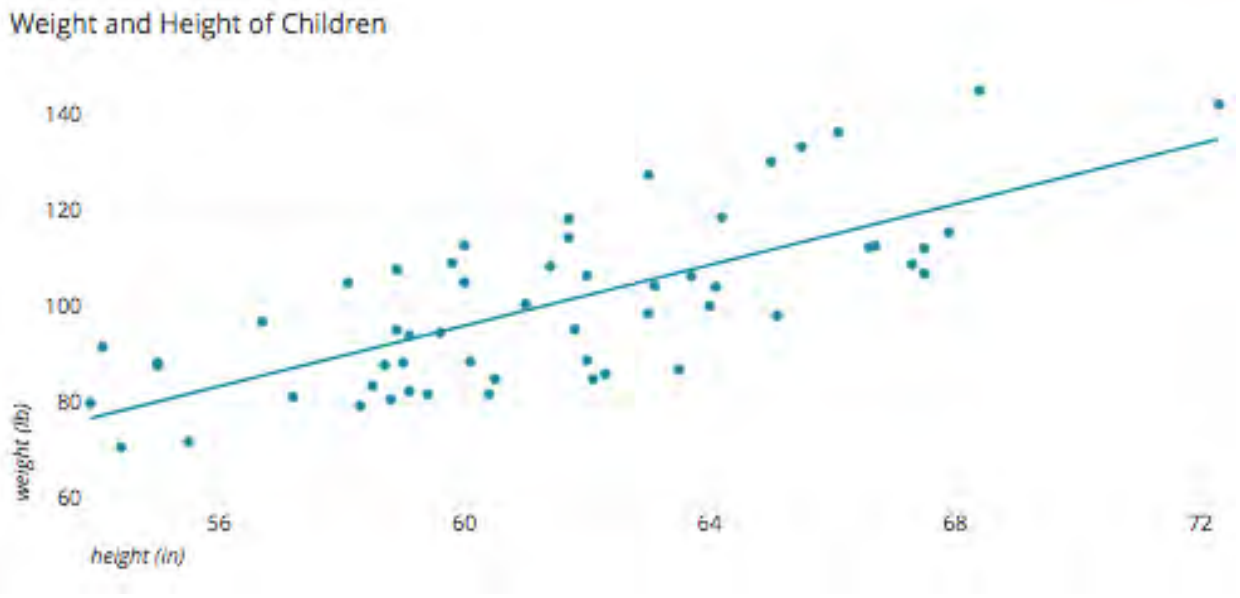



IQR
Q1
Q3
Q1 - 1.5*IQR
Q3 + 1.5*IQR
Median
−4σ
−3σ
−2σ
−1σ
0σ
1σ
2σ
3σ
4σ
-2.698σ
-0.6745σ
0.6745σ
2.698σ
Probability Density
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.35%
24.65%
50%
24.65%
0.35%

# Graphical summaries

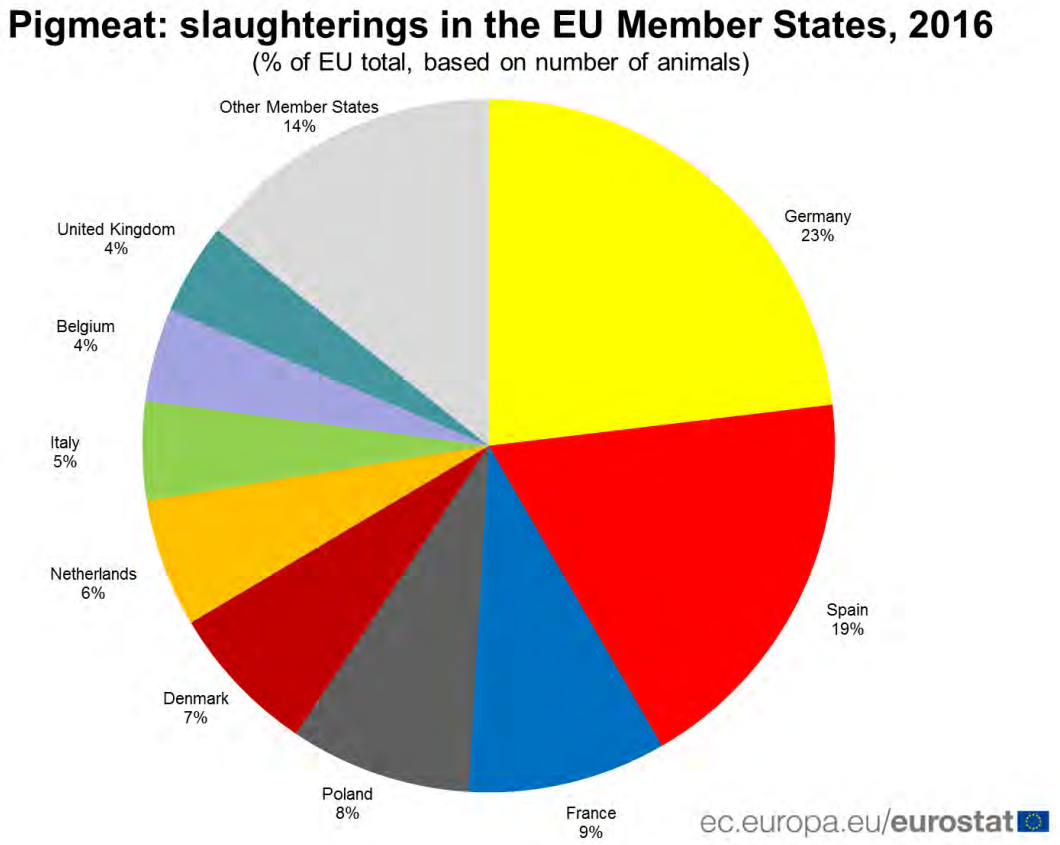
- Scatter Plots
  - Used to plot 2 numeric variables against each other
  - Used to demonstrate presence or absence of a relationship between 2 variables



# Graphical summaries

- Pie Charts
  - Only shows relative frequency – doesn't show values



# Types of data

- Categorical
  - dead/alive, male/female, pregnant/nonpregnant etc
  - Summarized by counts, proportions, percentages
  - e.g, number of abnormal cells in a microscope field
- Measurement
  - Continuous data
  - Summarized using means, medians usually with some estimation of variation among subjects (standard deviation, standard error of the mean)

# Formal experimental designs

- Controlled experiment - where different treatment groups are compared
  - Completely randomized
  - Randomized block
  - Latin square
  - Split-plot
  - Repeated measures
  - Sequential
  - Factorial – to obtain more information from same input of resources
- Aim is to control some of the variation so as to make the experiment more powerful

# Factorial treatments

- Usually provide extra biological information at little extra cost
- Single factor design – varies one independent variable e.g., administration of 4 compounds or 4 levels of one compound
- Factorial design – varies two independent variables (e.g. administration of 4 compunds to both sexes – a 4 x 2 design.
- Used to explore interrelationships between independent variables such as treatment, sex, strain

# A well-designed experiment should

- **Avoid bias**
  - When subjects in different groups have different environments
  - When subjects have not been blocked appropriately prior to treatment allocation (e.g., pregnancy, stage of lactation, parity, age etc)
  - Ensure units are allocate dat random to treatments
  - Ensure all subsequent manipulations are done in a random order
  - Blinding when applying treatment/collecting data
  - Double-blinding – neither patient nor doctor knows the group to which the patient belongs
  - Where suspected after a study, results should provide caveats to interpretation

# A well-designed experiment should

- **Have high power**
  - The probability (%) of being able to detect a treatment effect if one truly exists
  - Experiments should be designed to have the highest possible power (>80%)
  - Use uniform material
  - Can be increased by increasing sample size

# A well-designed experiment should

- **Have a wide range of applicability**
  - Each experiment is done with a particular set of ‘fixed’ effects (e.g., age, sex, strain, diet, caging, physical and social environments etc)
  - Results need to be generalized to a broader range of conditions
- **Be simple**
  - Detailed protocols
  - Compliance
- **Be capable of being analysed statistically**
  - Method of statistical analysis needs to be planned at same time as experiment is  planned

# Randomization

- Fundamental to all controlled studies
- Protects from making false conclusions
  - writing numbers or treatment designations on pieces of paper
  - Computer programs will generate random numbers or place a group oif numbers in random order
  - With Randomized Block design, experiment divided into number of mini-experiments and randomization done within block

# Replication

- The more times something is repeated, the greater the confidence of getting a genuine result
- Allows the identification of outliers

# Sample size

- Goldilocks approach – just the right size
- Power analysis - depends on mathematical relationship between
  - The sample size – usually what is being calculated
  - The effect size of biological interest or clinical relevance
    - Change in mean, median or proportion between treatment groups
    - Larger the effect size, the smaller the experiment needs to be to detect it
    - Where multiple outcome measures, need to account for the most important one
  - The standard deviation among the experimental subjects
  - The desired power of the experiment
    - usually set between 80-90%; higher power requires larger experiements
  - The significance level (type I error rate) to be used
    - Usually set at 5% (0.05)
  - The alternative hypothesis

# Completely Randomized Design

- Simplest
- Subjects allocated to treatment strictly at random
- Relatively unaffected by unequal numbers in each group (but highest precision with equal numbers)
- Where experimental material is heterogeneous (e.g., variably body weight), may be inefficient – RB or LS

# Randomized Block

- Takes account of variation in characteristics which may affect outcome
- Split experimental material into series of mini-experiments each involving a few subjects on each treatment
  - Block 1: parity 1 cows, Block 2: parity 2 cows etc
- Blocking increases precision

# Latin Square

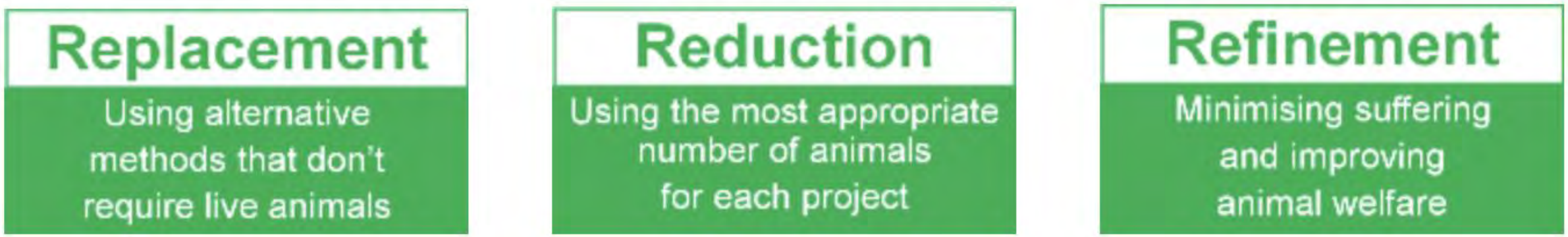
- Can be used to control two sources of variation that cannot be included together in one block of a RCB
- e.g.,….

# The 3Rs (Russell and Burch, 1959)

National Centre for the Replacement Refinement & Reduction of Animals in Research

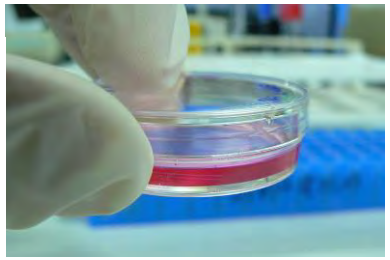
- There is general consensus in our society that, until satisfactory alternatives have been developed, animal research is necessary for the advancement of medical and scientific knowledge. Our laws reflect this situation in allowing animal research under certain constraints and conditions.
- Must comply with all the European and Irish Law relating to research.
  - European Communities (Amendment of Cruelty to Animals Act 1876) Regulations 2002 (S.I. 566/2002), EU Directive 2010/63/EU (SI no 543 of 2012 as amended)

# The 3Rs (Russell and Burch, 1959)



# The 3Rs (Russell and Burch, 1959)

- **Replacement** - Is it possible to use an alternative?
  - Lower organisms (e.g., *Drosophila*, *C. elega*ns)
  - In vitro (e.g., cell lines)
  - Mathematical modeling / computer simulation



  - Replacement refers to the use of alternative methods which substitute the use of animals for scientific purposes. Where replacement is not possible, animal use must only be permitted where justified and where the expected benefits outweigh the potential adverse effects.
  - In cases where there is no alternative to the use of live animals, the goal is to ensure that the highest standards of animal welfare and care are applied.

# The 3Rs (Russell and Burch, 1959)

- **Refinement**
  - Where replacement isn't an option
  - Aim is to minimize pain, suffering, lasting harm – ensure animal welfare is prioritised
  - Comfortable housing, adequate space, good diet, trained staff, free of disease, use of appropriate anaesthesia and analgesia, humane endpoints

  - to include enrichment materials (e.g. toys and nesting material)

# The 3Rs (Russell and Burch, 1959)

- **Reduction**
  - Minimizing numbers of animals used in each experiment
  - Principle is to ensure that the appropriate number of animals is used
  - Reduction should start with good experimental design and planning
  - Correct experimental design and statistical analysis main factor in reduction
  - Involves understanding and controlling variation with efficient experimental designs, appropriate statistical analysis and careful interpretation
  - requirement to maximise the amount of data emerging from animal experiments by judicious experimental design

# Examples of justification

- To help discover or develop new treatments or preventative strategies for disease control in human beings, animals or plants;
- To improve the welfare of animals and to assist in the improvement of production conditions for animals reared for agricultural purposes;
- For research purposes into the detection, assessment, regulation or modification of physiological conditions in humans, animals or plants;
- To satisfy European and global regulatory requirements in the manufacture or testing of the quality, safety or engaged of drugs, vaccines, foodstuffs, feedstuffs or products;
- To preserve a particular species, or gain insights into their normal behaviours;
- For higher education;
  For forensic reasons

# ARRIVE Guidelines

- Animal Research: Reporting of In Vivo Experiments
- developed as part of an NC3Rs initiative
- intended to to improve the design, analysis and reporting of research using animals – maximising information published and minimising unnecessary studies

| | ITEM | RECOMMENDATION |
|---|---|---|
| Title | 1 | Provide as accurate and concise a description of the content of the article as possible. |
| Abstract | 2 | Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study. |
| **INTRODUCTION** | | |
| Background | 3 | a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.<br><br>b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology. |
| Objectives | 4 | Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. |
| **METHODS** | | |
| Ethical statement | 5 | Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research. |
| Study design | 6 | For each experiment, give brief details of the study design including:<br><br>a. The number of experimental and control groups.<br><br>b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when).<br><br>c. The experimental unit (e.g. a single animal, group or cage of animals).<br><br>A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out. |
| Experimental procedures | 7 | For each experiment and each experimental group, including controls, provide precise details of all procedures carried out.<br><br>For example:<br>a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s).<br><br>b. When (e.g. time of day).<br><br>c. Where (e.g. home cage, laboratory, water maze).<br><br>d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used). |
| Experimental animals | 8 | a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range).<br><br>b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc. |
| Housing and husbandry | 9 | Provide details of:<br><br>a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish).<br><br>b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment).<br><br>c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment. |
| Sample size | 10 | a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group.<br><br>b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used.<br><br>c. Indicate the number of independent replications of each experiment, if relevant. |
| Allocating animals to experimental groups | 11 | a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done.<br><br>b. Describe the order in which the animals in the different experimental groups were treated and assessed. |
| Experimental outcomes | 12 | Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes). |
| Statistical methods | 13 | a. Provide details of the statistical methods used for each analysis.<br><br>b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron).<br><br>c. Describe any methods used to assess whether the data met the assumptions of the statistical approach. |
| **RESULTS** | | |
| Baseline data | 14 | For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing (this information can often be tabulated). |
| Numbers analysed | 15 | a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]).<br><br>b. If any animals or data were not included in the analysis, explain why. |
| Outcomes and estimation | 16 | Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval). |
| Adverse events | 17 | a. Give details of all important adverse events in each experimental group.<br><br>b. Describe any modifications to the experimental protocols made to reduce adverse events. |
| **DISCUSSION** | | |
| Interpretation/ scientific implications | 18 | a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.<br><br>b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2].<br><br>c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research. |
| Generalisability/ translation | 19 | Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology. |
| Funding | 20 | List all funding sources (including grant number) and the role of the funder(s) in the study. |

# Steps in good experimental design

- Formulation of an hypothesis
- Choice of the experimental unit and need for independent replication
- Controlling variability
- Choice of treatments (independent variables)
- Choice of dependent variables (things to be measured)
- Choice of design
- Determination of sample size
- Statistical analysis
- Interpretation
- Reporting

# Controlling variability

- The more uniform the animals, the fewer are needed
  - Uniform weight, age, sex, parity, stage of lactation, stage of the oestrous cycle
- Where this is not possible – use blocking
  - E.g. stratify by weight, parity etc (RCB)
- Apply treatments uniformly to all animals - Avoid bias
- Random variability (random effects)
  - Uncontrolled inter-individual differences
  - Contribute to noise
- Fixed effects
  - Sex, strain, age, diet, litter size, etc

- Most statistical tests compare size of the effect (the biological signal) relative to the amount of variability in the data (noise)
- control
  - Untreated
  - Sham-treated
  - Placebo treated
  - Vehicle control
  - Postive/negative control

# Interpretation of P values

- Absence of evidence is not evidence of absence!
- A non-significant difference may mean that
  - The treatment has no effect
  - The experiment is incapable of detecting an effect (noise obscuring treatment effect experiment too small)
- P value
  - is not the probability that the null hypothesis is true
  - is the probability that a result at least as extreme as the one actually observed could have been found if the null hypothesis were true

# Analysis of Variance - ANOVA

- For simple experiments comparing two means = Student's t-test
- More complex experiments (e.g. RB, repeated measures, factorial designs)
- ANOVA divides total variation in the dependent variable into parts
  - Variation is quantified as sums of squared deviations from group or overall means
  - First, overall mean across all treatment groups is calculated
  - Then, deviations of each group from overall mean are quantified by adding up the squares of these deviations (the Treatment 'Sum of Squares')
  - Finally, the remaining unexplained ('error') deviations of each individual data point from its treatment mean are squared and added up to give the Error Sums of Squares

- Give details on red tape – AREC, HPRA, Lyons, Welfare
- Give an example of a field trial – Sanchez/Federico/sexing trial
- Excel file
- Screen shot HPRA licence, AREC forms etc – flow diagram

- End of Study Report, Unforeseen events report

# Animal Research Ethics Committee (AREC)

- ensures that all ethical issues arising in connection with UCD research and teaching activities involving animals are identified and reviewed and that the use of animals can be ethically justified
- Appropriate decisions are taken to ensure that all procedures are conducted to the standards of international regulation and best practice and in accordance with the principals of  the 3Rs

# AA1 - Application to the Animal Research Ethics Subcommittee (AREC), UCD

## for approval for the use of live animals in research

(to be submitted via **Infohub/SISweb** only)

This form **must** also be accompanied by the HPRA project authorisation application form. (HPRA PAF)

**PI's Declaration (double-click to tick the box):**

☐ I have read the latest version of **AREC Policies and Guidelines.**

☐ I confirm that there are neither outstanding **End of Study Reports** nor **non-compliance** issues pertaining to any previous AREC protocols under my supervision and I acknowledge that the Committee reserves the right not to consider this application unless all such outstanding issues are resolved.

☐ I confirm that I have checked this application and it has met the standards for submitting to the AREC. I acknowledge that failure to complete this form correctly may result in delay of ethical review.

☐ I have read the NC3R's ARRIVE Guidelines and confirm that this application complies with them.

☐ **I have consulted with the UCD Biomedical Advisor** **Yes** ☐ **No** ☐

**Notes:**

- **Applications that are incorrectly completed or do not adhere to word limits will be returned without review.**
- **In particular, please read and follow the instructions for Section 4: Lay summary.**
- **Please DELETE ALL instruction notes throughout this form before submitting it for review.**

# Animal Welfare Body (AWB)

- Advising scientists and staff dealing with animals on matters related to animal welfare; the application of the 3Rs; rehoming schemes;
- Ensuring best practice is in place in order to maximise the welfare of animals;
- Monitoring the development and outcome of projects to identify/advise where further contributions to the 3Rs could be made;
- Facilitating programmes for the sharing of organs and tissues of euthanised animals;
- Providing the HPRA with records (on request) of any advice provided to scientists or staff and the decisions taken based on that advice.

# Health Products Regulatory Authority (HPRA)

- role is to protect and enhance public and animal health by regulating medicines, medical devices and other health products
- Directive 2010/63/EU was transposed into Irish law in December 2012 by SI No 543 of 2012 (as amended*). This legislation aims to improve the welfare of animals used for scientific purposes and to promote the principles of the 3Rs
- Scientific Animal Protection
- **Project authorisation**
- **Individual authorisation**
- **Authorised premises**

- Our Role
- Veterinary Medicines Information
- Safety Information
- Regulatory Information
- Veterinary News & Events
- Special Topics
- Contact details
- Scientific Animal Protection


## I want to:

Submit an Application | Learn about the 3Rs | Contact the HPRA

HPRA
An tÚdarás Rialála Táirgí Sláinte
Health Products Regulatory Authority

# Application for a Project Authorisation under Scientific Animal Protection Legislation

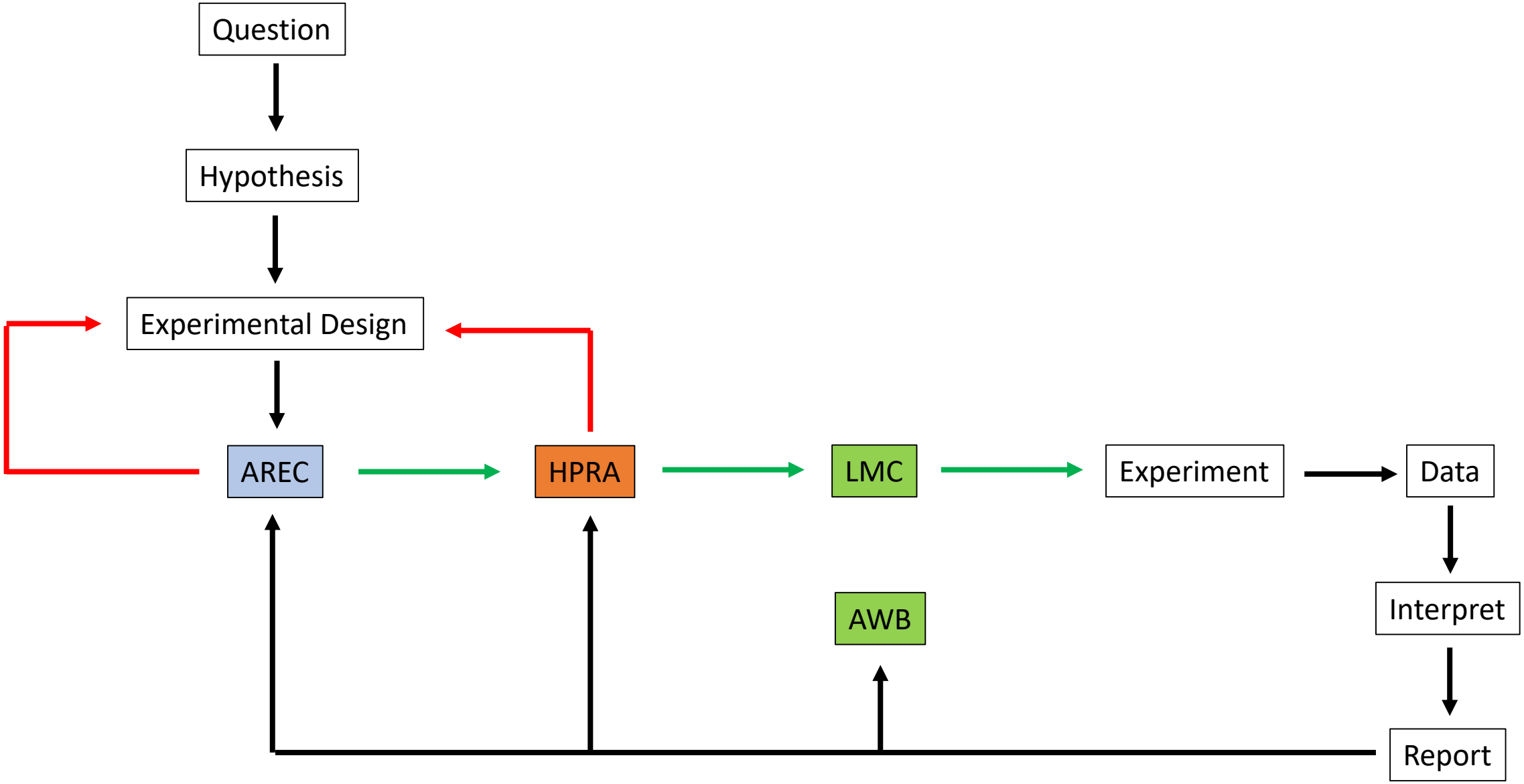
For details on completing this application form, refer to the 'Guide to Project Applications under Scientific Animal Protection Legislation' available at www.hpra.ie.

## SECTION A: PROJECT TITLE AND PROPOSED PROJECT AUTHORISATION HOLDER (I.E. USER)

| | |
|---|---|
| PROJECT TITLE<br>(≤ 500 characters) | |
| Name of proposed project authorisation holder (i.e. user) | |
| Address | |

# Lyons Management Committee (LMC)

- Controls access to resources (cows, sheep, pigs etc) locally


Question
Hypothesis
Experimental Design
AREC
HPRA
LMC
Experiment
Data
Interpret
Report
AWB

# Conclusion

- Science is fun!