The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

| Item | Recommendation | Section/line number, or reason for not reporting |
|------|----------------|-----------------------------------------------|
| Study design | For each experiment, provide brief details of study design including:  
  a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.  
  b. The experimental unit (e.g. a single animal, litter, or cage of animals). | Methods/Line 100-105, 112-121  
  Line 73-95 |
| Sample size |  
  a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.  
  b. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done. | Figure legends/Line 397-443  
  N/A |
| Inclusion and exclusion criteria |  
  a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly.  
  b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.  
  c. For each analysis, report the exact value of n in each experimental group. | Methods/Line 112-129  
  Methods/Line 112-129  
  Figure legends/Line 397-443 |
| Randomisation |  
  a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.  
  b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly. | Methods/Line 112-129  
  Methods/Line 112-129 |
| Blinding | Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis). | Methods/Line 131-207 |
| Outcome measures |  
  a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).  
  b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. | Methods/Line 131-207  
  N/A |
| Statistical methods |  
  a. Provide details of the statistical methods used for each analysis, including software used.  
  b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met. | Methods/Line 209-212  
  Methods/Line 209-212 |
| Experimental animals |  
  a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.  
  b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. | Methods/Line 100-105, 112-121  
  Figure legends/Line 397-443  
  Methods/Line 100-105, 112-121  
  Figure legends/Line 397-443 |
| Experimental procedures | For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:  
  a. What was done, how it was done and what was used.  
  b. When and how often.  
  c. Where (including detail of any acclimatisation periods).  
  d. Why (provide rationale for procedures). | Methods/Line 131-207  
  Methods/Line 131-207  
  Methods/Line 131-207  
  Methods/Line 131-207 |
| Results | For each experiment conducted, including independent replications, report:  
  a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).  
  b. If applicable, the effect size with a confidence interval. | Results/Line 215-271  
  Results/Line 215-271 |
### The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

| Item                      | Recommendation                                                                 | Section/line number, or reason for not reporting |
|---------------------------|--------------------------------------------------------------------------------|--------------------------------------------------|
| **Abstract**              | Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions. | Abstract/Line 39-60                              |
| **Background**            | a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.  
b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology. | Introduction/Line 65-80  
Introduction/Line 92-95 |
| **Objectives**            | Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested. | Introduction/Line 82-95                           |
| **Ethical statement**     | Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification. | Methods/Line 103-105                              |
| **Housing and husbandry**| Provide details of housing and husbandry conditions, including any environmental enrichment. | Methods/Line 100-102                              |
| **Animal care and monitoring** | a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.  
b. Report any expected or unexpected adverse events.  
c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this. | Methods/Line 100-103  
N/A  
Methods/Line 100-103 |
| **Interpretation/scientific implications** | a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.  
b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results. | Results/Line 215-271  
Results/Line 215-271 |
| **Generalisability/translation** | Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate). | Discussion/Line 310-313                            |
| **Protocol registration** | Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered. | Introduction/Line 93-95; Methods/Line 112-121 |
| **Data access**           | Provide a statement describing if and where study data are available. | Please see the Data Sharing Statement Form         |
| **Declaration of interests** | a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.  
b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study. | Conflicts of Interest/Line 325-326  
Acknowledgments/Line 316-320 |

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