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. Methods/line 114-116. |
| | a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. | b. Methods/line 108-245. |
| | b. The experimental unit (e.g. a single animal, litter, or cage of animals). | |
| **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. | a. Figure Legend/line 623, 633, 641, 653,671, 679. |
| | b. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done. | b. Figure Legend/line 623-24, 633-34, 642, 653-54,671-72, 680. |
| **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. | a. Methods/line 212-213. |
| | 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. | b. There were no exclusions. |
| | c. For each analysis, report the exact value of n in each experimental group. | c. Figure Legend/line 623, 633, 641, 653,671, 679. |
| **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. | a. No randomization. |
| | 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. | |
| **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). | NA. |
| **Outcome measures** | a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). | a. Methods/line 108-256. |
| | b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. | b. NA. |
| **Statistical methods** | a. Provide details of the statistical methods used for each analysis, including software used. | a. Methods/line 259-265 |
| | 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. | b. Figure Legend/line 623, 633, 642, 653,671, 680. |
| **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. | a. Methods/line 212. |
| | b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. | b. Methods/line 208-209. |
| **Experimental procedures** | For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: | a. Methods/line 108-133, 139-142, 145-152, 155-174, 177-178, 180-201, 204-204, 207-245, 248-255. |
| | a. What was done, how it was done and what was used. | b. Methods/line 116,181,187, 195-196,199,201,213,216,218,219. |
| | b. When and how often. | c. Methods/line 208-209. |
| | c. Where (including detail of any acclimatisation periods). | d. Methods/line 208-209. |
| | d. Why (provide rationale for procedures). | |
| **Results** | For each experiment conducted, including independent replications, report: | a. Figure Legend/line 623, 633, 649, 652,671, 679. |
| | 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. NA. |
| | b. If applicable, the effect size with a confidence interval. | |
### 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 37-68.                          |
| **Background**            | a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.                                                               | a. Introduction/line 74-94.                   |
|                           | b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.                                                                   | b. Introduction/line 99.                     |
| **Objectives**            | Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.                                                                                           | Introduction/line 92-94.                      |
| **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 204-207.                         |
| **Housing and husbandry** | Provide details of housing and husbandry conditions, including any environmental enrichment.                                                                                                                   | Methods/line 209-210.                         |
| **Animal care and monitoring** | a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.                                                                                           | a. Methods/line 213.                          |
|                           | b. Report any expected or unexpected adverse events.                                                                                                                                                         | b. NA.                                       |
|                           | 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. | c. Methods/line 213-214.                     |
| **Interpretation/ scientific implications** | a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. | a. Results/line 269-276, 279-284, 287-293, 298-304, 306-339, 342-347. |
|                           | b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.                                                      | b. Discussion/line 445-447.                   |
| **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). | Conclusions/line 457-460.                     |
| **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. | A protocol was prepared before the study without registration. |
| **Data access**           | Provide a statement describing if and where study data are available.                                                                                                                                          | Data Sharing Statement/line 474.              |
| **Declaration of interests** | a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.                                                                                   | a. Conflicts of Interest/line 476-478.        |
|                           | b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.                                                                 | b. Acknowledgments/line                      |

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*As the checklist was provided upon initial submission, the line number reported may be changed due to copyediting and may not be referable in the published version. In this case, the section may be used as an alternative reference.*