ARRIVE

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	1	For each experiment, provide brief details of study design including:	Methods/Line 206
		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 206
Sample size	2	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.	Methods/Line 219-221
		b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Each experiment was repeated three times to count the number of experimental animals
Inclusion and exclusion criteria	3	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.	Methods/Line 210-217
		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.	There were no excluded
		c. For each analysis, report the exact value of *n* in each experimental group.	Methods/Line 219-221
Randomisation	4	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.	N/A.The Randomisation Sequence was not involved in this study.
		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.	N/A.The Randomisation Sequence was not involved in this study.
Blinding	5	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. No double or triple blindness in clinical trials was involved in this study
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).	Methods/Line 210-217
		b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	This paper does not address the hypothesis testing studies
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used.	Methods/Line 299-307
		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.	This paper does not address the hypothesis testing studies
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.	Methods/Line 124-125
		b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Methods/Line 125
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:	Methods/Line 235-237
		a. What was done, how it was done and what was used.	Methods/Line 226
		b. When and how often.	Methods/Line 126-128
		c. Where (including detail of any acclimatisation periods).	
		d. Why (provide rationale for procedures).	It was designed according to relevant basic experimental methods
Results	10	For each experiment conducted, including independent replications, report:	Results/Line 339
		a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).	Methods/Line 307
		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	11		Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract/Line 37-45
Background	12	a.	Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.	Abstract/Line 30-36
		b.	Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Abstract/Line 41
Objectives	13		Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Abstract/Line 51-52
Ethical statement	14		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.	Footnote/Line 491-496
Housing and husbandry	15		Provide details of housing and husbandry conditions, including any environmental enrichment.	Methods/Line 126-128
Animal care and monitoring	16	a.	Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.	Methods/Line 130-131
		b.	Report any expected or unexpected adverse events.	Methods/Line 212
		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 133-138
Interpretation/ scientific implications	17	a.	Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.	Discussion/Line 448-456
		b.	Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Conclusions/Line 465-467
Generalisability/ translation	18		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).	No other species were studied in depth
Protocol registration	19		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	20		Provide a statement describing if and where study data are available.	Data Sharing statement
Declaration of interests	21	a.	Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.	Footnote/Line 484-486
		b.	List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	Acknowledgments/Line 476-480

NC 3Rs — National Centre for the Replacement Refinement & Reduction of Animals in Research

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