



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: 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).	Isolation of heart / line 5-6 single animal
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. b. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	Laboratory animals / line 1; Isolation of heart / line 5-6 The principles of 3R, by analogy with previous studies
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 <i>a priori</i> . 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 <i>n</i> in each experimental group.	Laboratory animals / line 1-6 All animals were included in the final analysis; no exclusions were made. For all experiments, (<i>n</i> = 8) rats per group.
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. 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.	Randomization was not applied; rats were allocated consecutively as they arrived at the laboratory. All procedures were conducted by the same researchers at the same time of day (4:00–6:00 p.m.)
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).	Only the researchers performing cardiac perfusion were aware of the group allocation.
Outcome measures	6 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.	page 8 / line 15 page 8 / line 15 page 5 / line 12
Statistical methods	7 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.	page 5 Statistical methods / line 1-7 page 5 Statistical methods / line 1-2
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. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	page 3 / Laboratory animals / line 1-2 Laboratory animals / line 2-3
Experimental procedures	9 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).	Equipment and perfusion solution / page 3-5 Equipment and perfusion solution / page 5 / line 4-5 Laboratory animals / line 2-3 Introduction / page 2-3
Results	10 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 / page 7-8 Results / page 8 / line 15-16