STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	Title: "Serum vitamin A and E improves neonatal necrotizing enterocolitis through activation of SOD/GPx pathway" (Observational human study + animal experiment)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1	Abstract: "Methods: The study measured serum vitamin A and E levels in healthy and NEC newborns/mice... Results: NEC subjects showed significantly lower vitamin A and E levels..."
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3	"Premature infants and newborns are susceptible to... necrotizing enterocolitis (NEC)... Evidence has confirmed an association between serum vitamin A and E levels and neonatal NEC... Vitamin A upregulates antioxidant pathways, increases SOD and GPx activity..."
Objectives	3	State specific objectives, including any prespecified hypotheses	3	"This study aims to elucidate the relationship between serum vitamin A and E levels and neonatal NEC. The findings may enhance our understanding... and provide new insights into the pathogenesis, prevention and treatment of NEC." Methods
Methods				
Study design	4	Present key elements of study design early in the paper	4	"Subjects and group intervention: This study enrolled 100 newborns... comprising 65 neonates with NEC (disease group) and 35 healthy newborns (healthy group)." + Animal model establishment (page 5-6)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4	"This study enrolled 100 newborns between January 2020 and January 2023" + "Department of Clinical Nutrition, West China Hospital of Sichuan University"
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4	"Inclusion criteria: (1) Family members cooperating... (2) Condition stable... (3) Completed clinical data... Exclusion criteria: (1) Previous functional disorders; (2) Congenital anomalies; (3) Spontaneous intestinal perforation."
		(*b*) *Cohort study*—For matched studies, give matching criteria and number	4	Not applicable (no matching reported)

		of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4,6	Outcomes: "NEC diagnosis... classified according to modified Bell's criteria" (page 4). Exposures: Serum vitamin A and E levels (page 4). Animal outcomes: Histopathological analysis, RT-qPCR, Western blot (page 6-7).
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4,6-7	Human: "Venous blood samples (2.5 mL) were collected... quantified using HPLC-MS/MS" (page 4). Animal: "ELISA kit... HE Staining... RT-qPCR... Western blot" (page 6-7).
Bias	9	Describe any efforts to address potential sources of bias	6	"the samples were evaluated through DRUCKER and double-blind methods" (Page 6, histopathological analysis)
Study size	10	Explain how the study size was arrived at	4,5	Human: "100 newborns... 65 NEC, 35 healthy" (page 4). Animal: "70 SD mice... 10 healthy controls, 60 for NEC model" (page 5) - No formal sample size calculation stated.

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Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7	"Continuous variables are presented as mean ± SD. One-way ANOVA followed by LSD post-hoc test. P < 0.05 considered significant." (Page 7)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7	"SPSS 21.0 and GraphPad Prism 8.0. One-way ANOVA with LSD post-hoc test."
		(*b*) Describe any methods used to examine subgroups and interactions	7	Not explicitly described for subgroups
		(*c*) Explain how missing data were addressed	Not reported	No mention of missing data handling
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5	Animal study: No loss to follow-up reported. Human: Cross-sectional, no sampling strategy detailed.
		(*e*) Describe any sensitivity analyses	Not reported	None mentioned
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4,5	Human: 100 enrolled (65 NEC, 35 healthy) all analyzed (Table 2). Animal: 70 total, 10 NC, 10 NEC, 10 Vit A, 10 Vit E, 10 Vit A+E, 10 A+E+DDW, 10 A+E+DDC (Page 5)
		(b) Give reasons for non-participation at each stage	Not reported	Not mentioned
		(c) Consider use of a flow diagram	Not included	No flow diagram provided
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4, Table 2	"46 males and 54 females, age range 7-28 days, mean 14.36±0.24 days" (page 4). Table 2: vitamin levels and pathological scores.
		(b) Indicate number of participants with missing data for each variable of interest	Not reported	Not mentioned
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Not applicable	Cross-sectional human study + animal experiment
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Tables 2,3,4; Figures 2-4	Human: Table 2 (vitamin levels, pathological degree). Animal: Table 3 (pathological scores), Table 4 (clinical scores), Figures 2-4 (histology, qPCR, Western blot)
		Case-control study—Report numbers in each exposure category, or summary measures of exposure		
		Cross-sectional study—Report numbers of outcome events or summary measures		
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Tables 2-4	Unadjusted mean ± SD and t-test P-values provided. No adjusted estimates (e.g., multivariate

				regression) reported.
		(*b*) Report category boundaries when continuous variables were categorized	5,6	NEC staging: "Stage I: intestinal dilation or mild obstruction; Stage II: dilation, pneumatosis; Stage III: pneumoperitoneum" (page 5). Animal scores: 0/1/2 for activity, hair, mental state (page 6).
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable	Not relevant

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Figures 3,4	Subgroup analyses by vitamin treatment and DDW/DDC co-administration reported.
Discussion				
Key results	18	Summarise key results with reference to study objectives	10-12	"NEC neonates exhibited significant deficiencies in vitamins A and E... vitamins A and E activated the SOD/GPx pathway... DDW synergistically enhanced therapeutic effects, DDC abolished benefits."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12	"whether serum vitamins A and E also mediate their effects through other signaling pathways remains unclear and warrants further investigation." (Partial - other limitations not fully discussed)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11, 12	"serum vitamins A and E activate the SOD/GPx pathway, thereby modulating inflammatory cytokine secretion... effectively ameliorating NEC-associated symptoms. These findings offer novel insights..."
Generalisability	21	Discuss the generalisability (external validity) of the study results	12	Not explicitly discussed
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13	"There was no funding."

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

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).	Groups compared: "NC group, NEC group, vitamin A group, vitamin E group, vitamin A+E group, vitamin A+E+DDW group, vitamin A+E+DDC group" – Materials and Methods – Animal intervention and grouping. Experimental unit: Individual mouse (implied by group sizes of n=10 and individual interventions)
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 *a priori* sample size calculation, if done.	Total and per-group numbers: Total 70 mice; NC group (10), NEC group (10), each treatment group (10) – Materials and Methods – Animals & Model establishment. How sample size was decided: Not reported. Sample size calculation: Not reported.
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. 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.	Not reported for animals. Not reported. Not reported.
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.	Not reported. Not reported.
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).	Histopathological analysis: Partially reported – "evaluated through DRUCKER and double-blind methods" for histological scoring. Blinding for other procedures (e.g., drug administration, behavior scoring, Western blot analysis) not described.
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.	Observation indicators: 1) Clinical scores (activity, hair, mental state); 2) Serum vitamin A/E levels; 3) HE staining histopathology; 4) RT-qPCR (TNF-α, IL-6, IL-1β); 5) Western blot (NF-κB, SOD, GPx, TNF-α, IL-6, IL-1β). Not specified as primary/secondary.
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.	Statistical analysis: SPSS 21.0 and GraphPad Prism 8.0; one-way ANOVA; LSD post-hoc test for pairwise comparisons. Not described.
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.	Animals & Model establishment: Species: Sprague Dawley (SD) mice; Age: 48 hours after birth; Weight: 5-10g; Source: Kunming Medical University; Sex: Not reported. Not reported.
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).	Model establishment & Animal intervention and grouping: Detailed procedures provided (hypoxia, formula feeding, LPS, drug administration). Animal intervention and grouping: Vitamin A 10 mg/kg, Vitamin E 10 mg/kg, Vitamin A+E 5 mg/kg each, DDC 25 mL/kg, DDW 10 mL/kg. Routes: gavage (vitamins), intraperitoneal (DDW/DDC). Model establishment: Specific times for feeding (8:00,12:00,16:00,20:00,23:00) and hypoxia (8:30,16:30,23:30) provided. No modifications reported.
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 – Tables 2,3,4 and Figures 3-4: All data presented as mean ± SD. Results – Tables 2,3,4 and Figures 3-4: Exact p-values provided (e.g., p=0.000) and t-values. Confidence intervals not provided.

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.	lusions are summarized. Sex not specified.
Background	12	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: Paragraphs 1 – 4. Background on NEC, vit amins A and E, SOD/GPx path way. Materials and Methods – Animals: SD mice used; relevance to human NEC discussed i n Introduction and Discussion.
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	) investigate the specific mechanisms..."
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.	Materials and Methods – Model establishm ent: "All animal experiments comply with the Guidelines for the Care and Use of L aboratory Animals and have been approved by the Animal Experiment Ethics Committee ." No protocol number provided.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	) light/dark cycle. No enrichment details.
Animal care and monitoring	16	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.	Not explicitl y described. No adverse event s reported. Humane endpoints not explicitly stated; m onitoring described in Observation indica tors (activity, hair, mental state scored 0 – 2).
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. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Discussion: Paragrap hs 4 – 6 interpret re sults. Limitations: Briefly mentioned in Conclus ion: "whether serum vitamins A and E als o mediate effects through other pathways remains unclear." No detailed bias or mo del limitation discussion.
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).	Discussion: Mentions human relevance (e.g., "These fi ndings offer novel insights and potential strategies f or prevention and treatment of neonatal NEC").
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.	Not reported.
Data access	20	Provide a statement describing if and where study data are available.	sponding author upon reasonable request."
Declaration of interests	21	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.	Conflict of interest: "The authors state that this research was conducted without any commercial or financial interests... No conflicts to declare." Funding: "There w as no funding."