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REVIEWER'S REPORT

Manuscript No.: IJAR-55939

Title: *CYTOGENETIC BIOMARKERS OF REPRODUCTIVE AGING: TELOMERE LENGTH AND CHROMATIN INTEGRITY IN SPERMATOZOA AND LEUKOCYTES OF FARMED DEER*

Recommendation:

Accept as it is .....

✓ Accept after minor revision.....

Accept after major revision .....

Do not accept (*Reasons below*) .....

Rating	Excel.	Good	Fair	Poor
Originality		✓		
Techn. Quality		✓		
Clarity		✓		
Significance	✓			

Reviewer Name: Dr S. K. Nath **Date:** 27.01.26

Detailed Reviewer's Report

Strengths of the Study

- **Originality and Relevance:** The study addresses an important gap in reproductive biology by exploring telomere dynamics in cervids, a relatively under-researched area, providing valuable insights into reproductive aging and conservation strategies.
- **Methodology:** Utilizes well-established quantitative PCR techniques for telomere measurement alongside comprehensive semen analysis, chromatin assessment, and oxidative stress evaluation.
- **Data Quality:** The study involves multiple parameters with robust statistical analyses, including nonparametric tests, regression models, and principal component analysis, enriching the data interpretation.
- **Contribution to the Field:** Offers novel comparative data across three deer species, revealing important breed-specific patterns and highlighting the potential of sperm telomere length as a reproductive biomarker.

Weaknesses of the Study

- **Sample Size:** The total sample size of 27 animals, with only nine per species, may limit statistical power and generalizability of the findings.
- **Study Design:** Cross-sectional rather than longitudinal, which constrains causal inferences about telomere dynamics over age.
- **Oxidative Stress Markers:** Systemic oxidative stress markers did not show correlation with telomere length; more localized tests (e.g., testicular oxidative stress) could be more informative.
- **Breed Comparisons:** Breed-specific differences are noted but may be confounded by small sample sizes and other unmeasured variables.
- **Presentation:** Some figures and tables could be presented more clearly; for example, figure labels and figure legends could be more detailed for better comprehension.

Reviewer Comments

- **Title and Abstract:** The title accurately reflects the study's focus but could be slightly more concise. The abstract effectively summarizes the research question, methodology, main findings, and implications, though it could emphasize the novelty by explicitly stating the contribution to conservation or reproductive management.

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- **Introduction and Objectives:** The introduction provides adequate background on telomeres and their relevance to aging and fertility. Clear objectives are laid out, although a brief mention of the potential application of findings to conservation practices would strengthen the framing.
- **Methodology and Statistical Analysis:** The methodologies, including qPCR for telomere quantification and semen analysis, are appropriate and well-described. The use of nonparametric tests and multivariate analysis is suitable given the sample size and data distribution. However, additional details about potential confounders or environmental variables would be beneficial. The statistical analysis appears sound but could be strengthened by including effect sizes and confidence intervals for key findings.
- **Results and Discussion:** Results are presented systematically, with clear correlations and breed differences articulated. The high correlation between leukocyte and sperm telomeres is notable. The discussion thoughtfully interprets findings, relating them to existing literature, though some speculative statements should be tempered. The section on oxidative stress results warrants further discussion, considering the systemic measurements may not reflect local testicular conditions.
- **Conclusion and Implications:** Conclusions are consistent with findings, emphasizing the potential of sperm telomere length as a reproductive biomarker. The implications for breeding and conservation are noted but could be further elaborated to suggest specific practical applications or future research directions.
- **Ethical Clearance:** The study states that it was approved by the Animal Ethics Committee of Universiti Putra Malaysia with a specific reference number. No concerns here.
- **Language and Presentation:** The manuscript is generally well-written with minimal typographical or grammatical errors. Some minor editing for clarity and consistency in figures and tables is recommended.
- **Figures, Tables, and Formatting:** Figures are appropriate but could benefit from higher resolution and clearer labels. Tables are informative but should be formatted for uniformity and clearer presentation. References are comprehensive and relevant.

Additional Note: Based on the review of the content provided and standard practices for scholarly publications, there are no indications or evidence within the text suggesting that this particular study has been previously published on the internet or elsewhere. To conclusively verify whether this work has been previously published, a dedicated plagiarism check or database search (e.g., PubMed, Google Scholar, or institutional repositories) would be necessary.