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Methods for assessing DNA fragmentation in bovine spermatozoa: A mini-review of Indonesian research

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ABSTRACT: This article reviews research methods and analyses of bovine sperm DNA fragmentation conducted in Indonesia. A literature review using Google Scholar identified 37 articles by Indonesian authors published since 2018, with a peak of eight articles in 2023. The primary techniques employed include Acridine Orange staining (17 articles), Sperm-Bos-Halomax kits (12 articles), Toluidine Blue staining (7 articles), and the TUNEL assay (1 article), each exhibiting methodological variations. This review provides a valuable reference for stakeholders aiming to develop standardised regulations for semen evaluation in cattle.

Keywords:
bovine, DNA fragmentation, method, spermatozoa

■ INTRODUCTION

The evaluation of bovine sperm quality now includes the assessment of DNA damage, a key factor influencing fertilisation success. DNA damage can occur due to disruptions in spermatogenesis, spermiogenesis, or oxidative stress (Baity *et al*. 2024). In Indonesia, semen evaluation typically relies on basic macroscopic and microscopic methods without considering DNA fragmentation (Satrio *et al*. 2022; Satrio *et al*. 2024). However, sperm DNA fragmentation is crucial for fertility assessment. Indonesian researchers have investigated various methods for analysing DNA fragmentation, focusing on accessibility and cost-effectiveness, including Toluidine Blue staining, Acridine Orange staining, Sperm-Bos-Halomax kits, and TUNEL assays. This article reviews the development of these methods in Indonesia and provides a reference for stakeholders working to establish bovine semen evaluation regulations.

■ MATERIALS AND METHODS

A literature review on sperm DNA fragmentation in cattle was conducted using Google Scholar. The review focused on studies by Indonesian authors in English and Bahasa Indonesia. The reviewed publications covered the entire period of relevant research up to the present.

■ BOVINE SPERM DNA FRAGMENTATION

Since 2018, Indonesian researchers have published 37 articles on bovine sperm DNA fragmentation (Table 1 and Supplementary Table S1). Publications peaked at eight articles in 2023, with the lowest output of three articles in 2021 (Figure 1). The breed-specific bovines data were presented in Supplementary Table S1.

Table 1 Assessment methods of bovine sperm DNA fragmentation

Assessment methods/ microscopic interpretation	Advantages (+) and disadvantages (-)	Articles	Variations between authors
Toluidine blue staining (TB) **Interpretation:** Bright blue sperm heads signify normal DNA; dark blue indicates fragmented DNA.	**(+)** simple, suitable for small sperm samples, and compatible with a light microscope **(-)** Lower accuracy from difficulty in distinguishing intact and damaged chromatin.	7	Minor differences in fixation and incubation times
Acridine orange staining (AO) **Interpretation:** Damaged DNA appears yellowish-green to reddish-orange, and intact DNA appears green.	**(+)** Simple and offers clear colour contrast for easy distinction. **(-)** requires a fluorescence microscope	17	Incubation times range from overnight to 5 minutes
Sperm-Bos-Halomax kit (SBH) **Interpretation:** Large halos indicate fragmented DNA, while small halos indicate intact DNA.	**(+)** Simplifies sperm head differentiation and microscope compatibility. **(-)** Complex, costly, and requires a larger sperm sample for reliable analysis.	12	Staining techniques include Wright's eosin methylene blue, Propidium iodide, and FluoGreen
TUNEL Assay **Interpretation:** Lacks detailed interpretation.	**(+)** Highly sensitive to single-cell DNA strand breaks across species. **(-)** expensive, complex, and time-consuming.	1	Lacks detailed evaluation procedures.

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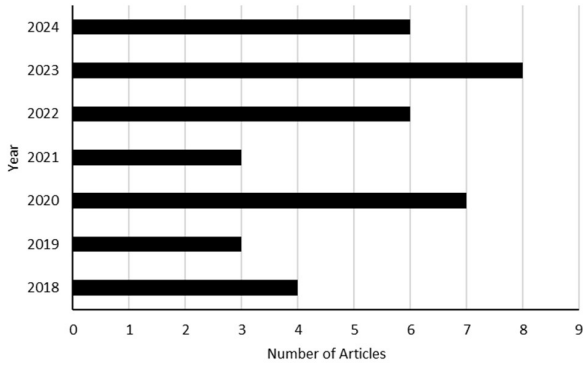

Figure 1 The number of articles published from Indonesia on bovine sperm DNA fragmentation.

■ COMMON TECHNIQUES

The most common techniques for analyzing sperm DNA fragmentation include acridine orange (AO) staining, the Sperm-Bos-Halomax (SBH) kit, toluidine blue (TB) staining, and the TUNEL assay. For AO staining, 17 studies consistently found that damaged DNA fluoresces yellowish-green to reddish-orange (Figure 2), while intact DNA appears green, although incubation times varied—overnight (Said *et al*. 2020), 12 hours (Arif *et al*. 2020), or 5 minutes (Pardede *et al*. 2021). The SBH kit yielded uniform interpretations (large halos = fragmentation; small halos = intact DNA), but the staining methods differed: Wright's eosin methylene blue (Satrio *et al*. 2022), propidium iodide (Pardede *et al*. 2022), eosin/methylene blue (Priyanto *et al*. 2018a), methylene blue alone (Prabowo *et al*. 2023), or Fluogreen (Baity *et al*. 2024). TB staining consistently showed bright blue heads (normal DNA) versus dark blue heads (fragmented DNA), although protocols varied—30-minute fixation/10-minute staining (Susilowati *et al*. 2021) or 60-minute fixation/5-minute staining (Pardede *et al*. 2020a). The TUNEL test (Prihantoko *et al*. 2020) lacks detailed evaluation criteria in the reviewed studies.

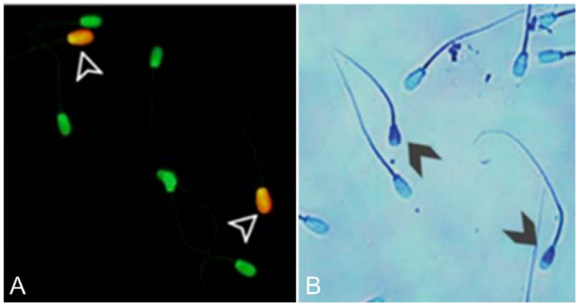

Figure 2. The photomicrograph shows sperm stained with acridine orange (AO) and toluidine blue (TB) methods. (A) In AO staining, damaged DNA appears yellowish-green to reddish-orange (white arrow), while intact DNA appears green (Pardede *et al*. 2022). (B) In TB staining, bright blue sperm heads indicate normal DNA, and dark blue (black arrow) shows DNA fragmentation (Pardede *et al*. 2020b).

■ FUTURE RESEARCH

Future research on bovine sperm DNA fragmentation in Indonesia should incorporate methods like sperm chromatin structure and COMET assays, compare their accuracy, explore fertility correlations, and investigate' omics' approaches for DNA fragmentation.

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■ REFERENCES

Arif AA, Maulana T, Kaiin EM, Purwantara B, Arifiantini RI, Memili E. 2020. Comparative analysis of various step-dilution techniques on the quality of frozen semen. Veterinary World. 13(11):2422-2428.

Baity AN, Maghfiroh NA, Fitriana SB, Prihantoko KD, Maharani D, Widayati DT. 2024. Effect of storage periods on DNA fragmentation of post-thawed Bali bull sperm. Advance of Animal Veterinary Sciences. 12(8):1456-1464.

Pardede BP, Agil M, Karja NWK, Sumantri C, Supriatna I, Purwantara. 2022. PRM1 gene expression and its protein abundance in frozen-thawed spermatozoa as potential fertility markers in breeding bulls. Veterinary Sciences. 9(3): 111.

Pardede BP, Agil M, Yudi Y, Supriatna I. 2020b. Relationship of frozen-thawed semen quality with the fertility rate after being distributed in the Brahman Cross breeding program. Veterinary World. 13(12):2649-2657.

Pardede BP, Maulana T, Kaiin EM, Agil M, Karja NWK, Sumantri C, Supriatna. 2021. The potential of sperm bovine protamine as a protein marker of semen production and quality at the nation artificial insemination center of Indonesia. Veterinary World. 14(9): 2473-2481.

Pardede BP, Supriatna I, Yudi Y, Agil M. 2020. Decreased bull fertility: age-related changes in sperm. E3S Web of Conferences, 1st ICVAES 2019. 151:01010.

Prabowo TA, Bintara S, Yusiati LM, Sitaresmi PI, Widayati D. 2023. Evaluation DNA fragmentation of local Indonesian cattle frozen sperm using Halomax® method. Biodiversitas. 24(4):2225-2230.

Prihantoko KD, Kusumawati A, Widayati DT, Pangestu M. 2020. Effects of storage duration on mitochondrial activity and DNA fragmentation of post-thawed spermatozoa from several ongole grade bull in Indonesia. Veterinary Practitioner. 21(2):264-268.

Priyanto L, Budiyanto A, Kusumawati A, Kurniasih, Arifiantini I. 2018. Perbandingan pemeriksaan kerusakan DNA spermatozoa post thawing antara Sperm-Bos-Halomax® dan Toluidine Blue. Jurnal Peternakan Sriwijaya. 7(1):30-39.

Said S, Maulana T, Setiorini S, Ibrahim G, Ramadhan M, Christophe E. 2020. Effect of addition an amino acid or its combination with EDTA on DNA integrity and morphometry sperm heads of freeze-dried bovine spermatozoa. Journal of the Indonesian Tropical Animal Agriculture. 45(3):189-196.

Satrio A, Karja NWK, Setiadi MA, Kaiin EM, Pardede BP, Purwantara B. 2024. Age-dependent variations in proteomic characteristics of spermatozoa in Simmental bull. Frontiers in Veterinary Science. 11:1393706.

Satrio FA, Karja NWK, Setiadi MA, Kaiin EM, Kurnia A, Purwantara B. 2022. Productivity and fresh semen characteristics of Simmental bulls of different ages. Jurnal Kedokteran Hewan. 16(1):23-28.

Susilowati S, ardjito T, Mustofa I, Widodo OS, Kurnijasanti R. 2021. Effect of green tea extract in extender of Simmental bull semen on pregnancy rate of recipients. Animal Bioscience. 34(2):198-204.