

SUPPORTING INFORMATION

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

Faisal Amri Satrio^{1,2,*}, Maghfira³, Ni Wayan Kurnia Karja⁴

¹ Veterinary Medicine Study Program, Faculty of Medicine, Padjajaran University, Bandung, Indonesia

² Department of Biomedical Science, Faculty of Medicine, Padjajaran University, Bandung, Indonesia

³ Animal Science Study Program, Faculty of Agriculture, Sultan Ageng Tirtayasa University, Serang, Indonesia

⁴ Division of Reproduction and Obstetrics, School of Veterinary Medicine and Biomedical Sciences, IPB University

[†] Electronic Supplementary Information (ESI) available.

See DOI: <https://dx.doi.org/10.29244/avl.9.1.29-30>

S1 Bovine sperm DNA fragmentation assessment methods

Table S1 shows the assessment methods that used in bovine sperm DNA fragmentation.

Table S1 Assessment methods of bovine sperm DNA fragmentation

Assessment methods/ microscopic interpretation	Advantages (+) and disadvantages (-)	Variations between authors	Number of articles	Breed/Authors
Toluidine blue staining 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.	minor differences in fixation and incubation times	7	• Brahman, Simmental, Limousin, Ongole (Priyanto <i>et al.</i> 2018a) • Simmental (Susilowati <i>et al.</i> 2021) • Bali, Madura (Safitri <i>et al.</i> 2022) • Brahman cross (Pardede <i>et al.</i> 2020b) • Indonesian local bulls (Pardede <i>et al.</i> 2020a) • Bali (Dwinofanto <i>et al.</i> 2018; Ardhani <i>et al.</i> 2020)
Acridine orange staining 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	Incubation times range from overnight to 5 minutes	17	• Bali, Simmental (Adiputra <i>et al.</i> 2022; 2023) • Limousin (Arif <i>et al.</i> 2020) • Bali (Fatmila <i>et al.</i> 2024; Hasbi <i>et al.</i> 2024) • Donggala (Gunawan <i>et al.</i> 2024) • Polled Bali (Gustina <i>et al.</i> 2024) • Holstein, Bali, Limousin (Handayani <i>et al.</i> 2021) • Polled Bali, Horned Bali (Hasbi <i>et al.</i> 2023) • Limousin, Holstein Friesian, Peranakan Ongole (Pardede <i>et al.</i> 2021; 2022) • Madura (Rosyada <i>et al.</i> 2023a; 2023b) • Holstein Friesian (Said <i>et al.</i>, 2020) • Pasundan (Rasad <i>et al.</i> 2020; Santoso <i>et al.</i> 2022) • Indonesian local bulls (Kusumawati <i>et al.</i> 2023)
Sperm-Bos-Halomax kit 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.	Staining techniques include Wright's eosin methylene blue (Satrio <i>et al.</i> , 2022), Propidium iodide (Pardede <i>et al.</i> , 2022), and Fluogreen (Baity <i>et al.</i> 2024).	12	• Simmental (Satrio <i>et al.</i> 2022b) • Indonesian local bulls (Kusumawati <i>et al.</i> 2023; Pardede <i>et al.</i> 2023; 2024) • Brahman, Simmental, Limousin, Ongole (Priyanto <i>et al.</i> 2018a) • Brahman (Priyanto <i>et al.</i> 2018b, 2019a, 2019b) • Bali, Madura, Aceh (Prabowo <i>et al.</i> 2023) • Limousin, Ongole, Holstein (Pardede <i>et al.</i> 2022) • Bali (Indriastuti <i>et al.</i> 2020; Baity <i>et al.</i> 2024)
TUNEL Assay Interpretation: Lacks detailed interpretation	(+) Highly sensitive to single- cell DNA strand breaks across species. (-) expensive, complex, and time-consuming	Lacks detailed evaluation procedures.	1	• Ongole Grade (Prihantoko <i>et al.</i> 2020)

S1 References

- Adiputra KDD, Maulana T, Kaiin EM, Sonjaya H. 2022. The semen quality of Bali and Simmental bulls reared in the technical implementation unit of the regional AI centre. *Advance in Animal Veterinary Sciences*. 10(12):2562-2570.
- Adiputra KDD, Sukandi S, Farida S, Sonjaya H, Hasbi H. 2023. Progressive motility, DNA fragmentation, intact plasma membrane, and acrosome status of frozen semen. *Hasanuddin Journal of Animal Sci-ence*. 4(2):109-118.
- Ardhani F, Mufidah H, Samsuriati R, Putra HP. 2020. The storage duration effects of Bali Bull's frozen semen at AI station on plasma membrane integrity, acrosome integrity, and spermatozoa DNA. *Jurnal Ilmu Peternakan Terapan*. 3(2):58-66.
- 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.
- Dwinofanto H, Rimayanti, Mustofa I, Susilowati S, Hernawati T. 2018. The effect of duration of preservation on the quality, MDA level, and DNA damage of post-thawed. *Iraq Journal of Veterinary Medicine Sciences*. 32(2):249-252.
- Fatmila DT, Pardede BP, Maulana T, Said S, Yudi Y, Purwantara B. 2024. Sperm HSP70 may not be an age-dependent gene but is associated with fertility. *Animal Reproduction*. 21(2):e20230048.
- Gunawan DD, Baharun A, Kaiin EM. 2024. Karakteristik integritas DNA dan defisiensi protamin semen beku sapi Donggala. *Jurnal Ilmiah Kajian Humaniora*. 8(8):29-40.
- Gustina S, Hasbi H, Sonjaya S, Baco S, Qhatiman H, Saputra W, Mutmainna M, Kaiin EM, Maulana T. 2024. Kualitas spermatozoa Sapi Bali Polled setelah pemberian pakan tersuplementasi taoge. *Jurnal Sain Veteriner*. 42(1):46-58.
- Handayani E, Supriatna I, Tumbelaka LITA, Kaiin EM. 2021. Analisis komparatif kualitas semen beku yang telah dan belum bersertifikat Standar Nasional Indonesia. *Jurnal Veteriner*. 22 (2):207-215.
- Hasbi H, Dagong MI, Zulkharnain Z, Baba S, Sonjaya H, Baco S, Gustina S, Maulana T, Gunawan M, Agung PP, Herlina N. 2023. Comparison of fresh and cryopreserved semen quality of polled and horned. *Iranian Journal of Applied Animal Science*. 13(1):33-41.
- Hasbi H, Iskandar H, Sonjaya H, Purwantara B, Arifiantini RI, Agil M, Pardede BP, Suyadi S, Septian WA, Samsudewa D, Damayanti E. 2024. Comparative developmental competence of in vitro embryos recovered from Bali cattle. *Veterinary World*. 17(3):593–601.
- Indriastuti R, Ulum MF, Arifiantini RI, Purwantara B. 2020. Individual variation in fresh and frozen semen of Bali bulls (*Bos sondaicus*). *Veterinary World*. 13(5):840-846
- Kusumawati A, Satrio FA, Indriastuti R, Rosyada ZNA, Pardede BP, Agil M, Purwantara B. 2023. Sperm head morphology alterations associated with chromatin instability and lack of protamine abundance in frozen-thawed sperm of Indonesian Local Bulls. *Animals*. 13: 2433.
- Pardede BP, Supriatna I, Yudi Y, Agil M. 2020a. Decreased bull fertility: age-related changes in sperm. *E3S Web of Conferences*, 1st ICVAES 2019.151: 01010
- 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, 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, Kusumawati A, Pangestu M, Purwantara B. 2023. Bovine sperm HSP-70 molecules: a potential cryo-tolerance marker associated with semen quality and fertility rate. *Frontiers Veterinary Sciences*. 10:1167594.
- Pardede BP, Karja NWK, Said S, Kaiin EM, Agil M, Sumantri C, Purwantara B, Supriatna I. 2024. Bovine nucleoprotein transitions genes and protein abundance as valuable markers of sperm motility and the correlation with fertility. *Theriogenology*. 215:86–94.
- Prabowo TA, Bintara S, Yusiati LM, Sitaesmi 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, Budiyo A, Kusumawati A, Kurniasih, Arifiantini I. 2018a. Perbandingan Pemeriksaan Kerusakan DNA Spermatozoa Post Thawing antara Sperm-Bos-Halomax® dan Toluidine Blue. *Jurnal Peternakan Sriwijaya*. 7(1):30-39.
- Priyanto L, Budiyo A, Kusumawati A, Kurniasih. 2018b. Tingkat kerusakan deoxyribonucleic acid spermatozoa memengaruhi profil protein spermatozoa pada semen beku Sapi Brahman. *Jurnal Veteriner*. 19(4):512-520.
- Priyanto L, Budiyo A, Kusumawati A, Kurniasih. 2019a. Kerusakan deoxyribonucleic acid spermatozoa memengaruhi tingkat kebuntingan Sapi Brahman. *Jurnal Veteriner*. 20(1):119-124.
- Priyanto L, Budiyo A, Kusumawati A, Kurniasih. 2019b. Pengaruh tingkat kerusakan deoxyribonucleic acid terhadap keguguran pada sapi. *Jurnal Peternakan Sriwijaya*. 8(1):28-35.
- Rasad SD, Solihati N, Winanun K, Yusrina A, Avicenna F. 2020. Effect of incubation time during sperm sexing process on sperm quality of Pasundan Bull. *Jurnal Ilmu Ternak dan Veteriner*. 25(3):112-119.
- Rosyada ZNA, Pardede BP, Kaiin EM, Tumbelaka LITA, Solihin DD, Purwantara B, Ulum MF. 2023a. Identification of heat shock protein70-2 and protamine-1 mRNA, proteins, and analyses of their association with fertility using frozen-thawed sperm in Madura. *Animal Bioscience*. 36(12):1796-1805.
- Rosyada ZNA, Yoelinda VT, Kaiin EM, Gunawan M, Ulum MF, Tumbelaka LITA, Solihin DD, Agil M, Gunawan A, Purwantara B. 2023b. Sperm osteopontin mRNA expression levels and its correlation on semen quality and fertility in Madura bulls. *Biodiversitas*. 24(1):563-570.
- Safitri BI, Wahjuningsih S, Yekti APA, Susilawati T. 2022. Effect of thawing technique on the quality and status of acrosome reactions. *Jurnal Ilmu dan Teknologi Peternakan Tropis*. 9(2):437-445.

- 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.
- Santoso, Gunawan A, Sumantri C, Arifiantini RI, Herdis. 2022. Differential Expressions of PRM1 and PRM2 Genes as Markers of Semen Quality in Pasundan Bulls. *Tropical Animal Sciences Journal*. 45(4):423-428.
- Satrio FA, Karja NWK, Setiadi MA, Kaiin EM, Kurnia A, Purwantara B. 2022a. Productivity and fresh semen characteristics of Simmental bulls of different ages. *Jurnal Kedokteran Hewan*. 16(1):23-28.
- Satrio FA, Karja NWK, Setiadi MA, Kaiin EM, Gunawan M, Purwantara B. 2022b. Post-Thaw Characteristics of the Simmental Sperm Function in Different Ages of Bulls. *Tropical Animal Sciences Journal*. 45(4):381-388.
- 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.
- 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.