Semen characteristics and production potential of Toraya Buffalo at UPT-PIBPS Pucak, South Sulawesi

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Abstract. This study aimed to evaluate the fresh semen characteristics, frozen semen production potential, and frozen semen quality of Toraya buffaloes at the Technical Implementation Unit for Artificial Insemination Services and Semen Production (UPT-PIBPS) Pucak, South Sulawesi, Indonesia. Two bulls, Sopai and Sadoko, were assessed to determine their reproductive potential. Fresh semen analysis showed that Sopai produced the highest number of ejaculates (84), with an average ejaculate volume of 1.88±0.19 mL and sperm concentration of 1133.91±31.13 million/mL. In contrast, Sadoko demonstrated a higher sperm concentration (1466.55±71.35 million/mL) and a greater number of potential straws per ejaculate (78.89) compared to Sopai (59.51). Analysis of frozen semen revealed significantly higher quality in Sopai's semen ($p<0.05$). The Sopai semen may be better suited for artificial insemination programs emphasizing frozen semen quality and cryopreservation efficiency, while Sadoko's higher sperm concentration and greater straw production potential could be advantageous for large-scale artificial insemination programs requiring high semen output. In conclusion, the fresh semen characteristics, frozen semen production potential, and overall quality of Toraya buffaloes at UPT-PIBPS Pucak meet the standards for frozen semen production. The consistent ejaculate volume, high motility, and adequate sperm concentration highlight their strong potential to support artificial insemination programs in Indonesia.

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1 Introduction

Semen characteristics and production potential serve as key indicators in determining the effectiveness of artificial insemination (AI) programs in livestock. In Indonesia, AI has emerged as a strategic method to improve livestock productivity, particularly in buffalo species (*Bubalus bubalis*). The Toraya buffalo (*Bubalus bubalis* carabensis), a local subspecies from South Sulawesi, possesses unique and significant genetic potential that warrants optimization. Beyond its vital role in local traditions and culture, this buffalo exhibits reproductive and adaptive characteristics that make it a superior candidate for national livestock development programs [1]

The application of modern reproductive technologies, such as frozen semen production, facilitates the widespread dissemination of the Toraya buffalo's superior traits through AI programs. Evaluating the characteristics of fresh semen is a crucial preliminary step in determining the reproductive quality of sires. Superior bulls are selected based on their pedigree, production traits, and reproductive performance [2]. High-quality semen must meet or exceed the minimum standards for fresh semen before being processed into frozen semen. Bull fertility is influenced by factors such as age, breed, body condition, nutrition, ejaculation capacity, and environmental conditions [3].

Cryopreservation technology plays a vital role in the long-term preservation of semen, facilitating the distribution of frozen semen to various regions to support breeding programs. However, the cryopreservation process poses challenges, including osmotic and oxidative stress on sperm, which can affect motility, viability, and membrane integrity [4]. The reproductive physiology of tropical buffalo, including Toraya buffalo, is influenced by environmental factors such as high temperatures, humidity, and fluctuating feed availability. These factors often present obstacles to achieving optimal reproductive performance [5] Dash. Nevertheless, the genetic adaptation of Toraya buffalo to tropical environments makes it a promising species for further development.

Studies on frozen semen in tropical buffalo have shown that the success of cryopreservation is influenced not only by the quality of fresh semen but also by specific parameters such as progressive motility, average path velocity (VAP), straight-line velocity (VSL), and curvilinear velocity (VCL) [6]. The Technical Implementation Unit for Artificial Insemination Services and Semen Production (UPT-PIBPS) in Pucak, South Sulawesi, plays a key role in advancing artificial insemination (AI) technology using superior buffalo bulls for frozen semen production. Sopai and Sadoko, two Toraya buffalo bulla at UPT-PIBPS, have been the focus of research assessing their reproductive potential.

The evaluation of fresh semen characteristics, including ejaculate volume, sperm concentration, progressive motility, and the number of straws per ejaculate, provides essential insights into semen production capacity. Likewise, frozen semen quality parameters, such as progressive motility and sperm velocity, serve as indicators of sperm resilience during the cryopreservation process [7]. This study aims to analyze the characteristics of fresh semen, the production potential of frozen semen, and the quality of frozen semen in Toraya buffalo at UPT-PIBPS, Pucak, South Sulawesi, Indonesia. The findings are expected to make a significant contribution to the development of more effective reproductive strategies, ultimately enhancing the productivity and sustainability of Toraya buffalo farming in Indonesia.

2 Material and methods

2.1 Animals

This study was conducted at UPT-PIBPS Pucak, Maros, South Sulawesi. The research involved two Toraya buffalo bulls aged 8–10 years. Over a period of two years (2022 and 2023), a total of 119 ejaculates were collected. The bulls were maintained under uniform management conditions following the standard operating procedures of UPT-PIBPS.

2.2 Semen collection and evaluation

Semen collection was performed once a week in the morning using an artificial vagina, adhering to the Standard Operating Procedure (SOP) of UPT-PIBPS. Immediately after collection, semen quality was analyzed in the laboratory through direct observation. Microscopic evaluations included sperm mass movement, individual motility, and sperm concentration, while macroscopic assessments covered semen volume, pH, and color. Semen volume (mL) was measured directly using a graduated collection tube. Sperm concentration was assessed using a spectrophotometer.

Frozen semen quality was evaluated from three different production batches. Sperm motility and kinematic parameters were analyzed using a computer-assisted semen analysis (CASA) system (SpermVision™ 3.7.8, Minitube, Germany), connected to a Carl Zeiss Microimaging GmbH device (Göttingen, Germany). The assessed parameters included total motility, Velocity Average Path (VAP), Velocity Curve Length (VCL), Velocity Straight Length (VSL), straightness (STR), linearity (LIN), amplitude of lateral head displacement (ALH), and beat-cross frequency (BCF). Additional analyses involved sperm abnormality and viability using the eosin-nigrosine staining method, as well as membrane integrity assessment via the hypoosmotic swelling (HOS) test [6].

Acrosome integrity was evaluated using fluorescence staining with FITC-PNA (Sigma, St. Louis, MO) in combination with propidium iodide (PI) [8]. Sperm DNA fragmentation was analyzed using the Sperm-Bos-Halomax® kit (Halotech DNA, SL; Campus de Cantoblanco, Madrid, Spain), following the manufacturer's guidelines.

2.3 Data classification

The total motile spermatozoa of each Toraya buffalo bull over two years were quantified to evaluate reproductive performance. Bull productivity was determined based on the Total Motile Spermatozoa per ejaculate using the formula adapted from [9]:
$Total\ Motile\ Spermatozoa\ per\ Ejaculate\ (\times 10^6) = Volume \times Concentration \times Indiv.\ Motility$
According to the Standard Operating Procedures (SOP) of UPT-PIPBS, a single semen straw (0.25 ml) contains 25×10^6 spermatozoa. Therefore, the estimated annual production of frozen semen straws was calculated using the formula:
$Number\ of\ Straws\ (Doses) = Total\ Motile\ Spermatozoa\ /\ 25\times10^6$

2.4 Statistical analysis

Frozen semen data were statistically analyzed using an unpaired t-test, processed with SPSS 24, and expressed as mean ± standard deviation (SD).

3 Results and discussions

The study results revealed significant differences in semen characteristics between Sopai and Sadoko bulls. Sopai produced a higher total number of ejaculations (84 times) compared to Sadoko (35 times). However, Sopai also had a greater number of rejected and aceepted ejaculation, indicating that despite being more productive in quantity (Table 1). This difference may be related to Sopai's higher ejaculation frequency, aligning with previous findings that increased ejaculation frequency can affect sperm concentration and overall quality [10]. The sperm motility of Sopai and Sadoko exceeds 65%, although this value remains below the SNI standard for frozen semen, which is set at 70%. However, according to SNI, if the progressive motility of spermatozoa in certain bulls is below 70%, a recovery rate value with a minimum threshold of 50% can still be applied [2].

Tabel 1. Toraya buffalo bulls fresh semen chacracteristics in UPT-PIBPS

Parameter	Bulls (Mean±SD)	
	Sopai	**Sadoko**
Total ejaculate (n)	84	35
Rejected ejaculate	18	6
Accepted ejaculate	66	29
Volume (mL)	1,97±0,04	2,07±0,26
Density (pH)	6.40±0.0	6.40±0.0
Consistency (1-3)	1.90±0.24	2.18±0.32
Mass movement (1-3)	1.87±0.25	2.21±0.37
Concentration (x10^6)	1073,54±62,42	1238,90±35,78
Motility (%)	66,61±4,80	65,13±6,89
Semen production (doses)	2959±695.09	1761±235.47
Total motile sperm (x10^6)	2134,26±462,13	5745,21±1778,50
Potential straw/ejaculate (doses)	85,37±18,49	229,81±61,97

In terms of fresh semen characteristics, Sadoko demonstrated superiority across several parameters (Table 1). Sadoko's semen consistency was higher (2.18 ± 0.32) compared to Sopai's (1.87 ± 0.25), indicating a denser sperm content. Additionally, Sadoko exhibited a higher sperm concentration (1238.90 ± 35.78 × 10^6/mL) than Sopai (1073.54 ± 62.42 × 10^6/mL) and superior sperm mass movement (2.21 ± 0.37 vs. 1.87 ± 0.25). These parameters collectively highlight better sperm vitality and quality in Sadoko. Baharun et al. [9] reported that fresh semen quality, including sperm concentration and motility, plays a pivotal role in optimizing the efficiency of artificial insemination programs. Therefore, Sadoko's superior fresh semen quality significantly enhances its potential for use in artificial insemination programs.

The findings also revealed a marked difference in the total motile sperm count, where Sadoko reached 5745.21 ± 1778.50 x10^6 sperm, substantially higher than Sopai's 2134.26 ± 462.13 x10^6 sperm. This directly impacts the potential frozen semen doses per ejaculate, with Sadoko producing an average of 229.81 ± 61.97 doses nearly three times more than Sopai, who produced only 85.37 ± 18.49 doses. TMS and straw production are influenced by semen quality, management practices, and breed variability, which affect sperm concentration, motility, and semen traits [11]. This disparity underscores Sadoko's greater productivity and suitability for semen freezing and artificial insemination applications.

Tabel 2. Frozen semen characteristic of superior toraya buffalo

Parameter	Bulls (Mean±SD)		P-value
	Sadoko	Sopai	
Progressive Motility, pMot (%)	52,76±3,94	47,54±2,46	0.123
Velocity Average Path, VAP (µm/s)	65,79±2,23	71,88±2,36	0.032*
Velocity Curve Length, VCL (µm/s)	95,45±4,20	106,36±3,45	0.025*
Velocity Straight Length, VSL (µm/s)	43,97±1,14	47,51±1,10	0.018*
Straightness, STR (%)	66,33±0,58	65,66±1,15	0.422
Linearity, LIN (%)	45,67±1,15	44,33±0,58	0.148
Amplitude of Lateral Head, ALH (µm)	5,35±0,11	5,98±0,37	0.047*
Beat Cross Frequency, BCF (Hz)	23,14±1,0	22,38±0,98	0.401
Viability (%)	56,22±1,32	47,81±8,58	0.169
Abnormality (%)	4,23±1,13	7,97±3,95	0.190
Membrane Integrity (%)	59,55±5,09	55,56±6,29	0.382
Acrosomal status (%)	88,31±5,09	90,08±0,74	0.582
DNA fragmentation Index, DFI (%)	3,88±1,62	3,0±1,40	0.516

Further evaluation of frozen semen parameters (Table 2) indicated that Sadoko exhibited higher progressive motility (pMot) (52.76 ± 3.94%) compared to Sopai (47.54 ± 2.46%), though this difference did not reach statistical significance (P=0.123). Conversely, sperm velocity parameters, including Velocity Average Path (VAP), Velocity Curve Length (VCL), and Velocity Straight Length (VSL), were significantly greater in Sopai than in Sadoko (P<0.05). The increased sperm velocity observed in Sopai may contribute to enhanced oocyte penetration capability [12].

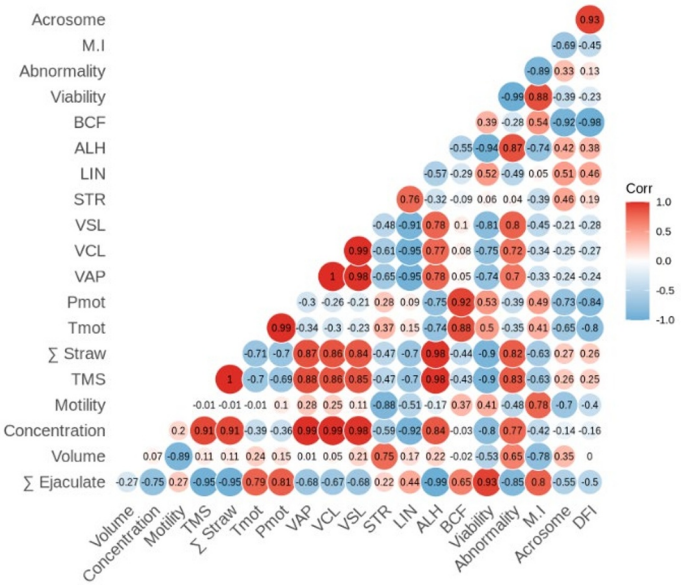

Fig. 1. Matrix correlation analysis of sperm characteristics and frozen semen potential production

Additionally, Sopai's amplitude of lateral head displacement (ALH) (5.98 ± 0.37 μm) was significantly greater than Sadoko's (5.35 ± 0.11 μm; P=0.047), indicating more active lateral sperm movement. These parameters, along with straightness (STR) and beat-cross frequency (BCF), reflect Sopai's superior sperm motility quality. Conversely, Sadoko showed higher sperm viability (56.22 ± 1.32%) compared to Sopai (47.81 ± 8.58%), although the variation was not statistically significant (P=0.169). Sopai's higher sperm abnormality rate (7.97 ± 3.95%) compared to Sadoko (4.23 ± 1.13%) [13] reported Morphological abnormalities often correlate with compromised DNA integrity, such as increased DNA fragmentation and affects embryo development and overall fertility.

The correlation analysis revealed significant relationships between sperm quality parameters and fertility (Fig. 1). Motility parameters such as VCL, VSL, and VAP exhibited very strong positive correlations ($r > 0.9$), highlighting their close association as indicators of sperm efficiency and quality [14]. Conversely, sperm abnormalities showed a strong negative correlation with motility parameters ($r \approx -0.8$), underscoring the detrimental impact of morphological defects on sperm movement efficiency.

Although sperm volume and concentration are important quantitative parameters, their correlations with motility were weak to moderate, indicating that quantity alone does not always reflect overall sperm quality. However, the positive correlation between the number of straws produced (∑ Straw) and total motility (Tmot) underscores the importance of motility quality in cryopreservation. This study offers a comprehensive analysis of the semen characteristics of Sopai and Sadoko bulls. The higher viability and lower sperm abnormality rates observed in Sadoko, along with the superior sperm velocity and lateral movement amplitude in Sopai, indicate that both bulls possess traits that can be effectively utilized in artificial insemination programs.

4 Conclusion

This study concludes that the fresh semen characteristics, frozen semen production potential, and overall quality of Toraya buffaloes at UPT-PIBPS Pucak meet the standards for frozen semen production. The stable ejaculate volume, high motility, and sufficient sperm concentration highlight their strong potential to support artificial insemination programs in Indonesia.

References

1. T. Maulana, H. Iskandar, S. Said, A. Gunawan, The Current Status and Potential Development of Genetic Resources of Indigenous Toraya Spotted Buffalo in Indonesia: A Systematic Review. World Vet. **13**(4), 617-625 (2023). https://doi.org/10.54203/scil.2023.wvj66
2. E.K. Klein, A. Swegen, A.J. Gunn, C.P. Stephen, R.J. Aitken, Z. Gibb, The future of assessing bull fertility: Can the 'omics fields identify usable biomarkers?. Biol. Reprod. **106**(5), 854–864 (2022). https://doi.org/10.1093/biolre/ioac031
3. M.M. Rojas-Downing, A.P. Nejadhashemi, T.M. Harrigan, S.A. Woznicki, Climate change and livestock: Impacts, adaptation, and mitigation. Clim. Risk Manag. **16**, 145-163 (2017). https://doi.org/10.1016/j.crm.2017.02.001
4. M.R. Ugur, A. Saber Abdelrahman, H.C. Evans, A.A. Gilmore, M. Hitit, R.I. Arifiantini, B. Purwantara, A. Kaya, E. Memili, Advances in Cryopreservation of Bull Sperm. Front. Vet. Sci. **6**, 268 (2019). https://doi.org/10.3389/fvets.2019.00268

5. S. Dash, A.K. Chakravarty, A. Singh, A. Upadhyay, M. Singh, S. Yousuf, Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. Vet world. **9**(3), 235–244 (2016). https://doi.org/10.14202/vetworld.2016.235-244
6. A.S. Amal, R. I. Arifiantini, M. A. Setiadi, and S. Said, Characteristics of the postthawed Balinese bull semen extended in three different extenders and equilibration times, J. Indonesian Trop. Anim. Agric. **44** (2), 135-145 (2019). https://doi.org/10.14710/jitaa.44.2.135-145
7. W.A. Khalil, M.A. El-Harairy, A.E.B. Zeidan, M.A.E. Hassan, O. Mohey-Elsaeed, Evaluation of bull spermatozoa during and after cryopreservation: Structural and ultrastructural insights. Int. J. Vet. Sci. Med. **6**, S4 9–S56 (2017). https://doi.org/10.1016/j.ijvsm.2017.11.001
8. R. Rajabi-Toustani, Q. S. Akter, E. A. Almadaly, Y. Hoshino, H. Adachi, K. Mukoujima, T. Murase, Methodogical improvement of fluorescein isothiocyanate peanut agglutinin (FITC-PNA) acrosomal integrity staining for frozen-thawed Japanese Black bull spermatozoa. J. Vet. Med. Sci. **81**, 649–702 (2019). https://doi.org/10.1292/jvms.18-0560
9. A. Baharun, I. Arifiantini, N.W.K. Karja, S. Said, Seminal plasma protein profile based on molecular weight and their correlation with semen quality of Simmental bull. J. Indonesian Trop. Anim. Agric. **46**(1), 20-28 (2021). https://doi.org/10.14710/jitaa.46.1.20-28
10. K.H. Parmar, F.S. Kavani, T.K. Patbandha, S.S. Parikh, Fresh and Post-Thaw Seminal Attributes of Jafarabadi Buffalo Bulls. Ind J. Vet. Sci. Biotech. **15**(3),45-48 (2020). https://doi.org/10.21887/ijvsbt.15.3.12
11. K. Bhave, T.P.J. Koilpillai, V. Ragothaman, S. Sontakke, G. Joshi, V. Ducrocq, Semen production and semen quality of indigenous buffalo breeds under hot semiarid climatic conditions in India. Trop. Anim. Health. Prod. **52**(5), 2529–2539 (2020). https://doi.org/10.1007/s11250-020-02284-9
12. S.G. Goodson, S. White, A.M. Stevans, S. Bhat, C.Y. Kao, S. Jaworski, D.A. O'Brien, CASA nova: A multiclass support vector machine model for the classification of human sperm motility patterns. Biol of Reprod. **97**(5), 698–708 (2017). https://doi.org/10.1093/biolre/iox120
13. G.B. Boe-Hansen, M.R.S. Fortes, N. Satake, Morphological defects, sperm DNA integrity, and protamination of bovine spermatozoa. Andrology. **6**(4), 627–633 (2018). https://doi.org/10.1111/andr.12486
14. B.M. Tanga, A.Y. Qamar, S. Raza, S. Bang, X. Fang, K. Yoon, J. Cho, Semen evaluation: methodological advancements in sperm quality-specific fertility assessment - A review. Anim. Biosci. **34**(8), 1253–1270 (2021). https://doi.org/10.5713/ab.21.0072