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Research Article

Optimalization of the Swim-Up Method for Bali Bull Sperm: Effect of Incubation Time and Medium on Sperm Kinetics

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Abstract: Bali bulls (Bos javanicus) are an essential part of Indonesia's indigenous livestock, prized for their ability to thrive in challenging environments, their natural disease resistance, and their strong reproductive capabilities. The aim of this study was to refine the swim-up technique for preparing Bali cattle sperm. We evaluated the effects of two commonly used media Tyrode's Albumin Lactate Pyruvate (TALP) and Brackett-Oliphant (BO) and various incubation times (30, 45, and 60 minutes) on sperm motility, progressive motility, and movement characteristics. Semen samples from Bali cattle were processed using the swim-up technique and then analyzed using a Computer-Assisted Sperm Analysis (CASA) system. The results were quite interesting: After 45 minutes of incubation, in both TALP and BO media, there was a significant increase in sperm motility and progressive motility. Unfortunately, after 60 minutes, these values actually decreased, likely due to oxidative stress and energy exhaustion of the sperm. Key movement parameters, such as Curvilinear Velocity (VCL) and average path velocity (VAP), also reached their peak values at 45 minutes. Equally important, both media TALP and BO, were shown to support sperm capacitation and viability. There were no significant differences between the two. Our findings highlight the importance of managing incubation time in the swim-up procedure. Forty-five minutes is the optimal duration for obtaining the highest quality sperm. This knowledge is invaluable, especially for assisted reproductive technologies such as ICSI. By following this optimized swim-up protocol, the chances of obtaining sperm with agile movement and superior kinematics are greater ultimately increasing the likelihood of successful fertilization. On a broader scale, this research not only supports the conservation of Bali cattle, but also contributes to maintaining their genetic diversity and supporting sustainable livestock farming practices in the tropics.

Keywords: Bali Bull, Swim-Up Method, Sperm Motility, Sperm Kinematics, Assisted Reproduction

Introduction

Bali bulls (Bos javanicus) are central to Indonesia's indigenous livestock systems. They cope well with heat and humidity, show natural tolerance to local diseases, and maintain reliable fertility, which makes them vital for smallholder livelihoods (Diansyah *et al*., 2025). As breeding programs seek faster genetic gains, the risk of losing native variation grows. This creates a clear need for reproductive technologies that both conserve and improve Bali bull genetics (Said, 2020).

Intracytoplasmic Sperm Injection (ICSI) is useful when gametes are scarce or sperm quality is reduced. Its success depends on selecting a single high-quality sperm cell, so the preparation method matters greatly (Ribas-

Maynou *et al*., 2022). The swim-up approach takes advantage of sperm motility: More active and morphologically normal cells move into the upper layer, leaving slower or damaged cells behind (Bouloorchi *et al*., 2024; Medica *et al*., 2024). Outcomes are shaped by the medium and the incubation period. TALP approximates conditions in the female reproductive tract and supports capacitation, while BO is formulated for in vitro use with energy substrates and pH control (Indhu *et al*., 2021; Mahmoud *et al*., 2023).

This study aims to observe the effect of medium type (TALP and BO) and incubation time (30, 45, and 60 minutes) on sperm motility and progressive motility in Bali bull semen. After collection, semen samples were stored in closed containers at 4 °C until testing. Kinematic parameters were analyzed using CASA after the swim-up procedure was performed. The aim is to deliver a practical, optimized protocol for sperm selection that supports ICSI and related ART, while also advancing conservation and sustainable use of this native breed.

Materials and Methods

Experimental Animals and Design

This study was conducted at the Animal Reproduction Laboratory, Faculty of Animal Husbandry, Hasanuddin University, Makassar. Sampling Sampling was performed five times on each of five Bali bulls. Semen collection was performed twice a week using an artificial vagina and in accordance with the protocol by Diansyah *et al*. (2023). Three incubation durations were used: 30 minutes (I1), 45 minutes (I2), and 60 minutes (I3). The incubation intervals were selected based on previous research by Lestari *et al*. (2018), which suggested that shorter incubation times could result in inandequate recovery of motile sperm, while longer incubation durations could cause oxidative stress, which negatively affect sperm energy balance. A control group of fresh semen was also used for comparison. To assess the impact of medium composition, all sampeles were processed using both TALP and BO media.

TALP Medium Preparation

TALP medium was prepared according to the method describe by Singina *et al*. (2016) with slight modification. First, NaCl, KCL, and $CaCl_{2.2}H_2O$ were dissolved in distilled water and homogenized, after which NaHCO3 and NaH2PO4 were added. Then, the solution was stirred continuously while adding HEPES, sodium pyruvate and phenol red, followed by the addition of sodium lactate and vigorous stirring with a vortex to ensure maximum dissolution. The pH is measured with a calibrated pH meter and adjusted to a pH range of 6-7. HEPES is added to stabilize the pH during the swim-up process, while bovine serum albumin (BSA, 5 mg/mL) is added to maintain sperm membarane integrity. The final medium is stored in a closed container at 4 degrees until use.

BO Medium Preparation

The preparation involved dissolving each ingredient NaCl, KCl, $MgCl_2$, NaH_2PO_4, $NaHCO_3$, $CaCl_2.2H_2O$, and sodium pyruvate in distilled water within a test tube. The first ingredient to be mixed is Nacl, KCl, $CaCl_2.2H_2O$, then homogenize after that the second ingredient is $NaHCO_3$ (Sodium Bicarbonate), NaH_2PO_4 (Sodium Dihydrogen Phosphate) then homogenize, after that the third ingredient is hepes, pyruvate, phenol red, then homogenize again and then mix the last solution, namely Na Lactate and then vortex until homogeneous. To ensure pH stability during the swim-up process, HEPES was incorporated. Similar to TALP medium, BSA at a concentration of 5 mg/mL was added to support sperm stability. The BO medium was stored in a closed container at 4°C until further use.

Sperm Selection (Swim Up)

Sperm selection was carried out using the swim-up method, following the protocol outlined by Diansyah *et al*. (2020). This method takes advantage of the natural motility of sperm, allowing the more active sperm to swim from the pellet at the bottom of the medium to the upper layer (Fig. 1). First, 50 µL semen aliquot was diluted with 10 mL of TALP medium and centrifuged at 1800 rpm for 10 minutes. The supernatant is separated to remove immotile sperm and debris, then the pellet is resuspended in 1 mL of TALP medium before being transferred to a 1.5 mL microtube. The suspension is then incubated at 37°C.

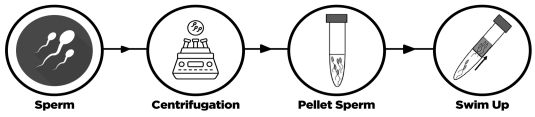

Fig. 1: Schematic flow of swim up method

Sperm Kinematics

Sperm kinematics analysis was performed using Computer-Assisted Sperm Analysis (CASA), specifically Sperm-Vision software (Minitub, Germany), according to the protocol by Raafi *et al.* (2021). The analysis was performed at 400x magnification, with a minimum of 200 sperm per sampel anayzed to ensure observation accuracy. The parameters measured included total motility, progressive motility (pMot), and three pairs of velocity and distance parameters, namely curvilinier velocity (VCL), curvilinier distance (DCL), average path velocity (VAP), average path distance (DAP), straight-line velocity (VSL), and straight-line distance (DSL). In

adittion, sperm movement characteristics were evaluated using linearity (LIN), straightness (STR), and wobble (WOB), as defined by Diansyah *et al*. (2022).

Data Analysis

Our research data is presented as mean ± standard deviation, allowing readers to see not only the mean but also the variability of the data. We performed statistical analysis using SPSS version 26. To determine whether there were differences between treatments, we applied One-way Analysis of Variance (ANOVA). If the ANOVA results showed significant differences, we continued with Duncan's test to determine which treatment specifically differed. All results were considered statistically significant if the p-value was less than 0.05.

Results

Sperm Motility and Progressive Motility Post-Swim-Up of Bali Bulls in Different Medium and Incubation Duration

Baseline evaluation of Bali bull semen, illustrated in Figure 1, revealed satisfactory motility and progressive motility prior to the swim-up, providing a benchmark to assess the procedure's effectiveness and the impact of incubation period and medium. Post-swim-up, as presented in Table 1 sperm motility and progressive motility varied significantly depending on the incubation duration (I1 = 30 minutes, I2 = 45 minutes, I3 = 60 minutes) and the medium used (BO and TALP). For both TALP and BO, the greatest motility and progressive motility values were recorded at 45 minutes (I2), followed by a decline at the 60-minute (I3) interval. Statistical analysis confirmed significant differences in values within each medium across incubation durations ($p<0.05$), while p-values of 0.364 for motility and 0.978 for progressive motility indicated no statistically significant differences between the BO and TALP media. As presented in Figure 1 and Table 1, sperm motility and progressive motility improved markedly after 45 minutes of incubation, whereas longer incubation caused a noticeable reduction in semen quality.

Sperm Distance Post-Swim-Up of Bali Bulls in Different Medium and Incubation Duration

The sperm distance of fresh semen from Bali bulls, as shown in Figures 2 and 3, provides baseline data for evaluating the effectiveness of the swim-up procedure and the effects of different incubation durations and media. Post-swim-up sperm distance (Table 2) showed variations across incubation durations (I1 = 30 minutes, I2 = 45 minutes, I3 = 60 minutes) and media (BO and TALP). Curvilinear distance (DCL) peaked at 45 minutes (I2) in both BO and TALP groups. However, no significant differences were found across incubation durations or between media ($p = 0.414$). For average path distance (DAP), significant differences were observed within each medium, with the highest value at 45 minutes (I2) and the DAP values decreased significantly in both media at 60 minutes (I3). When comparing BO with TALP in terms of DAP, no significant differences were observed ($p = 0.547$). For straight-line distance (DSL), the highest values were also recorded at 45 minutes (I2) in both media, although differences were not statistically significant ($p = 0.718$). The data in Figure 2 and Table 2 indicate that the swim-up procedure enhances sperm distance parameters, particularly at 45 minutes (I2) of incubation, while extended incubation to 60 minutes (I3) leads to a decline in these values.

Table 1: Sperm motility and Progressive motility post-swim-up of Bali bulls in different medium and incubation duration

Parameter	Medium	Incubation			p-Value
		I1	I2	I3	
Motility	BO	83.76±5.13a	87.80±2.6a	79.27±5.97^{b}	0.364
	TALP	84.43±3.44a	89.17±2.42a	71.51±24.27^{b}	
pMot	BO	70.35±8.27a	76.72±4.59b	65.94±7.52^{c}	0.978
	TALP	72.13±3.59a	78.86±3.58b	67.24±6.58^{c}	

Note: Means in a row with different superscripts differ on the same parameter significantly at $p<0.05$. I1 = 30 minutes; I2 = 45 minutes; I3 = 60 minutes

Table 2: Sperm distance post-swim-up of Bali bulls in different medium and incubation duration

Parameter	Medium	Incubation			p- Value
		I1	I2	I3	
DCL	BO	49.365±7.43	54.965±6.29	48.473±5.31	0.414
	TALP	51.955±6.36	49.581±17.39	48.453±5.54	
DAP	BO	25.715±4.30^{a}	27.595±2.55^{b}	24.698±3.44^{a}	0.547
	TALP	27.477±2.62^{a}	31.231±4.34^{b}	25.847±3.37^{a}	
DSL	BO	17.502±3.89	18.036±2.54	16.544±3.31	0.718
	TALP	18.443±2.51	20.032±2.95	16.933±2.54	

Note: Means in a row with different superscripts differ on the same parameter significantly at $p<0.05$. I1 = 30 minutes; I2 = 45 minutes; I3 = 60 minutes

Table 3: Sperm velocity post-swim-up of Bali bulls in different medium and incubation duration

Parameter	Medium	Incubation			p-Value
		I1	I2	I3	
VCL	BO	120.03±18.51[a]	134.96±16.33[b]	119.92±12.03[a]	0.583
	TALP	127.95±13.02[a]	134.76±14.28[b]	117.86±16.16[a]	
VAP	BO	62.94±9.83[a]	68.11±5.85[b]	61.48±7.47[a]	0.91
	TALP	67.28±7.00[a]	72.83±8.99[b]	64.04±8.17[a]	
VSL	BO	42.96±8.98	44.69±5.86	41.41±7.37	0.692
	TALP	44.18±5.91	48.81±7.58	41.57±6.60	

Note: Means in a row with different superscripts differ on the same parameter significantly at p<0.05. I1 = 30 minutes; I2 = 45 minutes; I3 = 60 minutes

Table 4: Sperm movement pattern post-swim-up of Bali bulls in different medium and incubation duration

Parameter	Medium	Incubation			p- Value
		I1	I2	I3	
LIN	BO	36.11±6.47	33.56±5.17	34.44±5.43	0.514
	TALP	34.44±4.41	36.12±4.5	35.33±5.85	
STR	BO	68.21±5.43	65.56±4.53	67.14±4.84	0.581
	TALP	65.55±7.21	66.67±3.70	64.78±7.47	
WOB	BO	52.67±5.63	50.78±4.49	51.22±4.17	0.49
	TALP	52.44±4.00	54.11±5.51	54.33±5.65	

Note: Means in a row with different superscripts differ on the same parameter significantly at p<0.05. I1 = 30 minutes; I2 = 45 minutes; I3 = 60 minutes

Sperm Movement Pattern Post-Swim-Up of Bali Bulls in Different Medium and Incubation Duration

The movement patterns of fresh sperm from Bali bulls, as depicted in Figures 4 and 5, provide baseline data to assess the impact of the swim-up procedure across different incubation durations and media. Post-swim-up analysis (Table 4) revealed no significant Variations in Linearity (LIN), Straightness (STR), or Wobble (WOB) across incubation durations or between BO and TALP media, as indicated by p-values of 0.514, 0.581, and 0.49, respectively. These findings suggest that the swim-up procedure maintains stable sperm movement patterns regardless of the incubation duration or medium used. Stable LIN, STR, and WOB values indicate that the swim-up procedure maintains key motility attributes without causing major alterations or deterioration.

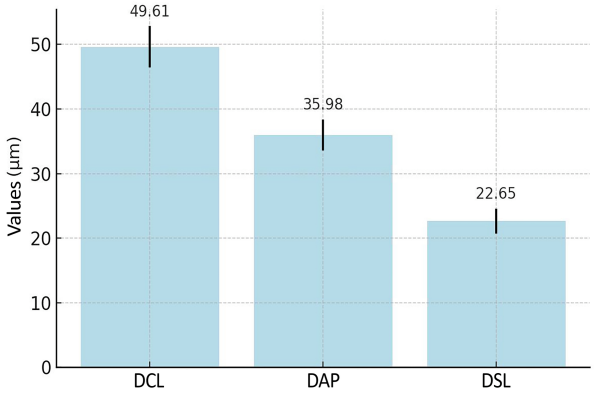

Fig. 3: Sperm distance of fresh semen Bali bulls

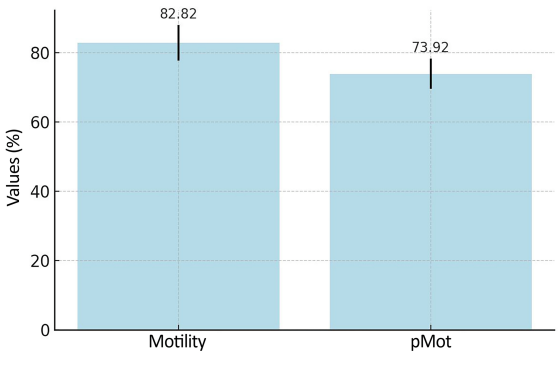

Fig. 2: Sperm motility and Progressive motility of fresh semen Bali bulls

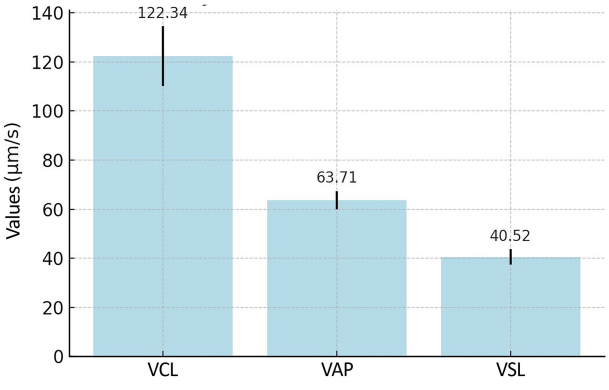

Fig. 4: Sperm velocity of fresh semen Bali bulls

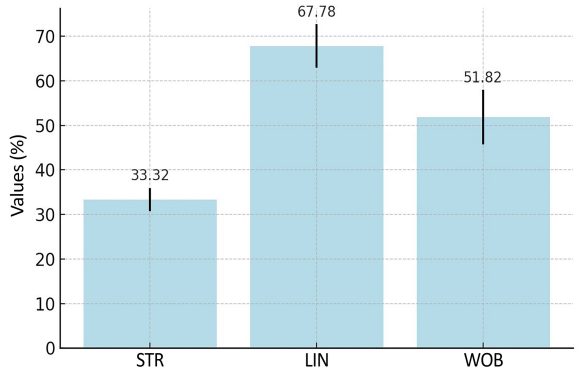

Fig. 5: Sperm movement pattern of fresh semen Bali bulls

Discussion

The swim-up method is effective in improving sperm quality, and our results could serve as a valuable reference for selecting optimal sperm for ICSI procedures and other assisted reproductive technologies. Both the TALP and BO methods showed similar result in Bali cattle (Bos javanicus) sperm. Although statistical analysis showed no significant differences ($p>0.05$), the swim-up method consistently improved sperm motility, progressive motility (pMot), and kinematic parameters. These findings confirm that the swim-up method is a promising method for producing high-quality sperm, especially for use in reproductive technologies such as Intracytoplasmic Sperm Injection (ICSI).

The swim-up method can effectively isolate sperm with high motility and normal morphology, which are key factors in determining fertilization success. These results are consistent whit the findings of previous studies by Baldini *et al*. (2021); Afriani *et al*. (2022). Optimal motility is achieved when sperm migrate toward the upper layer of the medium, whit a significant increase in distance and speed sperm have sufficient energy reserves and better kinematic performance.

Comparative analysis shows that both madia offer similar conditions, namely, favourable for sperm capacitation whitout compromising cell viability. Both media contain essential ions and substrates that support ATP Production and calcium signalling, which influence critical processes for sperm hyperactivation and oocyte penetration (Swain *et al*., 2022; Cordero-Martinez *et al*., 2022). Additionally, the addition og Bovine Serum Albumin (BSA) in both media helps stabilize the plasma membrane and eliminate factors that can inhibit capacitation (Balu *et al*., 2022; Mogielnicka-Brzozowska and Cichowska, 2024).

Kinematic parameters, such as VCL and VAP (Table 3), showed optimal results at an incubation duration of 45 minutes, indicating that the selected sperm had good mitochondrial integrity and sufficient ATP levels. These two parameters are essential factors for maintaining sperm viability during ICSI (Rahmat *et al.,* 2023; Handarini *et al*., 2024). However, sperm quality declined after 60 minutes of incubation, which was likely due to the accumulation of reactive oxygen species and depletion of energy reserves, consistent whit the findings of Gualtieri *et al*. (2021).

The mechanism behind the success of the swim-up method in the sexing process involves complex biological processes such as calcium flow, ATP production, and changes in membrane dynamics, which are influenced by the ionic an metabolic properties of TALP and BO media (Munoz *et al*., 2022; Tourmente *et al*., 2022). Bovine Serum Albumin (BSA) plays a key role in eliminating cholesterol from the sperm membrane, thereby increasing membrane flexibility and preparing the sperm for the acrosome reaction (Fasano *et al*., 2022)

The result of this study show very significanly results, especially for reproductive technology applications such as ICSI and IVF. For the bali cattle population, optimization of sperm selection protocol is essential for effective genetic conservation and selective breeding programs. The advantages and reproducibility of the swim-up method make it an auspicions choice for both laboratory and clinical setting, whit consistent result across various ART applications (Aydos and Aydos, 2021; Zhang *et al*., 2024)

DGC separates sperm based on density and is generally capable of producing sperm with better motility and overall quality. Several previos studies, including one conducted by Charles *et al*. (2024), show that the motility results from DGC are comparable to those from the swim-up method. However, the swim-up method is more straightforward and involves less handling, making it more suitable for routine use. Both methods have their own advantages, and further comparative studies, especially for species such as Bali cattle that have unique sperm characteristics, would be very beneficial.

While the study offers strong evidence for the swim-up method's effectiveness, some p-values (e.g., 0.49 for motility and 0.514 for progressive motility) were borderline insignificant. This suggests trends in sperm motility, but the weak statistical power in these cases warrants caution in drawing strong conclusions. To address this, applying a Bonferroni correction for multiple comparisons could help mitigate potential type I errors and ensure a more cautious interpretation of the data.

Further research is needed to explore the molecular and biochemical changes occurring during the swim-up process, such as changes in capacitation markers, oxidative stress levels, and DNA integrity. Comparative studies with alternative techniques, such as DGC and

microfluidics, could refine and integrate methods for different ART applications. Long-term studies assessing the outcomes of embryos derived from swim-up-selected sperm will further validate the method's utility.

Conclusion

The swim-up technique proved to be an efficient approach for sperm selection, especially for ICSI, with 45 minutes identified as the optimal incubation time to enhance motility and kinematic performance. BO and TALP media were equally effective in sustaining sperm preparation, offering reliable and consistent outcomes. The improvements in motility and kinematics achieved through this method support better fertilization and embryo development in ICSI. The outcomes of this study offer a strong basis for refining sperm preparation practices, aiding Bali bull conservation and genetic enhancement, and advancing sustainable reproduction and livestock management strategies.

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The authors did not receive support from any organization for the submitted work.

Author's Contributions

Athhar Manabi Diansyah and Muhammad Yusuf: Contributed in supervised the experiment, conception and design the study and improvement of the manuscript.

Sahiruddin and Masturi: Contributed in conception, data curation, data analysis and improvement of the manuscript.

Rahmat: Contributed in supervised the experiment, conception and design the study. Drafted the manuscript and data analysis.

Andi Muhammad Alfian and Ahmad Alfaruqi Syahrandi Adam: Collecting data and drafted the manuscript.

Muhammad Fajar Amrullah: Drafted the manuscript and data analysis.

Ethics

The Animal Ethics Commission of the National Research and Innovation Agency approved this study's animal models and experimental designs with certificate number 050/KE.02/SK/03/2023. The procedure for producing frozen semen complies with the Indonesian National Standard SNI 4869-1: 2021.

Novelty Statement

This study optimized the swim-up method for Bali cattle sperm preparation by assessing the effects of medium composition and incubation duration on sperm motility and kinematics. Unlike previous studies on other livestock species, this study specifically compared TALP and BO media for Bali bull sperm and identified the optimal incubation time. Additionally, this study provides a more efficient protocol for assisted reproductive technologies, which is crucial for the conservation and genetic improvement of Bali bulls.

References

Afriani, T., Udin, Z., Hellyward, J., Purwati, E., Rastosari, A., & Wahyudi, D. (2022). Separation of Bull Spermatozoa Bearing X- and Y-Chromosome by using Albumin Gradient and Swim-up Technique in Pesisir Cattle. *Journal of Animal Health and Production*, *10*(3), 337–342 https://doi.org/10.17582/journal.jahp/2022/10.3.337.343

Aydos, K., & Aydos, O. S. (2021). Sperm Selection Procedures for Optimizing the Outcome of ICSI in Patients with NOA. *Journal of Clinical Medicine*, *10*(12), 2687.https://doi.org/10.3390/jcm10122687

Baldini, D., Ferri, D., Baldini, G. M., Lot, D., Catino, A., Vizziello, D., & Vizziello, G. (2021). Sperm Selection for ICSI: Do We Have a Winner? *Cells*, *10*(12), 3566.https://doi.org/10.3390/cells10123566

Balu, R., Ramachandran, S. S., Mathimaran, A., Jeyaraman, J., & Paramasivam, S. G. (2022). Functional significance of mouse seminal vesicle sulfhydryl oxidase on sperm capacitation*in vitro* *Molecular Human Reproduction*, *28*(9), 1–11. https://doi.org/10.1093/molehr/gaac025

Bouloorchi, T., M., Saeidpour, Z., Habibi, Z., Javadizadeh, S., Firoozabadi, S. A., & Badieirostami, M. (2024). Microfluidics as an emerging paradigm for assisted reproductive technology: A sperm separation perspective. *Biomedical Microdevices*, *26*(2), 1–13. https://doi.org/10.1007/s10544-024-00705-2

Charles, D. K., Lange, M. J., Ortiz, N. M., Purcell, S., & Smith, R. P. (2024). A narrative review of sperm selection technology for assisted reproduction techniques. *Translational Andrology and Urology*, *13*(9), 2119–2133. https://doi.org/10.21037/tau-24-195

Cordero-Martínez, J., Jimenez-Gutierrez, G. E., Aguirre-Alvarado, C., Alacántara-Farfán, V., Chamorro-Cevallos, G., Roa-Espitia, A. L., Hernández-González, E. O., & Rodríguez-Páez, L. (2022). Participation of signaling proteins in sperm hyperactivation. *Systems Biology in Reproductive Medicine*, *68*(5–6), 315–330. https://doi.org/10.1080/19396368.2022.2122761

Diansyah, A. M., Santoso, S., Herdis, H., Yusuf, M., Priyatno, T. P., Maulana, T., Toleng, A. L., Dagong, M. I. A., Said, S., Iskandar, H., Nurlatifah, A., Lestari, P., Affandy, L., & Baharun, A. (2025). Identification of reproductive performance in Bali-polled bulls using computer-assisted semen analysis and plasma seminal proteomics. *Veterinary World*, *18*(1), 102–109. https://doi.org/10.14202/vetworld.2025.102-109

Diansyah, A. M., Yusuf, M., & Kaiin, E. M. (2020). The quality of sperm post-immobilization at some parts of FH sperm using laser diodes. *IOP Conference Series: Earth and Environmental Science*, *492*(1), 012074. https://doi.org/10.1088/1755-1315/492/1/012074

Diansyah, A.M., Yusuf, M., Toleng, A.L., and Dagong, M.I.A. (2022). Characteristic and Kinematics of Bali-Polled Bull Sperms. *Advances in Animal and Veterinary Sciences*, *10*(8), 1787–1796. https://doi.org/10.17582/journal.aavs/2022/10.8.1787.1796

Diansyah, A. M., Yusuf, M., Toleng, A. L., Dagong, M. I. A., Maulana, T., and Hasrin, B. A. 2023. The sperm post-thawing quality and proteomic seminal plasma on fertility performance of Bali-polled bull. *Advances in Animal and Veterinary Sciences*, *11*(4), 517–525. https://doi.org/10.17582/journal.aavs/2023/11.4.517.525

Fasano, C., D'Andolfi, G., Di Matteo, L., Forte, C., Dale, B., & Tosti, E. (2022). Comparison of sperm preparation methods to improve the recovery of mature spermatozoa in sub-fertile males. *Zygote*, *30*(5), 664–673. https://doi.org/10.1017/s0967199422000132

Gualtieri, R., Kalthur, G., Barbato, V., Longobardi, S., Di Rella, F., Adiga, S. K., & Talevi, R. (2021). Sperm Oxidative Stress during *in vitro* Manipulation and Its Effects on Sperm Function and Embryo Development. *Antioxidants*, *10*(7), 1025. https://doi.org/10.3390/antiox10071025

Handarini, R., Baharun, A., Rahmi, A., Sudrajat, D., Anggraeni, A., Nurcholis, N., Iskandar, H., Maulana, T., Kaiin, E., Anwar, S., & Said, S. (2024). Correlation of sperm motility, acrosome integrity, protamine deficiency, and DNA fragmentation in proven and unproven Friesian Holstein bulls. *Journal of Advanced Veterinary and Animal Research*, *11*(3), 796. https://doi.org/10.5455/javar.2024.k831

Indhu, M. S., Ramamoorthy, M., Pandey, S., Mathesh, K., Mahawar, M., Sarkar, M., Ghosh, S. K., Taru Sharma, G., & Bhure, S. K. (2021). Improved quality and fertilizability of cryopreserved buffalo spermatozoa with the supplementation of methionine sulfoxide reductase A. *Andrology*, *9*(6), 1943–1957. https://doi.org/10.1111/andr.13080

Lestari, S. W., Lestari, S. H., & Pujianto, D. A. (2018). Sperm quality after swim up and density gradient centrifugation sperm preparation with supplementation of alpha lipoic acid (ALA): A preliminary study. *AIP Conference Proceedings*, 056716. https://doi.org/10.1063/1.5023962

Mahmoud, M., Abd-allah, S., B.R., A.-H., & A.A.Y., K. (2023). Assessment of Developmental Competence of Holstein Bulls Spermatozoa upon Addition of Aloe Vera Raw Extract during In Vitro Capacitation. *Journal of Applied Veterinary Sciences*, *8*(5), 43–53. https://doi.org/10.21608/javs.2023.219422.1249

Medica, A. J., Gibb, Z., & Aitken, R. J. (2024). Optimizing equine sperm quality: an alternative to single layer centrifugation for sperm isolation. *Reproduction and Fertility*, *5*(4), 275–285. https://doi.org/10.1530/raf-23-0081

Mogielnicka-Brzozowska, M., & Cichowska, A. W. (2024). Molecular Biomarkers of Canine Reproductive Functions. *Current Issues in Molecular Biology*, *46*(6), 6139–6168. https://doi.org/10.3390/cimb46060367

Munoz, E. L., Fuentes, F. B., Felmer, R. N., Yeste, M., & Arias, M. E. (2022). Extracellular vesicles in mammalian reproduction: a review. *Zygote*, *30*(4), 440–463. https://doi.org/10.1017/s0967199422000090

Raafi, M., Yusuf, M., Toleng, A. L., Diansyah, A. M., Surahman, & Sahiruddin. (2021). Movement patterns of sperms at different bull breeds using computer-assisted sperm analysis (CASA). *IOP Conference Series: Earth and Environmental Science*, *788*(1), 012137. https://doi.org/10.1088/1755-1315/788/1/012137

Rahmat, R., Yusuf, M., Latief Toleng, A., Herdis, H., Manabi Diansyah, A., & Hasrin, H. (2023). Freeze Dry Albumin at Different Concentrations of Sexing Medium. *Advances in Animal and Veterinary Sciences*, *12*(2), 249–258. https://doi.org/10.17582/journal.aavs/2024/12.2.249.258

Ribas-Maynou, J., Barranco, I., Sorolla-Segura, M., Llavanera, M., Delgado-Bermúdez, A., & Yeste, M. (2022). Advanced Sperm Selection Strategies as a Treatment for Infertile Couples: A Systematic Review. *International Journal of Molecular Sciences*, *23*(22), 13859. https://doi.org/10.3390/ijms232213859

Said, S. (2020). Perbibitan Sapi Potong Lokal Indonesia Berbasis Bioteknologi Reproduksi Mendorong Percepatan Swasembada Daging Nasional. *LIPI Press., Indonesia, Jakarta*, *79*(10), 653-1.

Singina, G., Lebedeva, I., Shedova, E., & Zinovieva, N. (2016). 162 Effects of prolactin and dithiothreitol on the quality and developmental capacity of in vitro matured bovine oocytes. *Reproduction, Fertility and Development*, *28*(2), 211. https://doi.org/10.1071/rdv28n2ab162

Swain, D. K., Sharma, P., Shah, N., Sethi, M., Mahajan, A., Gupta, S., Mishra, A. K., & Yadav, S. (2022). Introduction to the pathways involved in the activation and regulation of sperm motility: A review of the relevance of ion channels. *Animal Reproduction Science*, *246*, 107052. https://doi.org/10.1016/j.anireprosci.2022.107052

Tourmente, M., Sansegundo, E., Rial, E., & Roldan, E. R. S. (2022). Capacitation promotes a shift in energy metabolism in murine sperm. *Frontiers in Cell and Developmental Biology*, *10*, 1–15. https://doi.org/10.3389/fcell.2022.950979

Zhang, X., Chao, S., Ye, N., & Ouyang, D. (2024). Emerging trends in sperm selection: enhancing success rates in assisted reproduction. *Reproductive Biology and Endocrinology*, *22*(1), 67. https://doi.org/10.1186/s12958-024-01239-1