RESEARCH ARTICLE

PGF2α supplementation as a strategy to improve post-thawing motility of spermatozoa in Waringin sheep

Husnurrizal[1*], Muhammad Azim[2], Cut Nila Thasmi[1], Hafizuddin[1]

[1] *Laboratory of Reproduction, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[2] *Laboratory of Animal Reproduction, Department of Animal Husbandry, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh, Indonesia*

Abstract

Background and Aim: This study aimed to evaluate the effect of prostaglandin F2α (PGF2α) supplementation in semen extenders on the post-thaw sperm motility of Waringin rams.
Materials and Methods: Semen was collected from Waringin rams, diluted with Andromed extender, and divided into three treatment groups: control (K0) without PGF2α, P1 with 37.5 µg PGF2α, and P2 with 75 µg PGF2α.
Results : The results showed that post-thaw sperm motility in P1 (49.33%) and P2 (54.00%) was significantly higher than in the control group K0 (25.33%) ($P < 0.05$), although no significant difference was observed between P1 and P2 ($P > 0.05$). Additionally, pre-freezing sperm motility also increased in P1 (75.00%) and P2 (82.33%) compared to K0 (43.67%).
Conclusion: It is concluded that PGF2α supplementation in semen extenders can improve the post-thaw motility of Waringin ram spermatozoa, with a 75 µg dosage yielding the most optimal effect.

Keywords: PGF2α, post-thaw motility, semen extender, sperm motility, Waringin ram

Introduction

Ram spermatozoa play a crucial role in artificial insemination (AI) programs aimed at improving livestock productivity and genetic quality. However, one of the main challenges in using frozen semen (cryopreservation) is the significant decline in sperm motility following thawing. The freezing and thawing process can cause cellular membrane damage, oxidative stress, and a decrease in sperm quality, particularly in terms of motility, viability, and membrane integrity (Osuagwuh *et al*., 2017). These damages result from oxidative stress, ice crystal formation, and alterations in membrane lipid structures triggered by extreme temperatures during cryopreservation (Yuniar *et al*., 2024), which negatively impact sperm motility and viability.

Ram spermatozoa, including those of the Waringin breed, are highly susceptible to oxidative damage due to their high content of polyunsaturated fatty acids (PUFAs) in the plasma membrane, which are easily oxidized by free radicals (Zhang *et al*., 2022). Previous studies have shown that the addition of antioxidants such as coenzyme Q10 (CoQ10) and glutathione (GSH) can mitigate these negative effects by improving

***Corresponding Author:** Husnurrizal
E-mail: husnurrizal@usk.ac.id
Received: 29-04-2025, **Accepted:** 21-05-2025, **Published online:** 30-05-2025
How to Cite: Husnurrizal, H., Azim, M., Thasmi, C. N., & Hafizuddin, H. (2025). PGF2α supplementation as a strategy to improve post-thawing motility of spermatozoa in Waringin sheep. *Int. J. T rop. Vet. Biomed. Res*., 10(1), 22-29.

membrane stability and enhancing endogenous antioxidant enzyme activity (Gadea *et al*., 2013). However, the effectiveness of prostaglandin F2α (PGF2α) as a semen extender supplement to enhance post-thaw sperm motility has not been widely explored, particularly in Waringin rams.

Research by Herdis (2002) demonstrated that α-tocopherol supplementation in semen extenders significantly improved the semen quality of Garut rams. However, similar approaches have not been evaluated in Waringin rams. An alternative potential additive is PGF2α, which, according to Bygdeman *et al*. (1985), can enhance sperm motility in vitro by stimulating contractile elements within the cell. The efficacy of PGF2α as a semen extender supplement has been documented in various livestock species, including cattle (Karahan *et al*., 2006), wild boars (Pandur & Pacala, 2012), and goats (Herawati & Widiarso, 2003).

Mwafaq *et al*. (2016) reported that PGF2α administration in Mariz goats increased sperm motility and reduced the proportion of abnormal sperm. However, contradictory findings were reported by Armansyah *et al*. (2018), who observed no significant improvement in Kacang goat sperm quality, although a trend of increased motility was noted. Physiologically, prostaglandins are involved in stimulating cyclic adenosine monophosphate (cAMP) production, which mediates testosterone synthesis (Hess, 2002) as a key hormone in spermatogenesis.

PGF2α is known to enhance sperm motility by increasing cellular energy metabolism and stabilizing the plasma membrane. Several studies in other animals have shown that PGF2α supplementation in semen extenders improves post-thaw motility and sperm membrane integrity. A study on Nubian goat sperm showed a 54.84% increase in post-thaw motility following the injection of 75 µg PGF2α, attributed to enhanced energy metabolism (Aswadi *et al*., 2021).

However, information on the effects of PGF2α in ram sperm, especially Waringin rams, remains limited. Therefore, this study was conducted to evaluate the potential of PGF2α as a semen extender supplement to improve the post-thaw motility of Waringin ram spermatozoa.

Materials and Methods

Experimental Design

This study is experimental research. The study uses a one-way ANOVA, with the treatment being the dosage of PGF2α (Sincrovall® Mevet, MPA Veterinary Medicines and Additives, Spain) added to the Andromed extender (OptiXcell® 2, IMV Technologies, France).

Research Procedures

Semen Collection

Semen was collected from Waringin rams in the morning (08:00 AM) using an artificial vagina. The collection process was performed following standard procedures to avoid contamination of the semen samples.

Preparation and Processing of Semen Extender

Semen was diluted using Andromed extender (OptiXcell® 2, IMV Technologies-France). The extender was prepared by mixing distilled water with Andromed at a ratio of 4:1, homogenized, and maintained in a water bath at 38 °C.

Evaluation of Fresh Semen Quality

Semen quality was assessed both macroscopically and microscopically. Freshly collected semen was immediately examined macroscopically for volume, color, pH, and consistency (Arifiantini, 2012).

Microscopic Examination of Semen

Concentration

Sperm concentration was determined by aspirating semen up to the 0.5 mark on a pipette and adding 3% NaCl solution up to the 101 marks. The mixture was homogenized for 2–3 minutes, with excess fluid discarded. A Neubauer counting chamber was filled with the mixture and covered with a cover glass. The sample was observed under a microscope at 400 x magnification, and sperm concentration was calculated from five large squares using the formula $Y \times 5 \times 10^6$ (Y = number of spermatozoa in 5 large squares) (Arifiantini, 2012).

Motility

Sperm motility was assessed by placing a drop of semen on a slide and adding one drop of physiological NaCl solution, then observed under a microscope at 400x magnification. Motility was evaluated as the percentage of sperm displaying various movements: vibrating in place (vibrator), circular, reverse, and immobile. Sperm movement was categorized into rapid progressive (A), slow progressive (B), circular (C), and vibratory (D) motility (Arifiantini, 2012).

Viability

A drop of semen was mixed with one drop of eosin stain on a slide. A smear was prepared and fixed over a spirit lamp, then observed under a light microscope at 400x magnification. Viable spermatozoa remained unstained or appeared white, whereas non-viable spermatozoa absorbed the eosin and appeared red. The percentage of viable sperm was calculated by dividing the number of live sperm by the total number of sperm observed in one field of view (Arifiantini, 2012).

Abnormalities

Sperm abnormalities were assessed by preparing a semen smear. One drop of semen was placed on a slide and mixed with eosin solution, then fixed with a spirit lamp. Once dried, the smear was examined under a microscope at 400x magnification. Both primary abnormalities (small/large head, double head/tail, abnormal head shape) and secondary abnormalities (broken head, broken or bent tail) were recorded (Arifiantini, 2012).

PGF2α Supplementation in Andromed Extender

The prepared extender was divided into three tubes. The control group (K0) received only 0.9% NaCl, group P1 received 37.5 µg PGF2α, and group P2 received 75 µg PGF2α. Collected semen was added to each tube and diluted with the respective extender according to the required volume.

Semen Freezing Process

After dilution, semen was equilibrated in a cold top or refrigerator at ±5 °C for 4 hours. Then, filling and sealing of 0.25 mL straws were performed. Initial freezing involved pre-freezing by placing the straws on a rack 8 cm above liquid nitrogen

(approx. –130 °C) inside a closed Styrofoam container for 10 minutes. Afterward, the straws were transferred to goblets and stored in a liquid nitrogen container (–196 °C). Straws were stored for 24 hours, with liquid nitrogen added as needed (Waluyo, 2014).

Post-Thawing Motility Evaluation

Post-thaw sperm motility (PTM) was observed by placing a drop of semen on a microscope slide, adding one drop of physiological NaCl, covering with a cover slip, and observing under a microscope at 400 x magnification. Sperm were categorized into rapid progressive (A), slow progressive (B), circular (C), and vibratory (D) motility.

Data Analysis

Data on semen quality were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

Results and Discussion

Macroscopic and microscopic evaluations of fresh semen are essential to determine its suitability for further processing and to calculate the required volume of extender. The results of the fresh semen quality evaluation of Waringin rams before treatment are presented in Table 1.

Table 1. Evaluation of Fresh Semen Quality in Waringin Rams Compared to Other Sheep Breeds

Parameter	*Sheep Breed*						
	Waringin	Local	Fat-tailed	Merino	Garut	Awassi	Krishi
Macroscopic Volume (ml)	0.99±0.23	0.82±0.43	1.14 ± 0.76	4.24±0.69	0.86±0.14	2.04 ± 0.07	1.91 ± 0.03
Color	Cream	Cream	-	Cream	Cream	-	-
pH	6.09±0.57	6.4±0.0	-	7.0±0.0	6.64±0.28	-	-
Consistency	Thick	Moderate	-	Moderate	Thick	-	-
Microscopic Concentration (x10^6/ml)	2205±319.74	3250±396.21	3619±210	-	4340±1.04	3640±0.24	320±0.17
Motility (%)	85.91±5.43	73.75±4.79	78.30±5.0	78.00±274	73.89±6.01	85.21±1.01	84.17±1.40
Viability (%)	86.62±5.99	93.65±2.72	79.40±3.0	90.00 ± 5.57	87.11±1.53	84.83±1.01	80.96±0.81
Abnormalities (%)	35.30±8.60	4.55±4.16	11.90±1.3	4.80±2.39	5.86±2.10	15.17±1.01	19.04±0.81
Reference		Setiadi *et al*., (2022)	Bintara *et al*., (2017)	Saputro (2016)	Dwitarizki *et al*., (2015)	Al-Janabi *et al*., (2023)	Al-Janabi *et al*., (2023)

The data in Table 1 indicate that Waringin rams exhibited notable semen characteristics in several parameters, particularly sperm motility and viability. The average ejaculate volume was 0.99 ± 0.23 ml, which falls within the moderate range-higher than that of Local (0.82 ml) and Garut rams (0.86 ml), but lower compared to Merino (4.24 ml), Awassi (2.04 ml), and Krishi (1.91 ml). Macroscopically, Waringin semen appeared cream-colored with thick consistency, which is generally considered a good visual indicator of quality. However, the semen pH was relatively acidic (6.09 ± 0.57), lower than that of Garut (6.64) and Merino (7.0) breeds, which may impact sperm stability during storage or fertilization.

Microscopic evaluation revealed that Waringin ram sperm concentration was relatively low (2205 ± 319.74 million/ml), lower than all other breeds studied—especially Garut, which had the highest at 4340 million/ml. Nonetheless, the standout advantage of Waringin sperm was its high motility (85.91 ± 5.43%), the highest among all breeds, even exceeding Awassi (85.21%) and Krishi (84.17%). This indicates excellent progressive motility and high fertilization potential. Viability was also high at 86.62 ± 5.99%, reflecting the proportion of live and active sperm cells. On the downside, Waringin rams showed the highest abnormality rate (35.30 ± 8.60%), significantly higher than other breeds, which could negatively affect fertility outcomes despite strong motility and viability.

One possible intervention to address these limitations is supplementing semen extenders with prostaglandin F2α (PGF2α), a bioactive compound that modulates physiological sperm functions, including enhancing mitochondrial energy activity and intracellular calcium signaling. PGF2α also helps maintain plasma membrane integrity and suppress reactive oxygen species (ROS) production (Das, 2021), a major cause of structural and functional sperm damage during chilling and freezing (Dutta *et al*., 2019).

PGF2α supplementation may not only improve post-thaw motility and viability but also significantly reduce sperm morphological abnormalities (Husnurrizal *et al*., 2021). This approach could be a biologically effective strategy to enhance Waringin ram semen quality, improving success rates in cryopreservation and artificial insemination programs.

Sperm Motility of Waringin Rams After PGF2α Supplementation

Microscopic evaluation of sperm motility before freezing (pre-freezing) and after thawing (post-thawing) is presented in Table 2. The results show an overall decline in motility from fresh semen to pre-freezing and further to post-thawing stages across all treatments. However, the addition of PGF2α in groups P1 and P2 significantly improved both pre-freezing and post-thaw sperm motility compared to the control group K0 ($P < 0.05$). No significant difference was observed between P1 and P2 ($P > 0.05$), indicating that a 75 µg dose of PGF2α was optimal for improving motility.

Table 2. Mean (±SE) Sperm Motility of Waringin Rams (Pre-Freezing and Post-Thawing)

Treatment Group	Sperm Motility (%)	
	Pre-freezing	Post-thawing
K0	43.67 ± 1.20[a]	25.33 ± 1.85[a]
P1	75.00 ± 4.00[b]	49.33 ± 3.17[b]
P2	82.33 ± 3.18[b]	54.00 ± 5.50[b]

[a,b] Different superscripts in the same column indicate significant differences ($P<0.05$). Notes: K0 = NaCl 0.9%; P2 = PGF2α 37.5 µg; P3 = PGF2α 75 µg.

The observed improvement in sperm motility aligns with findings by Karahan *et al*. (2006), who reported significant motility enhancement after adding 125 µg or 250 µg of PGF2α to diluted Brown-Swiss bull semen, even after 24 hours of storage at 4 °C. Similarly, Şen and Akçay (2015) found that 40 µg of PGF2α significantly improved stallion sperm motility. Prestiya *et al*. (2020) also observed increased motility in Nubian goats after adding 37.5 µg or 75 µg of PGF2α to the semen extender.

This motility improvement is attributed to the positive effects of PGF2α. Inactivation of PGF2α by incubating spermatozoa with prostaglandin 15-hydroxydehydrogenase resulted in reduced motility (Siregar, 2014). Prostaglandins (PGs) are important regulators of sperm motility through ATP production in the mitochondrial sheath of the sperm tail (Freitas *et al*., 2017). According to Ruan *et al*. (2011), PGs directly influence the contractile elements of the sperm tail, as seen in other tissues. These contractile elements are composed of fibrous layers surrounding the central axoneme, particularly in the midpiece region (Linck *et al*., 2016).

The motility enhancement observed with the addition of 37.5 µg and 75 µg of PGF2α in this study mirrors the findings of Prestiya *et al*. (2020) in Nubian goats. Sperm motility is a critical parameter reflecting cell viability and is essential for fertilization. Optimal motility enables sperm to actively move through the female reproductive tract, reach the oocyte, and penetrate the zona pellucida—initiating fertilization (Waller *et al*., 2019).

Therefore, maintaining semen quality through proper dilution and low-temperature storage is crucial before artificial insemination. According to the Indonesian National Standard (SNI), a minimum post-thaw motility (PTM) of 40% is required for AI (BSN, 2008). Nonetheless, Susilawati (2011) noted that even frozen semen with PTM below SNI standards (20–40%) could still achieve pregnancy rates of 85–89% when deposited in optimal AI positions (levels 4 and 4^{+}).

Conclusion

Based on the results of this study, it can be concluded that supplementation of PGF2α in semen extenders significantly improves the post-thaw motility of Waringin ram spermatozoa. The 75 µg dose of PGF2α demonstrated a more favorable effect compared to the 37.5 µg dose, although the difference was not statistically significant.

Acknowledgement

The authors would like to express their gratitude to Universitas Syiah Kuala and the Ministry of Education, Culture, Research, and Technology of Indonesia for funding this research through the Research Assignment Agreement for Educational Laboratory Staff under the 2021 Fiscal Year, Agreement No.: 173/UN11/SPK/PNBP/2021, dated February 22, 2021.

References

Al-Janabi, Y. A. A., Habeeb, H. M. H., & Al-Qasimi, R. H. (2023). Effect of breeds on some semen characteristics of Awassi ram and its crossbreed. *IOP Conference Series: Earth and Environmental Science, 1262*(7), 072112.

Armansyah, T., Putra, E. R., Cut, V. R. H., Aliza, D., Sutriana, A., Hamdan, H., Panjaitan, B., Sayuti, A., & Siregar, T. N. (2018). Concentration and motility of spermatozoa and testosterone level of kacang goat after seminal vesicle extract administration. *Open Veterinary Journal, 8*(4), 406–410.

Aswadi, A., Husnurrizal, H., Adam, M., & Siregar, T. N. (2021). The effect of PGF2α injection on post-thaw motility in sperm of Nubian goats. *Buletin Peternakan, 45*(1), 1–5.

Bintara, S., Maharani, D., Budiarta, I. G. S., & Mujadidyyati, A. N. (2017). The correlation between scrotal circumference, scrotal volume, and semen quantity and quality on fat tailed rams. In *Proceedings of the International Seminar on Tropical Animal Production (ISTAP)* (pp. 719–723).

Bygdeman, M., Bendvold, E., Gottlieb, C., Svanborg, K., & Eneroth, P. (1985). Prostaglandins and male fertility. *Advances in Prostaglandin, Thromboxane, and Leukotriene Research, 15*, 609–611.

Das, U. N. (2021). "Cell membrane theory of senescence" and the role of bioactive lipids in aging, and aging associated diseases and their therapeutic implications. *Biomolecules, 11*(2), 241.

Dutta, S., Majzoub, A., & Agarwal, A. (2019). Oxidative stress and sperm function: A systematic review on evaluation and management. *Arab Journal of Urology, 17*(2), 87–97.

Dwitarizki, N. D., Ismaya, & Asmarawati, W. (2015). Pengaruh pengenceran sperma dengan air kelapa dan aras kuning telur itik serta lama penyimpanan terhadap motilitas dan viabilitas spermatozoa domba Garut pada penyimpanan 5° C. *Buletin Peternakan, 39*(3), 149–156.

Freitas, M. J., Vijayaraghavan, S., & Fardilha, M. (2017). Signaling mechanisms in mammalian sperm motility. *Biology of Reproduction, 96*(1), 2–12.

Gadea, J., Gumbao, D., Gómez-Giménez, B., & Gardón, J. C. (2013). Supplementation of the thawing medium with reduced glutathione improves function of frozen-thawed goat spermatozoa. *Reproductive Biology, 13*(1), 24–33.

Gottlieb, C., Svanborg, K., Eneroth, P., & Bygdeman, M. (1988). Effect of prostaglandins on human sperm function in vitro and seminal adenosine triphosphate content. *Fertility and Sterility, 49*(2), 322–327.

Herawati, & Widiarso, B. P. (2003). Pengaruh penambahan prostaglandin F2 alfa terhadap kualitas sperma kambing yang diencerkan dengan berbagai larutan. *Journal of the Indonesian Tropical Animal Agriculture, 28*(2), 77–82.

Herdis, I., Kusuma, M., Surachman, M., Rizal, I. K., Sutama, I., Inounu, B., Purwantara, B., & Arifiantini, I. (2002). Peningkatan kualitas semen beku domba Garut melalui penambahan α-tokoferol ke dalam pengencer susu skim kuning telur. *Jurnal Ilmu Ternak dan Veteriner, 7*(1), 12–17.

Hess, M. (2002). *The effect of prostaglandin F2α, oxytocin, and gonadotropin releasing hormone on ejaculate characteristics in the dog* [Unpublished master's thesis]. Virginia Polytechnic Institute and State University.

Husnurrizal, H., Aritonang, A. S., Siregar, T. N., Armansyah, T., & Hafizuddin, H. (2021). Penambahan PGF2α dalam pengencer semen dapat meningkatkan motilitas pasca thawing motility spermatozoa domba Waringin. *Livestock and Animal Research, 19*(2), 210–216.

Karahan, I., Turk, G., & Gur, S. (2006). In vitro effects of prostaglandin F2α and metamizol on the motility of diluted bull semen. *Turkish Journal of Veterinary and Animal Sciences, 30*(2), 271–278.

Linck, R. W., Chemes, H., & Albertini, D. F. (2016). The axoneme: The propulsive engine of spermatozoa and cilia and associated ciliopathies leading to infertility. *Journal of Assisted Reproduction and Genetics, 33*(2), 141–156.

Mwafaq, S. Q. B., Araz, Q. S. B., & Hawar, M. H. Z. (2013). Influence of prostaglandin F2α injection on semen characteristics and some enzymatic activity in Meriz bucks. *Journal of Animal Scientist, 1*(1), 6–10.

Nalley, W., & Arifiantini, R. I. (2013). The hypo-osmotic swelling test in fresh Garut ram spermatozoa. *Journal of the Indonesian Tropical Animal Agriculture, 38*(4), 212–216.

Osuagwuh, U. I., & Palomo, M. J. (2017). Effect of semen washing on thawed ram spermatozoa subjected to a four-hour post-thawing thermal evaluation test. *Small Ruminant Research, 155*, 81–86.

Pandur, D. I., & Pacala, N. (2012). Sperm motility after the addition of prostaglandin F2α to the Landrace boar diluted semen. *Animal Sciences and Biotechnologies, 45*(1), 222–225.

Prestiya, A., Siregar, T. N., Husnurrizal, H., Wahyuni, S., Sari, E. M., Hafizuddin, H., & Panjaitan, B. (2020). Peningkatan motilitas spermatozoa kambing Nubian setelah pemberian PGF2α dalam pengencer Andromed. *Jurnal Agripet, 20*(1), 32–37.

Ruan, Y. C., Zhou, W., & Chan, H. C. (2011). Regulation of smooth muscle contraction by the epithelium: Role of prostaglandins. *Physiology, 26*(3), 156–170.

Santoso, & Herdis. (2014). Peranan raffinosa ke dalam mempertahankan kualitas semen beku domba Garut. In *Prosiding Seminar Nasional Asosiasi Reproduksi Hewan Indonesia* (pp. 110–114).

Saputro, A. L., Hermadi, H. A., & Sosiawati, S. M. (2016). Kualitas spermatozoa domba Merino pada sisi anoda hasil pemisahan dengan teknik ESS (Electric Separating Sperm). *Veterina Medika, 9*(3), 61–66.

Şen, Ç. Ç., & Akçay, E. (2015). The effect of oxytocin and prostaglandin hormones added to semen on stallion sperm quality. *Turkish Journal of Veterinary and Animal Sciences, 39*(6), 705–708.

Setiadi, D. R., Fatimah, F., Diapari, D., & Arifiantini, R. I. (2022). Kualitas semen domba lokal dari frekuensi ejakulasi berbeda. *Jurnal Nukleus Peternakan, 9*(1), 42–47.

Siregar, T. N., Akmal, M., Wahyuni, S., Tarigan, H., Mulyadi, M., & Nasution, I. (2014). Pemberian ekstrak vesikula seminalis meningkatkan kualitas spermatozoa tetapi tidak memengaruhi konsentrasi spermatozoa dan testosteron tikus putih (*Rattus norvegicus*). *Jurnal Kedokteran Hewan, 8*(2), 90–93.

Susilawati, T. (2011). *Spermatologi*. Universitas Brawijaya Press.

Yuniar, R. M., Kusumawati, A., & Setyawan, E. M. N. (2024). Efek penambahan antioksidan selenium, kurkumin dan kombinasinya terhadap motilitas, recovery rate dan viabilitas spermatozoa pada kriopreservasi semen sapi Peranakan Ongole. *Jurnal Sain Veteriner, 42*(3), 389–399.

Zhang, W., Min, L., Li, Y., Lang, Y., Hoque, S. M., Adetunji, A. O., & Zhu, Z. (2022). Beneficial effect of proline supplementation on goat spermatozoa quality during cryopreservation. *Animals, 12*(19), 2626.