talenta PUBLISHER

Jurnal Peternakan Integratif
Journal homepage: https://talenta.usu.ac.id/jpi/workflow/index/16319/3

Effect of Virgin Coconut Oil as a Natural Additive on the Quality of Murrah Buffalo (*Bubalus Bubalis*) Liquid Semen Using Tris-Egg Yolk Extender

D T Fatmila*[6869344], A Trisna, K J Simanjuntak, W H Sutanto

Animal Science Study Program, Faculty of Agriiculture, Universitas Sumatera Utara, Medan 20155, Indonesia
*Corresponding Author: diantriafatmila@usu.ac.id

ARTICLE INFO

Article history:
Received October 6, 2025
Revised October 30, 2025
Accepted October 30, 2025
Available online October 30, 2025

E-ISSN: 2808-2753

How to cite:
D T Fatmila, A Trisna, K J Simanjuntak, W H Sutanto,. "Effect of Virgin Coconut Oil as a Natural Additive on the Quality of Murrah Buffalo (Bubalus Bubalis) Liquid Semen Using Tris-Egg Yolk Extender". Jurnal Peternakan Integratif, Vol. 13, No. 02,pp.57-64October.2025,doi: 10.32734/jpi.v13i2.23047

ABSTRACT

Artificial insemination (AI) is an important technology in increasing the population and productivity of Murrah buffalo in Indonesia, however its success is also influenced by the quality of the semen during storage. The addition of virgin coconut oil (VCO) as a natural antioxidant in Tris-egg yolk extender has the potential to increase the stability of spermatozoa cells against oxidative stress. This study aims to evaluate the effect of VCO on the quality of Murrah buffalo liquid semen during 0, 24, and 48 hours storage at 3–5 °C. The study used a Completely Randomized Design (CRD) with five treatments (T0: control without VCO, T1: 8% VCO, T2: 10% VCO, T3: 12% VCO, and T4: 14% VCO) and four replicates. The results of the study showed that the addition of VCO at a dose of 8% (T1) had a positive effect on maintaining semen quality for up to 48 hours. The highest motility was recorded at T1 (57.00±1.54%) which was significantly different ($P<0.05$) with other treatments. T1 treatment (55.37±4.16) ($P<0.05$) significantly improved sperm viability at 24 to 48 hours. T2 (5.17±1.29) and T1 (4.80±0.71) treatments significantly improved sperm viability at 24 hours of storage, while T1 (6.24±0.88) was more effective in maintaining viability for up to 48 hours. Similarly, intact plasma membranes were maintained at T0 (41.75±0.68) and T1 (40.79±2.02) with significant differences ($P<0.05$) with other treatments at 48-hour storage. Overall, the addition of VCO in optimal concentrations was able to slow down the degradation of semen quality during storage in Murrah buffalo, demonstrating its potential as an effective and environmentally friendly natural additive antioxidant in sustainable Murrah buffalo liquid semen extender.

Keywords: Virgin Coconut Oil, Murrah Buffalo, Semen, Extender

http://doi.org/ 10.32734/jpi.v13i2.23047

1. Introduction

Murrah buffalo (*Bubalus bubalis*) is a type of slaughter and dairy cattle that has great potential in increasing animal protein production in Indonesia. In order to accelerate population improvement and reproductive efficiency, artificial insemination (AI) is one of the most reliable technologies. However, the success of AI is highly dependent on the quality of the semen used, especially during the storage and transportation process. During storage at cold temperatures (*chilling*), the quality of semen may decrease due to oxidative stress that damages the structure and function of spermatozoa [1].

The use of extender such as Tris-egg yolk has become standard in semem processing due to its content capable of maintaining the integrity of the membrane and the viability of spermatozoa. Egg yolks act as a

protector against low temperatures through their phospholipid and lipoprotein content [2]. However, these extender have not been able to completely prevent damage due to oxidative reactions, which are characterized by decreased motility, viability, and increased damage to the spermatozoan membrane. Therefore, the addition of additives that are antioxidants is needed to increase the efficiency of the extender.

In recent years, a number of studies have shown that the addition of natural antioxidants in extender can improve the quality of extender during storage. Research by Khalil et al. [3] proves that external antioxidant supplementation such as thymoquinone in extender can improve the quality of buffalo semen after freezing. In addition, Ramazani et al. [4] reported that L-proline and fulvic acid were able to improve the quality of frozen semen of buffalo when added to Tris-egg yolk extender. On the other hand, *virgin coconut oil* (VCO) is a natural antioxidant rich in medium-chain fatty acids and phenolic compounds, such as lauric acid and vitamin E, which have great potential in warding off free radicals and maintaining the stability of spermatozoan membranes.

Although the potential of VCO as a natural additive in semen extender has been studied in other species [5]; [6]. Studies on its use in Tris-egg yolk extender for Murrah buffalo liquid semen are still very limited. Therefore, this study was conducted to evaluate the effect of the addition of VCO in Tris-egg yolk on the quality of liquid semen of Murrah buffalo during cold storage. The results of this study are expected to provide scientific and practical information in the formulation of more effective and environmentally friendly extender to support sustainable Murrah buffalo productivity improvement programs.

2. Materials and methods

2.1. Animals

This study used 1 bull Murrah buffalo (ID 131507), aged 9 years with a body weight of 690 kg. The buffalo is intensively raised and provided with feed in the form of forage and concentrates according to daily nutritional needs and additional feed in the form of bean sprouts. The health of buffalo is checked regularly. This study did not involve invasive procedures on animals. The entire process of handling and storing semen is carried out in a method that does not cause stress or injury to animals. The semen storage procedure has been carried out in accordance with the standard operating procedures applicable at the AI Technical Unit North Sumatra and refers to the provisions of the Indonesian National Standard (SNI) No. 4869-2:2021 [7] related to livestock handling and semen production in bull buffalo.

2.2. Time and location

This research was carried out at the UPTD Laboratory of the AI Technical Unit North Sumatra, located on Jl. Gatot Subroto, Km. 7, No. 225 Medan, North Sumatra 20127. The research implementation time begins in May-July 2025.

2.3. Semen collection

Fresh semen is collected 1 time a week using an artificial vagina. After collection, fresh semen is immediately evaluated macroscopically and microscopically to ensure that the quality of fresh semen meets SNI criteria.

2.4. Manufacture of semen thinners

The extender used in this study is Tris-egg yolk. The ingredients used to make Tris-egg yolk extender are shown in Table 1.

Table 1. Tris-egg yolk extender ingredients.

Ingredient	Amount
Tris aminomethane	6.098 g
Citric acid	3.40 g
Fructose	2.50 g
Penicillin	0.20 g
Streptomycin	0.20 g
Glycerol	20 mL
Aquabidest	6 mL
Yolk	80 mL

2.5. Experimental design

This study used a Completely Randomized Design (CRD) with five semen extender treatments and four replicates for each treatment. The treatment consists of variations of VCO concentrations added to the egg yolk tris[5].

T0	:	100% Tris-egg yolk
T1	:	Tris-egg yolk 100% + 8% virgin coconut oil
T2	:	Tris-egg yolk 100% + 10% virgin coconut oil
T3	:	Tris-egg yolk 100% + 12% virgin coconut oil
Q4	:	Tris-egg yolk 100% + 14% virgin coconut oil

2.6. Dilution and storage

Semen that meets the SNI criteria is diluted with each extender formulation according to the calculation of extender needs. After that, the semen is put in a straw and stored at a cold temperature (3-5 °C) in the refrigerator. Evaluations are performed at the 0^{th}, 24^{th}, and 48^{th} hours during storage.

2.7. Evaluation of the quality of liquid semen

2.7.1. Sperm motility.

Sperm motility was observed using a light microscope with a magnification of 400×. A total of one drop of semen was incubated on the glass of a warm object (37 °C) and the movement of spermatozoa was observed randomly in five field of view. The result is expressed in the percentage of progressively active spermatozoa [8]; [9].

2.7.2. Sperm viability.

The viability of spermatozoa was analyzed using eosin-nigrosine staining. A mixture of semen and dye solution (1:1) is made on the glass object, dried, and then observed under a 400×magnification microscope. Spermatozoa that do not absorb dye are considered alive (viable), while those that absorb dye are considered dead. A total of 200 spermatozoa were counted to determine the percentage of viability [8]; [9].

2.7.3. Sperm abnormalities.

Abnormalities were observed from the same eosin-nigrosine preparation. A total of 200 spermatozoa were observed to calculate the percentage of abnormal morphology, including abnormalities of the head, neck, and tail. The results are presented as a percentage of total abnormalities [8]; [9].

2.7.4. Intact plasma membrane.

The hypotonic swelling test (HOS test) is used to evaluate the integrity of the plasma membrane. A total of 10 µL of semen was mixed with 100 µL of hypotonic solution (Na-citrate 0.73%) and incubated at 37 °C for 30 minutes. Spermatozoa with a circular tail show an intact plasma membrane. A total of 200 spermatozoa were counted to determine the percentage [8]; [9].

2.8. Data analysis

The data obtained was analyzed using Analysis of Variance (ANOVA). If there is a noticeable difference ($P<0.05$), then it is followed by Duncan's Multiple Range Test (DMRT). Data were analyzed using SPSS statistical software version 29.0. The data is presented in ± Standard error mean (SEM) average values.

3. Result and discussion

3.1. Fresh semen

Fresh semen is collected using artificial vaginas. Murrah buffalo fresh semen data is presented in Table 2. Fresh semen that has been collected is evaluated macroscopically and microscopically [10]. Table 1 shows that the fresh semen collected has quality that meets SNI standards for further processing. Based on macroscopic and microscopic evaluation, it is known that the quality of fresh semen of Murrah buffalo is in normal condition, in accordance with studies conducted by Bhakat et al. [11]. High-quality fresh semen is associated with the quality of preserved semen and the success of artificial insemination [12]; [13].

Table 2. Quality of fresh semen of Murrah buffalo

Parameters		Result
Macroscopic		
a.	Volume	6 mL

b.	Color	Milky-white
c.	Odor	Distinctive
d.	Consistency	Thick
e.	pH	6.5
Microscopic		
a.	Sperm concentration	$851x10^6$ cell/mL
b.	Mass motility	2+
c.	Sperm motility	65%
d.	Sperm viability	70%
e.	Sperm abnormalities	10%

3.2. Spermatozoa motility

The average spermatozoa motility in Murrah buffalo liquid semen observed at different observation times is shown in Table 3.

Table 3. Sperm motility of Murrah buffalo in liquid semen

Treatment	0 hours	24 hours	48 hours
T0	$60.75±1.71^{c}$	$58.33±1.15^{b}$	$54.58±1.98^{c}$
T1	$59.85±1.07^{c}$	$58.25±1.26^{b}$	$57.00±1.54^{c}$
T2	$57.50±2.52^{bc}$	$52.20±1.26^{a}$	$46.60±4.34^{b}$
T3	$55.40±2.06^{ab}$	$46.38±5.94^{a}$	$40.50±1.94^{a}$
Q4	$52.45±2.92^{a}$	$46.25±6.24^{a}$	$39.13±6.42^{a}$

Note: Different letters in each column indicate significant differences at the 0.05 level

The results showed that the addition of VCO in Tris-egg yolk extender was able to affect the spermatozoa motility of Murrah buffalo during liquid storage ($P<0.05$) 0, 24, and 48 hours between treatments. The T1 treatment (suspected optimal VCO dose) showed the highest motility at 48 hours (57.00±1.54%) even exceeded the control (T0: 54.58±1.98%), while the treatment with higher doses (T2-T4) experienced a significant decrease in motility. These findings support the results of a study by Blegur et al. [14], who reported that the addition of 6% VCO to Balinese cattle semen was able to maintain the best motility during liquid storage. The effectiveness of VCO at optimal doses is attributed to the content of natural antioxidants such as lauric acid and vitamin E [15] – [17]. Antioxidants function to protect the plasma membrane of sperm from damage due to oxidative stress during storage [18]; [1].

A sharper decrease in motility in T2-T4 suggests that too high a concentration of VCO can have toxic effects or disrupt the physicochemical balance of the storage medium. A similar study by Zein [19] on PO cow semen showed that VCO doses above 8% actually decreased motility. The addition of large amounts of lipids can increase viscosity and decrease the efficiency of the diffusion of oxygen and nutrients to sperm cells. The high viscosity of semen has the consequence of decreased motility in spermatozoa [20].

3.3. Spermatozoa viability

The average viability of cold-stored Murrah buffalo spermatozoa is shown in Table 4. Based on Table 3, the viability of Murrah buffalo sperm in liquid semen showed significant differences between treatments at 24- and 48-hours storage time ($P<0.05$). The percentage of sperm viability at T1 (61.70±1.10) after 24 hours of storage, gave the highest average although at 0-hour observation the highest average was held by T3 (71.53±3.69). After 48 hours, sperm viability decreased on all treatments, but T1 still showed the highest viability (55.37 ± 4.16) and significant differences ($P<0.05$) between treatments. These results demonstrate the effectiveness of T1 in maintaining long-term viability. In contrast, T4 consistently showed the lowest viability at 0, 24, and 48 hours of storage, indicating that these treatments were less effective or even negatively impacted sperm viability during storage.

Table 4. Sperm viability of Murrah buffalo in liquid semen

Treatment	0 hours	24 hours	48 hours
T0	65.83±3.12	$62.36±2.48^{c}$	$50.36±5.68^{c}$

T1	65.82±8.46	61.70±1.10^{bc}	55.37±4.16^{bc}
T2	70.87±3.90	50.95±6.57^{a}	45.20±6.67^{ab}
T3	71.53±3.69	55.18±5.66^{ab}	44.00±3.44^{ab}
T4	63.82±4.86	54.66±5.35^{ab}	40.78±5.50^{a}

Note: Different letters in each column indicate significant differences at the 0.05 level

The above results show the consistency of the results of the Silva et al. study [21] which reported that fat supplementation from palm oil improved the viability of Murrah's frozen sperm and improved motility progression compared to controls. In addition, a study conducted by Tarig et al. [22] showed that the combination of VCO and lecithin at certain levels resulted in higher sperm viability than controls after 2472 hours of refrigeration storage. A possible mechanism is the protection of the sperm cell membrane against oxidative stress and lipid peroxidation, maintaining the integrity of the plasma and mitochondrial membranes, so that the living cell maintains its function longer on liquid storage. However, too much VCO or high lipid additives can cause osmotic disruption or the accumulation of excess fat that triggers cell damage, so the optimal dose is important to determine [23]; [24].

3.4. Spermatozoa abnormalities

The average abnormalities of Murrah buffalo sperm at liquid storage for 0, 24, and 48 hours are shown in Table 4. Based on the results of the study, the abnormalities of Murrah buffalo sperm in liquid semen showed significant differences between treatments at 24- and 48-hours storage time ($p<0.05$). At 0 hours, there was no significant difference between treatments ($P>0.05$), indicating that all treatments had relatively equal levels of sperm abnormalities at baseline.

Table 5. Sperm abnormality of Murrah buffalo in liquid semen

	0 hours	24 hours	48 hours
T0	5.94±1.72	6.85±1.48^{ab}	7.40±0.27^{b}
T1	8.66±2.92	4.80±0.71^{a}	6.24±0.88^{a}
T2	8.65±1.89	5.17±1.29^{a}	8.53±0.52^{c}
T3	6.80±1.57	9.48±1.99^{b}	6.39±0.38^{ab}
Q4	8.65±3.80	7.96±4.53^{ab}	6.43±0.99^{ab}

Note: Different letters in each column indicate significant differences at the 0.05 level

After 24 hours, the T1 (4.80 ± 0.71) and T2 (5.17 ± 1.29) treatments showed significantly lower levels of sperm abnormalities ($P<0.05$) than the other treatments. This shows the effectiveness of both treatments in inhibiting the increase in abnormalities during liquid storage. At 48 hours, T1 remained the lowest abnormality rate (6.24 ± 0.88), which was significantly lower than T2 (8.53 ± 0.52). This indicates that T1 is most effective in maintaining the morphological quality of sperm during liquid storage.

The use of antioxidant additives can contribute to the morphological stability of sperm during storage, by preventing membrane damage and cell degradation [1]. VCO supplementation along with lecithin may improve sperm morphology (reduce abnormalities) in cold semen compared to controls without additives [22]. The reduction of abnormalities during liquid storage is likely related to the ability of VCO to optimally dose to strengthen defences against oxidative stress and maintain the integrity of the sperm cell membrane. This is in line with studies reported by Ajibare et al. [25], VCO supplementation improves sperm quality through antioxidant and anti-inflammatory effects that support normal spermatozoa development and fight oxidative stress on cells. However, at high or suboptimal doses, lipid additives can cause additional damage or changes in the osmolarity of the medium so that abnormalities increase [24].

3.5. Intact plasma membrane of spermatozoa

The average plasma membrane of intact spermatozoa in Murrah buffalo liquid semen observed at different observation times is shown in Table 6. Based on the results of the study, the viability of the Murrah buffalo sperm plasma membrane in liquid semen showed a significant difference between treatments, especially after storage for 48 hours ($p<0.05$). At 0 and 24 hours, there was no significant difference ($P>0.05$) between all treatments, indicating that the initial condition of plasma membrane viability was relatively equivalent and had not been significantly affected by treatment. After 48 hours, the T0 (41.75 ± 0.68) and T1 (40.79 ± 2.02) groups had significantly higher percentages of intact plasma membranes than the other

treatments. This indicates that T0 and T1 are more effective in maintaining the integrity of the sperm plasma membrane during liquid storage, which is important for maintaining sperm physiological function and fertility.

Table 6. Intact membrane plasm of sperm of Murrah buffalo in liquid semen

Treatment	0 hours	24 hours	48 hours
T0	60.87±1.62	54.28±0.49	41.75±0.68^{c}
T1	61.56±2.66	52.49±2.56	40.79±2.02^{c}
T2	61.57±2.10	53.09±0.66	35.48±1.33^{b}
T3	60.21±2.48	51.53±3.55	32.80±2.31^{a}
Q4	59.16±1.93	52.55±1.62	35.61±1.95^{b}

Note: Different letters in each column indicate significant differences at the 0.05 level

The integrity of the sperm plasma membrane is susceptible to damage due to oxidative stress, temperature changes, and lipid peroxidation during liquid storage or cryopreservation [26] – [28]. The results of this study are also in line with research on celastrol nanoemulsions in buffalo semen thinners which found that the addition of-nano-antioxidants significantly improved the function of the plasma membrane of spermatozoa post-thawing, including maintaining an intact membrane more than controls [29]. The mechanism of protection is likely related to the ability of antioxidants to ward off free radicals and prevent lipid peroxidation changes in the sperm cell membrane [1]; [18]; [20].

4. Conclusion

The addition of VCO as a natural additive antioxidant in Tris-egg yolk extender has a noticeable effect on the quality of Murrah buffalo liquid semen during storage for up to 48 hours. Treatment with a VCO dose of 8% (T1) was shown to be most effective in maintaining motility (57.00%), viability (55.37%), as well as reducing the rate of spermatozoa abnormalities (6.24%) and maintaining the integrity of the sperm plasma membrane (40.79%) compared to other treatments. On the other hand, too high doses of VCO (T3 and T4) tend to reduce the quality of semen. As such, VCO has the potential to be used as a natural additive in extenders to extend shelf life and maintain the quality of liquid semen, provided it is used at optimal doses. Follow-up research is recommended to identify the best concentrations of VCO and test their impact on fertility in vivo.

References

[1] Silvestre, M. A., Yániz, J. L., Peña, F. J., Santolaria, P., & Castelló-Ruiz, M. (2021). Role of antioxidants in cooled liquid storage of mammal spermatozoa. *Antioxidants*, *10*(7), 1096.

[2] Chang, F., Zhang, B., Liu, H., Fan, H., Xie, R., Li, J., Hu, Q., & Ruan, C. (2025). Effect of Centrifuged Chicken Egg Yolk on the Cryopreservation of Boar Semen. *Animals*, *15*(4), 599.

[3] Khalil, W. A., Hassan, M. A., El-Harairy, M. A., & Abdelnour, S. A. (2023). Supplementation of thymoquinone nanoparticles to semen extender boosts cryotolerance and fertilizing ability of buffalo bull spermatozoa. *Animals*, *13*(18), 2973.

[4] Ramazani, N., Mahd Gharebagh, F., Soleimanzadeh, A., Arslan, H. O., Keles, E., Gradinarska-Yanakieva, D. G., Arslan-Acaröz, D., Zhandi, M., Baran, A., Ayen, E., & Dinç, D. A. (2023). The influence of L-proline and fulvic acid on oxidative stress and semen quality of buffalo bull semen following cryopreservation. *Veterinary Medicine and Science*, *9*(4), 1791-1802.

[5] Ma, S., Liang, Q., Li, Z., He, F., Wang, Y., Zhuang, Z., Zhou, J., Zhao, Y & Chen, F. (2025). The Cryoprotective Effect of Virgin Coconut Oil on Boar Sperm: Enhancing Sperm Quality and Reproductive Performance. *Animal Reproduction Science*, 107998.

[6] Brito, B. F., Pontes, K. D. S., Leite, S. O. L., Cabral, L. A. R., Sousa, M. S., Salgueiro, C. C. D. M., & Nunes, J. F. (2022). Extra virgin coconut oil as a cryoprotector for cryopreservation of caprine semen. *Biopreservation and Biobanking*, *20*(2), 163-167.

[7] [BSN] Badan Standardisasi Nasional 2021 SNI Semen Beku – Bagian 2: Kerbau No 4869-2:2021 Jakarta: Badan Standardisasi Nasional.

[8] Susilawati, T. (2011). *Spermatology*. Universitas Brawijaya Press.

[9] Álvarez-Rodríguez, M. (Ed.). (2025). *Spermatology: Methods and Protocols* (Vol. 2897). Springer Nature.

[10] Wahjuningsih, S., Ihsan, M., Siswoyo, D. A., Fatmila, D. T., & Firmawati, A. (2021). Extract of cincau (Mesona palustris B.) supplementation in semen extender improves boer goat sperm cryopreservation. *Journal of Advanced Veterinary Research*, *11*(4), 247-253.

[11] Bhakat, M., Mohanty, T. K., Gupta, A. K., Prasad, S., Chakravarty, A. K., & Khan, H. M. (2015). Effect of season on semen quality parameters in Murrah buffalo bulls.

[12] Carvalho, F. E., Ferraz, J. B. S., Pedrosa, V. B., Matos, E. C., Eler, J. P., Silva, M. R., Guimarães, J. D., Bussiman, F. O., Silva, B. C. A., Cançado, F. A., Mulim, H. A., Espigolan, R., & Brito, L. F. (2023). Genetic parameters for various semen production and quality traits and indicators of male and female reproductive performance in Nellore cattle. *BMC genomics*, *24*(1), 150.

[13] Tamargo, C., Garriga, F., Yeste, M., Pinart, E., Muiño, R., Carbajo, M. T., & Hidalgo, C. O. (2024). Predictive Indicators of Cryotolerance and Fertility in Bovine Sperm: Evaluating Fresh Semen Quality to Improve AI Outcomes With Frozen–Thawed Sperm. *Reproduction in Domestic Animals*, *59*(11), e14742.

[14] Blegur, J., Nalley, W. M., & Hine, T. M. (2020). Pengaruh Penambahan virgin coconut oil dalam pengencer tris kuning telur terhadap kualitas spermatozoa sapi bali selama preservasi (infiluence addition virgin coconut oil in tris egg yolk on the quality of bali bull spermatozoa during preservation). *Jurnal Nukleus Peternakan*, *7*(2), 130-138.

[15] Zeng, Y. Q., He, J. T., Hu, B. Y., Li, W., Deng, J., Lin, Q. L., & Fang, Y. (2024). Virgin coconut oil: A comprehensive review of antioxidant activity and mechanisms contributed by phenolic compounds. *Critical Reviews in Food Science and Nutrition*, *64*(4), 1052-1075.

[16] Marina, A. M., Che Man, Y. B., Nazimah, S. A. H., & Amin, I. (2009). Chemical properties of virgin coconut oil. *Journal of the American Oil Chemists' Society*, *86*(4), 301-307.

[17] Nitbani, F. O., Tjitda, P. J. P., Nitti, F., Jumina, J., & Detha, A. I. R. (2022). Antimicrobial properties of lauric acid and monolaurin in virgin coconut oil: a review. *ChemBioEng Reviews*, *9*(5), 442-461.

[18] Llavanera, M., Delgado-Bermúdez, A., Olives, S., Mateo-Otero, Y., Recuero, S., Bonet, S., Fernández-Fuertes, B., Yeste, M., & Barranco, I. (2020). Glutathione S-transferases play a crucial role in mitochondrial function, plasma membrane stability and oxidative regulation of mammalian sperm. *Antioxidants*, *9*(2), 100.

[19] Zein, M. D. (2024). *Pengaruh Penambahan Virgin Coconut Oil dalam Pengencer Semen Beku terhadap Motilitas dan Viabilitas Spermatozoa Sapi Peranakan Ongole (PO)* (Doctoral dissertation, Universitas Gadjah Mada).

[20] De Luca, M. N., Colone, M., Gambioli, R., Stringaro, A., & Unfer, V. (2021). Oxidative stress and male fertility: role of antioxidants and inositols. *Antioxidants*, *10*(8), 1283.

[21] Silva, L. K. X., Lourenço, J. D. B., Silva, A. O. A. D., Sousa, J. S. D., Silva, A. G. M. E., Reis, A. N. D., Miranda, M. D. S., Santos, S. D. S. D., Ohashi, O. M., Martorano, L. G., Filho, G. N. D. R., Faturi, C., Morais, E. D., Mares, E. K. L., & Garcia, A. R. (2020). Increased quality of in natura and cryopreserved semen of water buffaloes supplemented with saturated and unsaturated fatty acids from the palm oil industry. *Animal Reproduction*, *17*(4), e20200522.

[22] Tarig, A. A., Wahid, H., Rosnina, Y., Yimer, N., Goh, Y. M., Baiee, F. H., Khumran, A. M., Salman, H., Assi, M. A., & Ebrahimi, M. (2017). Effect of different concentrations of soybean lecithin and virgin coconut oil in Tris-based extender on the quality of chilled and frozen-thawed bull semen. *Veterinary world*, *10*(6), 672.

[23] Nwafor, J. A., Uduma-Ukpai, C., Okechukwu, J., Uchewa, O., Ekaikite, O., & Ikpeze, S. (2021). Biotherapeutic sufficiency of virgin coconut oil on paraquat-mediated reproductive dysfunction. *Journal of the Anatomical Society of India*, *70*(2), 106-112.

[24] Kinkar, D., Sarkar, D., Debbarma, A., Rahman, M., Swain, S., & Karunakaran, M. (2020). Review on recent advancement in semen additives for improving cryopreservation of bull semen. *J. Entomol*, *8*, 1493-1505.

[25] Ajibare, A. J., Akintoye, O. O., Folawiyo, M. A., Babalola, K. T., Omotuyi, O. I., Oladun, B. T., Aransi-ola, K. T., Odetayo, A. F., & Olayaki, L. A. (2024). Therapeutic potential of virgin coconut oil in mitigating sodium benzoate-model of male infertility: Role of Nrf2/Hmox-1/NF-kB signaling pathway. *Iranian Journal of Basic Medical Sciences*, *27*(5), 543.

[26] Partyka, A., Łukaszewicz, E., & Niżański, W. (2012). Effect of cryopreservation on sperm parameters, lipid peroxidation and antioxidant enzymes activity in fowl semen. *Theriogenology*, *77*(8), 1497-1504.

[27] Zhang, B., Wang, Y., Wu, C., Qiu, S., Chen, X., Cai, B., & Xie, H. (2021). Freeze-thawing impairs the motility, plasma membrane integrity and mitochondria function of boar spermatozoa through generating excessive ROS. *BMC veterinary research*, *17*(1), 127.

[28] Peris-Frau, P., Soler, A. J., Iniesta-Cuerda, M., Martín-Maestro, A., Sánchez-Ajofrín, I., Medina-Chávez, D. A., Fernández-Santos, M. R., García-Álvarez, O., Maroto-Morales, A., Montoro, V., & Garde, J. J. (2020). Sperm cryodamage in ruminants: understanding the molecular changes induced by

the cryopreservation process to optimize sperm quality. *International journal of molecular sciences*, *21*(8), 2781.

[29] Khalil, W. A., Elkhamy, S. A., Hegazy, M. M., Hassan, M. A., Abdelnour, S. A., & El-Harairy, M. A. (2025). The cryoprotective effects of celastrol nanoemulsion on post-thawed attributes and fertilizing ability of cryopreserved buffalo semen. *Veterinary Research Communications*, *49*(4), 214.