MEDIA KEDOKTERAN HEWAN VOL 36(3)
https://e-journal.unair.ac.id/MKH
ISSN Online: 2775-975X pISSN : 0215-8930

# The Effect of Kemangi Leaf (*Ocimum Basilicum)* Extract in The Egg Yolk Skim Milk Diluent on The Plasma Membrane and Motility of Boerawa Goats' Sperm

**Farah Ghifara Hafidha[1*], Budi Utomo[2], Soeharsono[3], Tita Damayanti Lestari[2], Nove Hidajati[4], Herry Agoes Hermadi[2]**

Corresponding email: farah.ghifara.hafidha.1743396-2022@fkh.unair.ac.id

[1]Master Student of Veterinary Agribusiness, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia,
[2] Division of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia,
[3] Division of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia,
[4] Divisions of Basic Veterinary Medical Sciences, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

**Received: October 29th, 2025**
**Accepted: February 9th, 2025**
**Published: September 10th, 2025**

## Abstract

The purpose of this research was to determine the effect of kemangi leaf (*Ocimum basilicum*) extract in the egg yolk skim milk diluent on the plasma membrane and motility of Boerawa goats' sperm at room temperature. The research used fresh samples of Boerawa goat semen collected by artificial vagina. The experimental design used was a completely randomized design, divided into four treatments with six replications. The treatments were the addition of kemangi leaf extract at the levels of 0% (P0), 1% (P1), 2% (P2), and 3% (P3). Diluted semen was stored at room temperature and observed at hours 0, 1, 2, and 3. Analysis of the data using *Multivariate Analysis of Variance* (MANOVA), then proceed to the Duncan Test to determine significant differences between treatments. The results showed that there was no significant difference ($p > 0.05$) in sperm plasma membrane, but a significant difference ($p < 0.05$) in sperm motility was observed when kemangi leaf extract was added. However, the addition of kemangi leaf (*Ocimum basilicum*) extract tends to provide a better value than without it. The addition of kemangi leaf extract at a concentration of 2% yields the highest results in sperm plasma membrane integrity and sperm motility of Boerawa semen at room temperature.

## Keywords

Boerawa goats, Motility, *Ocimum basilicum*, Plasma membrane, Sperm.

DOI: 10.20473/mkh.v36i3.2025.273-281

## Introduction

Boerawa goats are a cross between Boer goats and Peranakan Ettawa (PE) goats. The results of the Boerawa goat cross are superior in terms of birth weight compared to the PE and Boer goats. Male gonads and spermatogenesis are essential for males to maintain normal reproductive function (Zhaol *et al.,* 2022). Improving fertility in goats has become a significant issue to address.

The use of liquid semen requires a diluent because semen stored at room temperature will undergo metabolism, which causes the quality of the semen to decrease (Putri *et al*., 2019). The various components of semen, when combined in a suitable medium, are essential for the semen to maintain its natural properties and prolong its life from the initial process to the freezing and post-thawing stages (Ardiana *et al.,* 2024). The diluents commonly used are tris, egg yolk, and skim milk, as they meet the dilution requirements, namely by containing nutrients for sperm, protecting the sperm membrane from cold shock, and acting as a buffer (Iskandari *et al.,* 2020).

A decrease in semen quality is indicated by a decline in sperm motility and increased damage. Morphology that influences sperm capacitation. The flavonoid content in kemangi leaf extract is useful in protecting sperm from free radicals, thereby preventing damage to sperm (Sholihin and Nur., 2024). The seminiferous tubule is a highly dynamic structure that undergoes permanent remodeling throughout life, enabling spermatogenesis (Clement *et al.,* 2021). Sperm damage can be observed by examining the integrity of the plasma membrane and the level of motility in live sperm. The pathological imbalance can be caused by multiple factors, including excessive production of endogenous or exogenous Reactive Oxygen Species (ROS), a depleted supply of antioxidants, inactivation or diminished production of antioxidant enzymes, or a combination of these factors (Evans *et al.,* 2021).

In this study, kemangi leaf extract (*Ocimum basilicum*) will be added to the plasma membrane and motility of Boerawa goat sperm at room temperature with the hypothesis that it can increase the durability of the intact plasma membrane of sperm and maintain the motility rate of Boerawa goat sperm that has been diluted with skim milk egg yolk and kemangi leaf extract added with several dosage levels to obtain the appropriate dosage.

## Materials and Methods

### Research Design

The research design used in this study was a Completely Randomized Design (CRD). This experiment was conducted with four treatments, each repeated six times and observed every hour for three hours.

### Research Sample

The sample used in this study was obtained from 4–5-year-old male Boerawa goats weighing approximately 50 kg, which were kept at the Teaching Farm of Universitas Airlangga in Gresik, East Java. The collection frequency was twice a week in one month.

### Place and Time of Research

This research was conducted from March to April 2022. The manufacture of kemangi leaf extract was conducted at the Faculty of Veterinary Medicine, Airlangga University, Surabaya. Implementation of the research Involved the Storage, processing, and testing of Boerawa goat semen quality at the Teaching Farm, Universitas Airlangga, Gresik, East Java.

### Research Materials

The materials for making kemangi leaf extract are basil leaves, distilled water, and 96% ethanol. The materials for making the diluent are 10 g of skim milk, 1 g of fructose, 5 mL of egg yolk, 1000 IU/mL penicillin, 1 mg/mL streptomycin, and distilled water. The materials for storing semen are Vaseline and warm water. The materials used for evaluating semen quality are eosin, nigrosin, and 3% Sodium Chloride.

### Procedure

### Making Kemangi Leaf Extract

The powder was extracted by maceration in 96% ethanol solvent for 3 × 24 hours, then filtered to separate the residue. With filtration and evaporation at a temperature of 56$^0$ °C until there is no dew on the evaporator, the solvent evaporation process is maximized to prevent toxicity to sperm. The process then undergoes freeze-drying.

### Semen Collection

The female goat is placed in a clamp cage as bait to stimulate the libido of the male goat. Preparation of the male goat and cleaning the prepuce. Before the male is accommodated, false mounting is done 2-3 times. In the 3rd mounting, the male prepuce is held to direct the penis into the artificial vagina.

### Fresh Semen Evaluation

Semen evaluation includes macroscopic and microscopic examination. Semen that has been collected, stored, and immediately examined both macroscopically and microscopically. Macroscopic examination includes color, odor, consistency, volume, and pH. After macroscopic examination, continued microscopic semen examination, which includes motility and concentration.

### Semen Dilution

The semen is diluted using skim milk egg yolk diluent. To prepare a skim milk egg yolk diluent of up to 100 ml, place 10 g of skim milk in a beaker, add distilled water to a total volume of 100 ml, and stir until the solution is homogeneous. Place the Erlenmeyer flask in a vessel containing water and heat it indirectly to a temperature of 92 $^0$C. After the temperature reaches 92 $^0$C, maintain the temperature for 10 minutes. Cool the milk slowly to room temperature, 36 $^0$C, according to the temperature of the semen. The milk solution is filtered using sterile gauze, similar to double filtration. Add the egg yolk, up to 5 ml, into the milk solution and stir both ingredients until homogeneous. Add 100 mg to 400 ml of diluent, penicillin 1000 IU, and streptomycin 0.1 mg, and gradually stir using a stirrer. Add 0.75 g of fructose and stir again until all ingredients are homogeneous. Put the diluent in a water bath at a temperature of 33 $^0$C.

### Sperm Motility Examination

Sperm motility examination is carried out by dropping one drop of diluted semen onto a glass slide, then adding one drop of physiologically balanced NaCl, and covering it with a cover glass. The sample is then observed using 400× magnification for individual motility and 100× magnification for mass motility. The criteria for assessment were categorized as follows: very good (+++/3), indicating fast and thick mass sperm motility, good (++/2) for slow but thick mass sperm motility, fair (+/1) denoting slow and thin mass sperm motility, and poor (0) indicating no observable waves (Junaedi *et al.*, 2024).

### Sperm Plasma Membrane Examination

Evaluation of intact sperm plasma membranes is done by counting the number of sperm with coiled and uncoiled tails. sperm

with an intact plasma membrane had circular tails, while damaged plasma membranes were indicated by straight sperm tails (Triyaningrum *et al*., 2024). Examination of the integrity or wholeness of the plasma membrane is carried out using the Hypoosmotic Swelling Test (HOS Test). Observations are made with preparations that have been dripped with a semen fluid mixed with HOS solution and then reviewed with eosin-nigrosin staining.

### Data Analysis

The data in this study are presented in the form of tables, showing averages and standard deviations. The pattern of the relationship between dose, treatment, and time is illustrated in a line diagram. The research hypothesis was tested using Multivariate Analysis of Variance (MANOVA). The test results indicated that the null hypothesis (H0) was rejected, as the F count exceeded the F table value (df, 0.05) or ($p < 0.05$). If a difference is identified, use Duncan's multiple range test at $p < 0.05$ to distinguish between the different pairs. Data processing was performed using the Statistical Package for the Social Sciences (SPSS) software, version 26, for Windows.

## Results and Discussion

Based on the research findings, the volume of fresh semen from Boerawa goats was normal, according the standard volume of semen in goats is an average of 1 ml (0.5–2 ml), the color of the semen is creamy white with a thick consistency, and pH 6.4–6.8 (Prastiya *et al*., 2021). The smell of fresh semen is normal, according to a study by Nur *et al*. (2022), which found that normal semen generally has a distinctive odor accompanied by the odor of the animal. The results of the pH of fresh semen Boerawa goats in this study were normal. The mean rank table is presented in Table 1.

**Table 1.** Average Result of Fresh Semen Quality Examination of Boerawa Goat

| Observation | $\bar{X} \pm SD$ |
|---|---|
| Volume | 1,03 ± 0,326 |
| Consistency | Thick |
| Color | Cream white |
| Aroma | Distinctive |
| pH | 6-7 |
| Mass movement | +++ |
| Individual movement | 89,17 ± 2,041 |
| Concentration | 3990 x $10^6$ ± 709,567 |

There is an extensive literature on the methodological requirements for measuring motility and obtaining acceptable values (a minimum acceptable value for sperm motility in control groups is generally considered to be 70%) (Dianne *et al*., 2018). This indicates that fresh goat semen, as observed, has high progressive movement. The percentage of individual motility of progressively moving sperm was assessed by taking one drop of semen using an ose. It can be used as a measure of the ability to fertilize an ovum. This concentration is still considered normal,

because the normal goat semen concentration is between 2.5-5.0 × $10^9$ sperm/ml.

**Table 1.** Average Percentage of Intact Plasma Membrane of Boerawa Goat Sperm with the Addition of kemangi leaf Extract During Room Temperature Storage.

| Treatment | MPU Observation ($\bar{X} \pm SD$) | | | |
|---|---|---|---|---|
| | 0 hours | At 1 hours | At 2 hours | At 3 hours |
| P0 (0%) | 45.20 ± 3.09 | 43.12 ± 3.76 | 35.07 ± 9.89 | 30.77 ± 11.25 |
| P1 (1%) | 49.96 ± 6.37 | 44.87 ± 3.92 | 39.79 ± 7.56 | 32.73 ± 13.41 |
| P2 (2%) | 51.29 ± 4.94 | 48.75 ± 6.91 | 43.23 ± 9.24 | 36.72 ± 11.49 |
| P3 (3%) | 50.29 ± 4.62 | 45.48 ± 7.71 | 41.88 ± 11.58 | 34.97 ± 12.79 |

Based on Table 2, it is stated that the addition of kemangi leaf extract was not significantly different ($p>0.05$) from the intact plasma membrane of Boerawa goat sperm at room temperature. Gautier and Christine (2022) stated that highly unsaturated acyl chains, however, render sperm particularly susceptible to the detrimental actions of reactive oxygen species and lipid peroxidation. The susceptibility of sperm to lipid peroxidation can increase due to cold stress. Living organisms are equipped with natural defense systems (antioxidants) to scavenge and neutralize the effects of ROS. Reports have verified the presence of a wide range of antioxidants in seminal plasma, which can protect sperm against the detrimental effects of ROS (Qamar *et al*., 2023).

**Table 2.** Average Percentage of Sperm Motility of Individual Boerawa Goats with the Addition of kemangi leaf Extract During Room Temperature Storage.

| Treatment | Motility Observation ($\bar{X} \pm SD$) | | | |
|---|---|---|---|---|
| | 0 hours | At 1 hours | At 2 hours | At 3 hours |
| P0 (0%) | 80.83 ± 2.04 $^{a}$ | 74.16 ± 3.76 $^{a}$ | 63.33 ± 8.75 $^{a}$ | 46.67 ± 15.05 |
| P1 (1%) | 84.16 ± 2.04 $^{b}$ | 77.5 ± 2.73 $^{ab}$ | 68.33 ± 5.16 $^{ab}$ | 56.67 ± 18.3 |
| P2 (2%) | 85.83 ± 2.04 $^{b}$ | 80.83 ± 3.76 $^{b}$ | 75.0 ± 3.16 $^{b}$ | 65.0 ± 12.64 |
| P3 (3%) | 86.67 ± 4.08 $^{b}$ | 80.83 ± 3.76 $^{b}$ | 75.83 ± 5.84 $^{b}$ | 63.33 ± 12.51 |

Based on Table 3 from the results of the MANOVA test, it was stated that the addition of leaf extract basil significantly differed ($p< 0.05$) in sperm motility of Boerawa goats at room temperature. Statistical calculations revealed that the treatment was continued using the Duncan Test. From the data between hours 0 and 2, it is evident that treatments P2 and P3 differ significantly from the control. However, there is no difference between P2 and P3, so the conclusion should state that treatments P2 and P3 show positive results and

have a significant impact on motility. To find out the difference for each hour, the ANOVA test was continued. Flavonoids, phenolic acids, and polyphenols have an oxidation-reducing capacity, as they can donate hydrogen atoms and deactivate singlet (or excited) oxygen, and chelate pro-oxidant metal ions (Aguirre *et al*., 2023), thereby preventing a decrease in sperm motility. Flavonoids can maintain sperm quality by enhancing sperm motility, thereby increasing sperm viability and count (Louis *et al*., 2019).

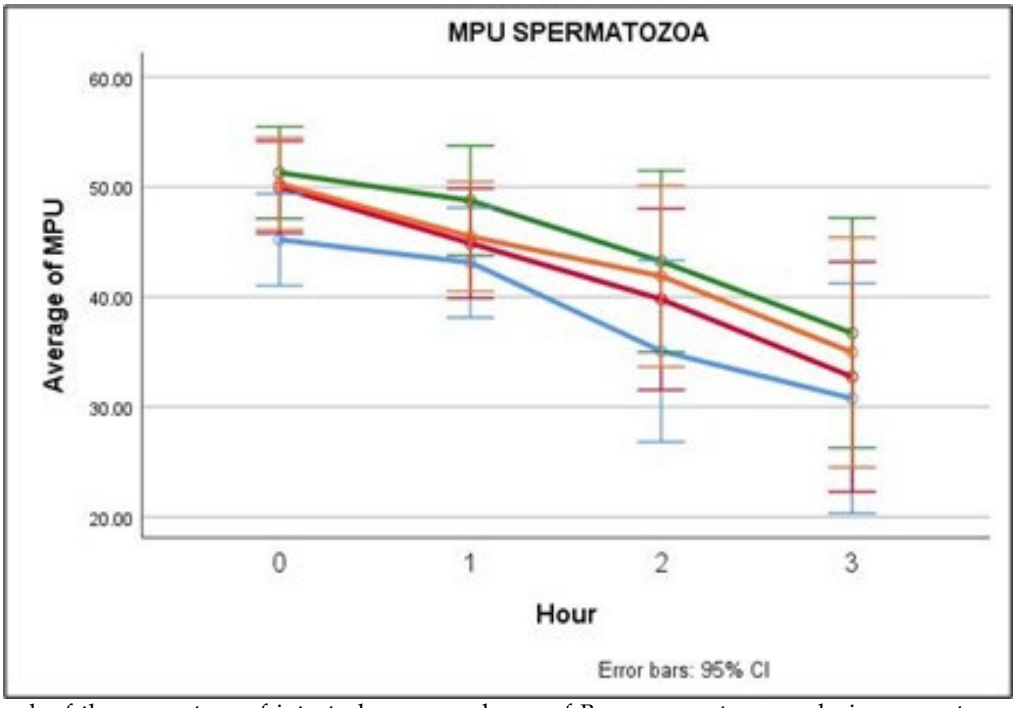


**Figure 1.** Graph of the percentage of intact plasma membrane of Boerawa goat sperm during room temperature storage. Color: Blue = P0 (0%) kemangi leaf extract, red = P1 (1%) kemangi leaf extract, green = P2 (2%) kemangi leaf extract, orange = P3 (3%) kemangi leaf

According to Bahmid *et al*. (2023), damage to the plasma membrane during the freezing and thawing process is caused by lipid peroxidation in long-stored sperm which can reduce viability and affect the preservation of semen for artificial insemination diluents which have an impact on membrane permeability disorders, decreased metabolic activity, damage to cell membranes, and can further reduce sperm motility. Physical and chemical changes caused by oxidative stress in the sperm membrane can reduce sperm function and even lead to death (Jumintomo *et al*., 2021). An intact plasma membrane will also protect the acrosome vesicle, located just below the plasma membrane of the cell at the tip of the sperm head, from mechanical damage, ensuring the acrosome vesicle remains intact and maintaining motility values (Hermawan, 2019).

Semen can still be considered good if it has a value of greater than 50%. Semen that can be frozen according to the Indonesian National Standard (SNI 4869-1:2021, Part 1) should

have a motility of 70% (Komariah *et al., 2023)*. In this case, according to the results of motility observations sperm that can be used for IB are at hour 0 and hour 1, for hour 2 that can be used for IB only at P2 and P3 because they have a motility value of more than 70%, at hour 3 it can no longer be done for IB because at hour 3 the average motility value is below 70%. Overall, P2 has the best value compared to other treatments. This is because the antioxidant content in P2 reaches its maximum value, enabling it to reduce free radicals and maintain sperm motility during room temperature storage.

**Figure 2.** Observation of Sperm Plasma Membrane. (Letters: A = normal sperm plasma membrane is marked by a circular tail and a bulging membrane and a clear head, B = damaged sperm plasma membrane is marked by a straight tail and a darker head)

## Conclusion

The addition of kemangi leaf extract (*Ocimum basilicum*) in skim milk egg yolk diluent from statistical results cannot maintain the integrity of the plasma membrane of Boerawa goat sperm stored at room temperature. The addition of kemangi leaf extract (*Ocimum basilicum*) to skim milk egg yolk, as indicated by statistical results, can maintain the percentage of motility of Boerawa goat sperm stored at room temperature, with the optimal dose being 2%.

## Approval of Ethical Commission

This study does not require ethical approval, as it utilizes animal semen, which is considered non-invasive and poses no risk to the animals involved. The semen was obtained from Educational Livestock Park Universitas Airlangga in Gresik, East Java, ensuring that all procedures adhered to relevant guidelines for the ethical treatment of animals.

## Acknowledgement

The authors express their sincere gratitude to the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, for its support of this study.

## Author's Contribution

FGH: Conducted the investigation, data curation, and wrote the original draft of the manuscript. BU: Responsible for the conceptualization. S: Methodology of the research and data curation. TDL, NH and HAH: Critical revisions and editing. All authors contributed to the supervision and overall project administration.

## Conflict of Interest

The authors declare that there are no conflicts of interest related to this research.

## Data Availability Statement

The data are not publicly available due to privacy restrictions.

## References

Ardiana, D., Yusuf, M., Zulkharnaim, Z., 2023. Effect of egg yolk powder as an alternative extender on the quality of bali bull sperm. *Hasanuddin J. Anim. Sci*., 6 (1), pp. 60-67. DOI: 10.20956/hajas.v6i1.26956

Aguirre, G.O., Angel, T.P.V., Jose, R.S.G., Adriana, S.Z., Angelica, A.O.F., Maria, R.S.C., Einar, V.B.P., Alfonso, J.C.C., 2023. Using plant-based compounds as preservatives for meat products: A review. *Heliyon*, 9, pp. e17071. DOI: https://doi.org/10.1016/j.heliyon.2023.e17071

Bahmid, N.A., Jamil, N.I., Yusuf, O.D.P., Farida, S., Gustina, S., 2023. Plasma membrane integrity and acrosomal integrity of fresh and frozen Bali bull semen based on different ejaculate volume. *IOP Conf. Series: Earth and Environmental Science*. DOI: 10.1088/1755-1315/1174/1/012034

Clement, F., Romain, Y., Nathalie, G., Laurine, G., Florian, G., Pascale, C., 2021. Cellular Endocrinology in Health and Disease Chapter 20- The follicle stimulating hormone signaling network in gonadal Cells. ISBN 978-0-12-819801-8 DOI: https://doi.org/10.1016/C2018-0-04934-8

Dianne, M.C., Robert, E., Chapin., 2018. Fundamentals of Toxicology Pathology Chapter 17- Male Reproductive System. Academic Press. DOI: https://doi.org/10.1016/B978-0-12-809841-7.00017-4

Evans, E.P.P., Jorien, T.M.S., Aldona, M., Claudia, R.S.M., Arturo, E.L., Thamir, H., Eus, G.J.M.A., Romana, S., Astrid, E.P.C., 2021. Male subfertility and oxidative stress. *Redox Biology*, 46 (2021), 102071.DOI:https://doi.org/10.1016/j.redox.2021.102071

Gautier, C., Christine, A., 2022. Fine feathers make fine birds – The mammalian sperm plasma membrane lipid composition and effects on assisted reproduction. *Animal Reproduction Science*, 246 (2022), 106884. DOI:https://doi.org/10.1016/j.anireprosci.2021.106884

Hermawan, B. D., 2019. Effect of addition of kemangi leaf extract (*Ocimum sanctum*) in diluent on the quality of boer goat semen during room temperature storage. [Skripsi]. Faculty of Animal Husbandry. Brawijaya University Malang.

Iskandari, N.N., Madyawati, S.P., Wibawati, P.A., Suprayogi, T.W., Prastiya, R.A., and Agustono, B., 2020. Comparison of tris egg yolk diluent and skim milk egg yolk on the percentage of motility, viability, and integrity of the plasma membrane of sapera goat sperm in storage temperature 5°C. *Journal of Veterinary Medicine,* 3(2), pp.196-202.

Jumintomo, J., Alkubaisy, S., Yanez Silva, D., Singh, K., Turkey, J.A., Mutia, S.S., Fakri Mustafa, Mikolaychik, I., Morozova, L., Derkho, M., 2021. Effect of Cystamine on Sperm and Antioxidant Parameters of Ram Semen Stored at 4°C for 50 Hours. *Razi Institute*, 76 (4), pp.1115-1123.

Junaedi, Nurul, I., Muhammad, H.N., Trinil, S., 2024. Supplementation of Glycine and Glucose into Egg Yolk Lactated Ringer Diluent on The Quality of Local Chicken Semen Stored at 5°C for 120 Hours. *Jurnal Medik Veteriner*, 7(1), pp.105–122. DOI: https://doi.org/10.20473/jmv.vol7.iss1.2024.105-122

Komariah, M.I, Zuhdi, M.A, Tahar, T., Maulana., 2023. Frozen Semen Quality of Simmental Cattle in Various Commercial Diluents. *Jurnal Ilmu Produksi Dan Teknologi Hasil Peternakan*, 11(1), pp. 41-47. DOI: https://doi.org/10.29244/jipthp.11.1.41-47

Louis, S.L., Salni, and Sri, N., 2019. Effect of kemangi leaf fraction (*ocimum americanum.I.*) on weight, diameter, epididymal thickness, motility and viability of sperm of male white rats (*ratus norvegicus*). *J Health*., 10, pp. 25 - 33.

Nur, E.N., Abdul, L.T., Yusuf, M., 2022. The quality of bali bull sexed sperm using soybean extract extender. *Hasanuddin J. Anim. Sci*., 4 (1), pp. 48-57. DOI: 10.20956/hajas.v4i1.19439

Putri, R.F, Hermawan, D.H., Suyadi., 2019. The quality of liquid semen of boer goats during room temperature storage with the addition of kemangi leaf extract (*Ocimum sanctum*). *Proceedings of the National Seminar on Animal Husbandry and Veterinary Technology*. pp.346 – 356.

Prastiya, RA., Rimayanti., MM. Munir., AP. Nugroho., 2021. The protective impacts of α-tocopherol supplementation on the semen quality of sapera goat preserved at 4 $^{0}$C. *Tropical Animal Science Journal*, 44(3), pp.261–266. DOI: https://doi.org/10.5398/tasj.2021.44.3.261

Qamar, A.Y., Ilyas, M. N., Sanan, R., Xun, F., Puntu, K.R., Seonggyu, B., Bereket, M.T., Islam, M.S., Sanghoon, L., Jongki, C., 2023. Role of antioxidants in fertility preservation of sperm. *National Library of Medicine*, 36(3), pp.385–403. DOI: 10.5713/ab.22.0325

Sholihin, A., and Ducha, N., 2024. Effect Of Kemangi (*Ocimum basilicum*) Extract in Spermatozoa Quality of Mice (*Mus musculus*) Exhibited to Cigarette Smoke. *LenteraBio: Berkala Ilmiah Biologi, 13*(2), pp. 289–299. DOI: https://doi.org/10.26740/lenterabio.v13n2.p289-299

Triyaningrum., Mokhamad, F., Ni Wayan Kurniani, K., Wahono, E.P., 2024. The quality of chilled ram semen after storage in extender with addition of melatonin at 4°C. *Jurnal Kedokteran Hewan*, 18(1), pp.34–39. DOI: https://doi.org/10.21157/j.ked.hewan.v18i1.34649

Zhaol, J., Peng-cheng, Y., Hua, Y., Zhi-bo, W., El-samahy, M.A., Feng, W., Yan-Li, Z., 2022. Dietary supplementation with metformin improves testis function and semen quality and increases antioxidants and autophagy capacity in goats. *Theriogenology*, 188(2022), pp. 79–89. DOI: https://doi.org/10.1016/j.theriogenology.2022.05.030