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# The Effect of Adding Moringa Leaf Extract to TCG Medium on the Post-Thawing Sperm Quality of Donggala Bull

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**ABSTRAK**

*This study aimed to evaluate the effect of Moringa (Moringa oleifera) leaf extract (MLE) diluted in Tris-Citric Acid-Glucose (TCG) extender on post-thawing quality of Donggala cattle sperm in. Semen samples were collected from Donggala bulls using an artificial vagina. The semen sample were then pooled and diluted with TCG supplemented with MLE. Three different concentrations of MLE (0 mg/mL, 0.5 mg/mL, 1.0 mg/mL, and 1.5 mg/mL) were tested to evaluate the post thawing motility and viability of Donggala cattle sperm. A standard cryopreservation procedure was used to freeze the semen and storagedin liquid nitrogen container. Sperm motility were valuated under light microscopy while sperm viability was assesed using eosin-nigrosin staining procedures. The results showed that post-thaw sperm motility and viability were significantly maintained compare to control group following the addition of MLE at 1.0 mg/mL ($p < 0.05$). The conclusion that moringa leaf extract at a concentration of 1.0 mg/mL in TCG medium provides optimal cryoprotection, improving the post-thaw quality of Donggala bull sperm. This natural additive may serve as a promising supplement in semen extenders to enhance artificial insemination outcomes and genetic conservation efforts.*

*Keywords: Donggala cattle, Moringa leaf extract, extraction methods, TCG extender, post-thawing sperm*

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## PENDAHULUAN

The success of artificial insemination in cattle is highly dependent on the quality of sperm produced through cryopreservation process. Despite its necessity, cryopreservation is known to cause cellular damage, especially because of oxidative stress brought on by the production of reactive oxygen species (ROS) during the freezing and thawing process. Sperm motility, membrane integrity, viability, and eventually fertility can all be harmed by these oxidative agents (Len, et. al., 2019)

Efforts to mitigate oxidative damage have led to the exploration of natural antioxidants as additives in cryoprotective media. Among these, *Moringa oleifera*, commonly known as Moringa, has garnered significant attention due to its rich phytochemical profile, including flavonoids, phenolics, and vitamin C, all known for their antioxidant properties. Incorporating Moringa leaf extract into semen extenders like Tris-Citric Acid-Glucose (TCG) medium could potentially improve the resilience of sperm cells against cryo-induced oxidative stress. This study aims to evaluate the effect of adding Moringa leaf extract to TCG medium on the post-thawing sperm quality of Donggala bulls. By assessing key parameters such as motility, viability, morphology, and membrane integrity, the research seeks to determine the potential of Moringa leaf extract as a natural antioxidant supplement to enhance cryopreservation outcomes in local cattle breeding programs.

## MATERI DAN METODE

### Experimental Animals and Semen Collection

This study was conducted using four clinically healthy Donggala bulls, aged 3–5 years, with proven fertility and maintained under uniform management and feeding conditions. Semen was collected using an artificial vagina twice a week over a period of 4 weeks, resulting in a total of 32 ejaculates. Only ejaculates meeting the minimum standard for motility (≥70%) and concentration (≥800 million sperm/mL) were used for further processing.

### Preparation of Moringa Leaf Extract

Fresh Moringa leaves were washed, air-dried at room temperature, and ground into powder. The powder was subjected to extraction using ethanol 70% in a 1:10 (w/v) ratio by maceration for 48 hours. The extract was filtered and concentrated using a rotary evaporator at 40°C until a semi-solid consistency was obtained. The extract was stored at 4°C in a dark container until use.

### Semen Dilution and Treatment Groups

The semen was diluted using a Tris-Citric Acid-Glucose (TCG) extender consisting of 3.028 g Tris (hydroxymethyl aminomethane), 1.675 g citric acid ,1.25 g glucose, 20% egg yolk, 7% glycerol, and Distilled water up to 100 mL.

The diluted semen was divided into four treatment groups:

T0 (Control): TCG without Moringa extract

T1: TCG + 0.5 mg/mL Moringa extract

T2: TCG + 1.0 mg/mL Moringa extract

T3: TCG + 1.5 mg/mL Moringa extract

Semen samples were equilibrated at 5°C for 4 hours, packed into 0.25 mL straws, and frozen in liquid nitrogen vapor for 10 minutes before being plunged into liquid nitrogen at −196°C for storage.

### Thawing and Evaluation of Sperm Quality

Frozen semen straws were thawed in a water bath at 37°C for 30 seconds. Post-thaw sperm quality was evaluated based on the following parameters:(Rios et., 2018)

- Motility: Assessed subjectively using light microscopy (400× magnification). by CASA system (computer-assisted sperm analyzer)
- Viability: Evaluated using eosin-nigrosin staining; 200 sperm cells per slide were counted.
- Abnormality: Determined using eosin-nigrosin staining; abnormalities were recorded.
- Membrane Integrity: Assessed using the Hypo-Osmotic Swelling (HOS) test.

Each parameter was evaluated in triplicate for each treatment group.

### Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) for post-hoc comparisons when significant differences were observed ($p < 0.05$). Statistical analysis was performed using SPSS version 25.0.

## RESULTS AND DISCUSSION

### Result

The study investigated the effect of adding various concentrations of Moringa leaf extract to TCG extender on the post-thaw sperm quality of Donggala bull. The evaluated parameters included sperm motility, viability, abnormality, and membrane integrity. The results are summarized in Table 1.

Table 1. Post-Thaw Sperm Quality of Donggala Bull in Different Treatment Groups

| Parameter | T0 (Control) | T1 (0.5 mg/mL) | T2 (1,0 mg/mL) | T3 (1,5 mg/mL) |
|---|---|---|---|---|
| Motility (%) | 52.3 ± 2.5$^{a}$ | 58.9 ± 2.0$^{b}$ | 65.4 ± 2.1$^{c}$ | 60.2 ± 2.3$^{b}$ |
| Viability (%) | 60.2 ± 2.0$^{a}$ | 66.7 ± 2.2$^{b}$ | 71.8 ± 1.8$^{c}$ | 67.1 ± 2.1$^{b}$ |
| Abnormality (%) | 78.4 ± 2.1$^{a}$ | 83.5 ± 1.7$^{b}$ | 87.6 ± 1.5$^{c}$ | 84.0 ± 1.9$^{b}$ |
| Membrane Integrity (%) | 56.1 ± 2.5$^{a}$ | 63.3 ± 2.0$^{b}$ | 68.7 ± 2.2$^{c}$ | 64.5 ± 1.8$^{b}$ |

*Note: Values are presented as mean ± standard deviation. Different superscript letters in the same row indicate significant differences ($p < 0.05$)*

### Motility

Sperm motility was significantly higher in all Moringa-treated groups compared to the control (T0). The T2 group (1.0 mg/mL) showed the highest motility (65.4%), indicating optimal preservation at this concentration.

### Viability

Sperm viability was also significantly improved in the Moringa-treated groups, with T2 yielding the highest viability at 71.8%, compared to 60.2% in the control group.

### Morphology

The percentage of morphologically normal sperm was significantly higher in the treatment groups, with the T2 group again showing the best result (87.6%), indicating better preservation of structural integrity.

## Membrane Integrity

Sperm membrane integrity, assessed via the Hypo-Osmotic Swelling (HOS) test, showed significant improvement in all treated groups. The T2 group had the highest membrane integrity at 68.7%, suggesting a strong protective effect from Moringa extract.

## Discussion

The results of this study demonstrated that the addition of MLE to the TCG medium can significantly maintain the post-thawing quality of Donggala bull sperm. Several key sperm parameters, including motility, viability, abnormality, and membrane integrity of the freeze-thawed sperm were significantly maintained after freezing and thawing. The group supplemented with 1.0 mg/mL MLE (T2) showed the highest sperm motility, viability, membrane integrity, and low abnormality among all treatments, indicating that this concentration offers the best cryoprotective effects. Sperm cells are severely stressed by cryopreservation, which has an impact on mitochondrial function and plasma membrane integrity, two factors that are essential for motility. One of the main causes of lipid peroxidation, mitochondrial damage, and reduced ATP synthesis—all of which eventually hinder sperm motility is the production of reactive oxygen species (ROS) during the freeze-thaw cycle (Agarwal et al., 2014); Syuhuud Arumbinang Wajdi et.al., 2021). Strong free radical scavengers found in seminal plasma, such as glutathione peroxidase, catalase, superoxide dismutase, and small molecular weight antioxidants including ascorbic acid and α-tocopherol, protect spermatozoa (Aitken and Baker, 2004; Sikka, 2004). The dangerous effects of oxygen free radicals were reduced by the antioxidants in the herbal additions. The advantageous synergistic qualities of herbal supplements and their many substances, as opposed to the single purified active fractions, are currently of great interest on a global scale (Seeram et al., 2004).

Moringa oleifera leaves are known to contain high concentrations of natural antioxidants, such as flavonoids, polyphenols, vitamin C, and carotenoids, which can effectively scavenge ROS and protect sperm cells from oxidative damage (Dina M. Shokrya et al., 2021). The presence of these compounds likely contributed to the improved motility observed in the Moringa-treated groups. Moringa leaves contain minerals, vitamins, amino acids, and antioxidants (Khalafalla et al. 2010). Antioxidants found in moringa include phenols, flavonoids, proanthocyanidins, flavonols, zinc, selenium, carotene, diet C, and diet E (Vergara-Jimenez et al. 2019). According to this analysis, MLME's phenolic content material is similar to others (Sreelatha and Padma 2009; Atawodi et al. 2010). Compared to Sreelatha and Padma (2009), our M. oleifera leaves' methanolic extract significantly reduced DPPH radicals. The polarity of the solvents and the location of the plant are likely the causes of this drop (Sreelatha and Padma 2009).

It is commonly known that antioxidants help preserve sperm motility during cryopreservation. Sajid Iqbal et al. (2021) claim that adding natural antioxidants to semen extenders can increase sperm motility and viability after thawing. According to Akhter et al. (2012), plant-derived antioxidants greatly improve sperm quality parameters like motility when added to extenders because they lessen oxidative stress. These findings suggest that Moringa leaf extract at optimal concentrations can protect spermatozoa during the freeze-thaw process by reducing oxidative stress. Moringa is known to contain high levels of antioxidants, such as flavonoids and vitamin C, which neutralize reactive oxygen species (ROS) and prevent lipid peroxidation of the sperm membrane. Antioxidants stabilize sperm membranes by reducing oxidative damage and preserving enzymatic activity essential for cell survival (José Maria Carrera-Chávez, 2020). This aligns with previous studies that have demonstrated increased viability when using plant-based antioxidants in semen extenders. This suggests that Moringa leaf extract helps preserve the ultrastructural integrity of sperm cells during the freeze-thaw process. Excessive ROS generation during cryopreservation is

known to damage both the DNA and structural components of sperm, and the application of natural antioxidants like Moringa may reduce this damage effectively. A functional membrane is crucial for sperm motility, fertilization, and interaction with the female reproductive tract. The antioxidants in Moringa leaf extract help maintain membrane fluidity and prevent premature acrosome reactions, thus improving fertility potential.

## CONCLUSION

Donggala bulls' post-thaw sperm quality was greatly maintained by adding Moringa leaf extract to the TCG semen extender, especially in terms of motility, viability, and membrane integrity. Because of the extract's antioxidant qualities, the most effective concentration was 1.0 mg/mL, which offered the best defence against cryo-damage. Higher concentrations might have negative effects and did not demonstrate any additional benefits. These results point to Moringa leaf extract as a natural supplement that may help Donggala bulls preserve their semen better. It may also find use in reproductive biotechnology and genetic conservation initiatives.

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## REFERENCES

Agarwal, A., Virk, G., Ong, C., & du Plessis, S. S. (2014). Effect of oxidative stress on male reproduction. *World Journal of Men's Health*, 32(1), 1–17. https://doi.org/10.5534/wjmh.2014.32.1.1

Aitken RJ and Baker MA, 2004. Oxidative stress and male reproductive biology. Reprod Fert Develop, 16: 581-588.

Akhter, S., Ansari, M. S., Andrabi, S. M. H., Ullah, N., & Qayyum, M. (2012). Effect of green tea extract on freezability, plasma membrane and acrosome integrity of buffalo bull spermatozoa. *Animal Reproduction Science*, 135(1–4), 144–149. https://doi.org/10.1016/j.anireprosci.2012.09.001

Bucak, M. N., Sarıözkan, S., Tuncer, P. B., Sakin, F., Alici, T., & Otlu, S. (2010). The effect of antioxidants on post-thawed bull sperm quality, lipid peroxidation and antioxidant activities. *Revue de Médecine Vétérinaire*, 161(12), 539–546.

Dina M. Shokrya , Mabrouk Attia Abd Eldaimb , Magdy R. Badr a , Hanem K. Khalifa c , Sahar H. Orabic ,Aziza M. Hassand and Ragab Dohreig. 2021. Enhancement impact of Moringa oleifera leaves extract–base extender oncryopreservation and fertilization of Barki ram sperms: comparative studywith vitamin E and selenium combination. ITALIAN JOURNAL OF ANIMAL SCIENCE2021, VOL. 20, NO. 1, https://doi.org/10.1080/1828051X.2021.1953411

José Maria Carrera-Chávez, Edson Eduardo Jiménez-Aguilar, Theisy Patricia Acosta-Pérez,José Alberto Núñez-Gastélum, Andrés Quezada-Casasola, Angélica María Escárcega-Ávila,Mateo Fabián Itza-Ortiz a and Ernesto Orozco-Lucero. 2020. Effect of Moringa oleifera seed extract on antioxidant activity and sperm characteristics in cryopreserved ram semen. JOURNAL OF APPLIED ANIMAL RESEARCH. VOL. 48, NO. 1, 114–120. https://doi.org/10.1080/09712119.2020.1741374

Holt, W. V. (2000). Basic aspects of frozen storage of semen. *Animal Reproduction Science*, 62(1–3), 3–22. https://doi.org/10.1016/S0378-4320(00)00152-4Iqbal, S., Naz, S., Bhutta, M. F., Sufyan, A., & Awan, M. A. (2021). Antioxidant effect of

*Moringa olifera* leaves extract in extender improves post-thaw quality, kinematics, lipid peroxidation, total antioxidant capacity and fertility of water buffalo bull semen. *Andrologia, 53*(10), e14300. https://doi.org/10.1111/and.14300

Kavita Khosla Chatley, Mukesh Bhakat, T.K. Mohanty, Raj Kumar, Ranjana Sinha, Abdul Rahim. 2025. The Impact of *Moringa oleifera* Leaf Extract on Microbial Load in Bovine Semen at Refrigerated Temperatures. Bhartiya Krishi Anusandhan Patrika, volume 40 issue 1 (march 2025) : 110-114. doi: 10.18805/BKAP770.

Khan, M. I. R., Naqvi, S. M. K., Dar, A. H., & Ahmad, S. (2011). Effects of different levels of vitamin E on motility and viability of spermatozoa in buck semen. *Iranian Journal of Applied Animal Science*, 1(4), 261–265.

Khalafalla, M.M., et al., 2010. Active principle from Moringa oleifera Lam leaves effective against two leukemias and a hepatocarcinoma. African Journal of Biotechnology, 9, 8467–8471Len, J. S., Koh, W. S. D., & Tan, S. X. (2019). The roles of reactive oxygen species and antioxidants in cryopreservation. *Bioscience Reports, 39*(8), BSR20191601. https://doi.org/10.1042/BSR20191601

Rios, A. P., Gascón, A., Martínez, J. V., Balasch, S., & Molina-Botella, I. (2018). Sperm preparation after freezing improves motile sperm count, motility, and viability in frozen-thawed sperm compared with sperm preparation before freezing-thawing process. *Journal of Assisted Reproduction and Genetics, 35*(2), 237–245. https://doi.org/10.1007/s10815-017-1050-2

Seeram NP, Adams LS, Hardy ML, et al., 2004. Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects. J Agric Food Chem, 52: 2512–2517

Sikka SC, 2004. Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. J Androl, 25: 5-1

Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of *Moringa oleifera* leaves. *Journal of Agricultural and Food Chemistry*, 51(8), 2144–2155. https://doi.org/10.1021/jf020444+

Syuhuud Arumbinang Wajdi, Budi Utomo, Rimayanti Rimayanti, Erma Safitri, Tri Wahyu Suprayogi, Wurlina Wurlina. 2021. Suppementation of Kelor Leaf (Moringa oleifera) Aqueous Extract Increase on Post-Thawed Limousin Bull Sperm Quality. Jurnal Medik Veteriner. https://doi.org/10.20473/jmv.vol4.iss2.2021.249-255

Vergara-Jimenez, M., et al., 2019. Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. Antioxidants (Basel, Switzerland), 6,4 91. 16

Watson, P. F. (2000). The causes of reduced fertility with cryopreserved semen. *Animal Reproduction Science*, 60–61, 481–492. https://doi.org/10.1016/S0378-4320(00)00099-3