International Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

Crossref

IJBSM August 2026, 17(8): 01-06 **Article** AR7105a

Research Article *Natural Resource Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2026.7105a

Cryoprotective Effects of α-Tocopherol, Quercetin and Their Combination on Sperm Functional Integrity during Cryopreservation of Murrah Buffalo (*Bubalus bubalis*) Semen

Avani Singh[1], Pavan Kumar Mittal[2], Govind Sahay Gottam[2], Barkha Gupta[2], Bhagat Singh Saini[2] and Deepak Sharma[3]

[1]Dept. of Veterinary Physiology and Biochemistry, Arawali Veterinary College, Sikar, Rajasthan (332 001), India
[2]Dept. of Veterinary Physiology and Biochemistry, Post Graduate Institute of Veterinary Education and Research, Rajasthan University of Veterinary and Animal Sciences, Jobner, Jaipur, Rajasthan (302 031), India
[3]Dept. of Veterinary Physiology and Biochemistry, Apollo College of Veterinary Medicine, Rajasthan University of Veterinary and Animal Sciences, Jobner, Jaipur, Rajasthan (302 031), India

Open Access

Corresponding drpavanmittal111@gmail.com

0000-0002-3084-5966

ABSTRACT

The study was conducted during August, 2022 to April, 2024 at frozen semen bank, Bassi, Jaipur and department of Veterinary Physiology and Biochemistry, Post Graduate Institute of Veterinary Education and Research (PGIVER) Jaipur, Rajasthan, India to study the cryoprotective impacts on the combined supplementation for superior protective effects over the individual treatments in cryopreserved Murrah Buffalo (*Bubalus Bubalis*) bull semen. Total ejaculates were collected and evaluated. Ejaculate was processed separately and divided into four equal aliquots designated as Control (no antioxidant), T_1 (α-tocopherol @ 0.65 mg ml^{-1}), T_2 (quercetin @ 75 µM), and T_3 (α-tocopherol @ 0.65 mg ml^{-1}+quercetin @ 75 µM). Samples were cooled to 5°C in a cold cabinet, packed in 0.25 ml French straws, frozen, and stored in liquid nitrogen (-196°C). Frozen straws were thawed individually at 37°C for 30 s before evaluation. Significant differences (p<0.05) were observed among the treatment groups for all evaluated semen quality parameters, including progressive motility (%), Live spermatozoa (%), Intact acrosome (%) and Abnormal spermatozoa (%) at all stages of cryopreservation, including post-dilution (PD), post-equilibration (PE), and post-thaw (PT) stages. Among the treatments, the combination group (T_3) exhibited significantly superior results compared to the individual antioxidant treatments (T_1 and T_2), whereas T_2 showed significantly better performance than T_1 for most of the evaluated parameters. The present study concluded that the combination of α-tocopherol and quercetin exerts the most pronounced protective effect against oxidative stress and cryoinjury, compared to their individual supplementation, thereby improving semen quality during cryopreservation in Murrah buffalo bulls.

KEYWORDS: Antioxidants, cryopreservation, membrane integrity, murrah, tocopherol, quercetin

***Citation* (VANCOUVER):** Singh et al., Cryoprotective Effects of α-Tocopherol, Quercetin and Their Combination on Sperm Functional Integrity during Cryopreservation of Murrah buffalo (*Bubalus bubalis*) Semen. *International Journal of Bio-resource and Stress Management*, 2026; 17(8), 01-06. HTTPS://DOI.ORG/10.23910/1.2026.7105a.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, author have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The author has declared that no conflict of interest exists.

RECEIVED on 02nd April 2026 RECEIVED in revised form on 12th July 2026 ACCEPTED in final form on 29th July 2026 PUBLISHED on 10th August 2026

1. INTRODUCTION

Artificial insemination (AI) is widely recognized as a key reproductive technology in modern livestock production, allowing efficient dissemination of superior germplasm and accelerating genetic improvement in bovine populations. However, the success of AI programs is fundamentally dependent on the biological competence of cryopreserved semen. Despite notable progress in semen extender formulations and cryobiological techniques, the freezing–thawing process continues to impose substantial physical and biochemical stress on spermatozoa, which often results in compromised post-thaw quality and reduced fertility performance (Yuan et al., 2022; Luo et al., 2023). Mature spermatozoa are highly specialized cells with minimal cytoplasmic content, a feature that limits their intrinsic antioxidant defence capacity. This structural characteristic makes them particularly prone to oxidative injury. During cryopreservation, sperm cells are subjected to sequential stressors, including rapid temperature shifts, osmotic changes, lipid phase transitions, and excessive production of reactive oxygen species (ROS). The combined effect of these factors leads to membrane destabilization, mitochondrial dysfunction and alterations in chromatin integrity, ultimately reducing sperm motility and survival (Bang et al., 2022; Aitken and Drevet, 2020). Among the multiple pathways contributing to cryodamage, oxidative stress plays a predominant role. The sperm plasma membrane contains abundant polyunsaturated fatty acids (PUFAs), which are essential for maintaining membrane fluidity and supporting key fertilization-related processes. However, this lipid composition also increases susceptibility to ROS-induced lipid peroxidation. Excessive ROS disrupts membrane permeability, alters ionic equilibrium, and may induce premature capacitation or acrosomal exocytosis, thereby impairing fertilization capability (Jiménez-Aguilar et al., 2021; Reyes et al., 2022). Preservation of plasma membrane stability and acrosomal structure is therefore critical for maintaining functional competence after thawing. The acrosome, located at the anterior region of the sperm head, contains enzymes necessary for penetration of the zona pellucida during fertilization. Cryopreservation-associated damage to the acrosomal membrane can result in premature acrosome reaction or leakage of enzymatic contents, reducing the fertilizing potential of spermatozoa. Accordingly, assessment of membrane functionality through the hypo-osmotic swelling test (HOST), along with evaluation of acrosomal integrity, provides reliable indicators of sperm quality following freezing and thawing. In recent years, antioxidant supplementation in semen extenders has been explored as a strategy to reduce ROS-mediated injury. α-Tocopherol (vitamin E), a lipid-soluble antioxidant, integrates into the sperm membrane and interrupts lipid peroxidation chain reactions by neutralizing peroxyl radicals. Its inclusion in cryopreservation media has been associated with improved membrane preservation and enhanced post-thaw motility in bovine and buffalo semen (Mittal et al., 2014; Kumar et al., 2018; Yuan et al., 2022). Quercetin, a bioactive flavonoid, possesses strong antioxidant and metal-chelating properties. In addition to directly scavenging reactive species, it modulates endogenous antioxidant systems, including enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Several studies have demonstrated the beneficial effects of quercetin supplementation on post-thaw sperm characteristics across different livestock species (Ahmed et al., 2019; Bang et al., 2022). The Murrah buffalo is an economically important dairy breed contributing substantially to milk production systems. Buffalo spermatozoa are generally regarded as more sensitive to cryo-induced oxidative stress compared to those of cattle, underscoring the importance of developing optimized, species-specific cryopreservation strategies. Improving semen preservation efficiency in this breed is therefore directly linked to enhanced reproductive efficiency and effective genetic dissemination. Based on these considerations, the present study was designed to examine the individual and combined effects of α-tocopherol and quercetin supplementation in a Tris–egg yolk–glycerol extender on plasma membrane and acrosomal integrity of Murrah buffalo bull spermatozoa at post-dilution, post-equilibration, and post-thaw stages. It was hypothesized that combined supplementation would provide superior protective effects compared to individual treatments, thereby strengthening resistance to oxidative cryodamage and improving post-thaw sperm functional quality.

2. MATERIALS AND METHODS

The study was conducted on four Murrah buffalo bulls aged 3–6 years and weighing 500–550 kg, maintained at the Biosecure Farm Complex and the Frozen Semen Bank, Bassi, Jaipur, located in the semi-arid region of northern India. Buffalo Bulls were housed in individual pens with concrete flooring and asbestos roofing, fed a balanced ration, and vaccinated against major contagious and infectious diseases.

Semen was collected twice weekly using a Danish-model artificial vagina on a dummy, between 7:00–8:00 AM during summer and 8:30–9:00 AM in other seasons, from August, 2022 to April, 2024. A total of 24 ejaculates (six from each of four Murrah buffalo bulls) were collected and evaluated individually at 37°C.

Each ejaculate was processed separately and divided into

four equal aliquots and extended with Tris–egg yolk–glycerol (TEYG) containing the following treatments: T_1 (α-tocopherol @ 0.65 mg ml^{-1}), T_2 (quercetin @ 75 μM), T_3 (α-tocopherol @ 0.65 mg ml^{-1}+quercetin @ 75 μM), and control (no antioxidant). Each pooled ejaculate was considered as a single experimental unit. For each treatment, three independent pools were prepared from the ejaculates of four Murrah buffalo bulls, and each pool was divided into four equal aliquots corresponding to the treatments (T_1, T_2, T_3 and Control). All analyses were performed in triplicate for each pool to ensure reproducibility. The concentrations of α-tocopherol (0.65 mg ml^{-1}) and quercetin (75 μM) were selected based on previous studies demonstrating optimal improvement in post-thaw sperm motility, acrosomal integrity and Hypo-osmotic swelling Test (HOST) during cryopreservation (Batool et al., 2024; Prajapati et al., 2022). Preliminary trials with varying concentrations also indicated that these levels provided maximal protective effects without inducing cytotoxicity.

Semen quality was assessed at pre-dilution, post-equilibration, and post-thaw stages. Progressive motility was evaluated using a phase-contrast microscope with a 40× objective on a thermostatically controlled stage at 37°C (Pardede et al., 2023). Sperm viability and abnormalities were determined via differential staining (Kanna and Shetty, 2023), counting a minimum of 200 spermatozoa per sample. Acrosome integrity was assessed using Giemsa staining (Manokaran et al., 2010).

Data were analyzed using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). The effects of treatments on semen quality parameters were assessed using a General Linear Model (multivariate analysis of variance). Post hoc pairwise comparisons were performed with Duncan's Multiple Range Test (DMRT). Results are presented as mean ± standard error (SE), and differences were deemed statistically significant at $p<0.05$.

3. RESULTS AND DISCUSSION

The mean (±SE) values of progressive motility, viability, acrosomal integrity, and morphological abnormalities of Murrah buffalo bull semen extended with Tris egg yolk glycerol (TEYG) supplemented with α-tocopherol (T_1), quercetin (T_2), and their combination (T_3) at post-dilution (PD), post-equilibration (PE), and post-thaw (PT) stages are summarized in Table 1. Analysis of variance revealed significant ($p<0.05$) differences among treatment groups for all evaluated parameters at each stage of cryopreservation.

Progressive motility was significantly ($p<0.05$) higher in antioxidant-supplemented groups compared to control across all stages. At PD, T_3 recorded the highest motility (73.91±0.63), followed by T_1 (68.94±0.56) and T_2 (66.00±0.62), whereas the control exhibited the lowest value (64.75±0.71). This superiority of T-3 was maintained at PE (66.58±0.56) and PT (61.53±0.50) stages.

The highest percentage of live spermatozoa was obtained

Table 1: Effect of different antioxidants and their combination on percentage of intact acrosomes and HOST positive spermatozoa of Murrah bull spermatozoa at each stage of cryopreservation

Semen attributes	Stages	Antioxidant treatment			
		Control (C)	α-Tocopherol T_1	Quercetin T_2	α-Tocopherol+ Quercetin T_3
Progressive motility (%)	PD	64.75^{a}±0.71	68.94^{b}±0.56	66.00^{a}±0.62	73.91^{c}±0.63
	PE	58.25^{a}±0.58	63.22^{b}±0.56	61.28^{c}±0.42	66.59^{d}±0.58
	PT	50.31^{a}±0.41	55.34^{b}±0.45	53.13^{c}±0.50	61.53^{d}±0.50
Live (%)	PD	80.47^{a}±0.41	84.27^{b}±0.38	82.67^{c}±0.33	85.95^{d}±0.32
	PE	76.72^{a}±0.39	78.98^{b}±0.44	77.70^{a}±0.41	80.11^{b}±0.45
	PT	57.13^{a}±0.57	60.94^{b}±0.63	58.72^{c}±0.55	63.41^{d}±0.51
Intact acrosome (%)	PD	82.13^{a}±0.37	84.38^{b}±0.32	82.89^{a}±0.31	86.34^{c}±0.33
	PE	78.94^{a}±0.40	81.92^{b}±0.30	80.91^{c}±0.29	83.91^{d}±0.24
	PT	70.31^{a}±0.45	74.67^{b}±0.42	71.95^{c}±0.42	76.36^{d}±0.42
Abnormal sperm%	PD	9.56^{a}±0.29	8.16^{b}±0.28	7.91^{b}±0.28	6.77^{c}±0.25
	PE	11.17^{a}±0.32	9.31^{b}±0.20	10.22^{c}±0.26	8.42^{d}±0.26
	PT	12.22^{a}±0.28	$10.88^{b,c}$±0.17	11.34^{b}±0.17	10.70^{c}±0.22

Means bearing different superscripts (a–d) within rows differ significantly ($p<0.05$); PD: Post-dilution; PE: Post-equilibration; PT: Post-thaw

in T_3 at PD (85.95±0.32), PE (80.11±0.45), and PT (63.41±0.51), differing significantly (p<0.05) from individual antioxidant treatments and control.

Semen samples receiving combined supplementation (T_3) showed the highest proportion of intact acrosomes at PD (86.34±0.33), PE (83.91±0.24), and PT (76.36±0.42), while control samples exhibited comparatively lower values.

The percentage of morphologically abnormal spermatozoa was significantly (p<0.05) reduced in antioxidant-treated groups. The lowest abnormality rates were recorded in T-3 at PD (6.77±0.25), PE (8.42±0.26), and PT (10.70±0.26), whereas the control group consistently showed higher values (9.56±0.29, 11.17±0.32, and 12.22±0.28, respectively).

3.1. Progressive motility

Progressive motility decreased significantly (p<0.05) from PD to PT stage in all treatments, indicating the adverse impact of cryopreservation on sperm function. The freeze–thaw process is known to induce oxidative stress, lipid peroxidation, and structural alterations in the sperm plasma membrane and flagellar apparatus, which ultimately compromise motility (Tironi et al., 2019; Prajapati et al., 2022). In the present study, semen supplemented with α-tocopherol (T_1) maintained higher motility than the quercetin (T_2) and control group, suggesting that vitamin E protected membrane lipids from oxidative damage during processing. Comparable improvement in post-thaw motility with α-tocopherol supplementation has been reported in bovine semen (Kumar et al., 2018; Yuan et al., 2022). Semen treated with quercetin (T_2) also retained relatively higher motility than the control, which may be associated with its strong antioxidant activity and protective effect on mitochondrial function (Prastiya et al., 2021). The combined supplementation (T_3) consistently showed the highest motility at all stages, indicating that the interaction of α-tocopherol and quercetin enhanced protection against cryo-induced oxidative damage. Similar beneficial effects of antioxidant combinations on sperm motility during semen cryopreservation have been reported in livestock species (Bang et al., 2022; Reyes et al., 2022).

3.2. Live spermatozoa percentage

Sperm viability declined significantly (p<0.05) from post-dilution to post-thaw stage in all groups, reflecting the susceptibility of spermatozoa to cryogenic stress during freezing and thawing. Such reduction is commonly associated with oxidative stress and membrane lipid peroxidation that impair sperm survival (Tironi et al., 2019; Prajapati et al., 2022). In the present study, α-tocopherol supplementation (T_1) maintained higher viability than the quercetin (T_2) and control group, indicating its protective role in stabilizing sperm membrane lipids. Similar improvements in post-thaw sperm survival with vitamin E supplementation have been reported in bovine semen (Kumar et al., 2018; Yuan et al., 2022). Quercetin-treated samples (T_2) also exhibited relatively better viability, likely due to their potent antioxidant and ROS-scavenging properties (Prastiya et al., 2021). The combined treatment (T_3) consistently produced the highest viability across all stages, suggesting a synergistic protective effect of α-tocopherol and quercetin during semen cryopreservation, which agrees with observations in livestock semen reported by Bang et al. (2022) and Reyes et al. (2022).

3.3. Acrosomal integrity

A significant (p<0.05) reduction in the proportion of intact acrosomes was observed from PD to PT stage in all groups, reflecting the susceptibility of sperm membranes to cryo-induced stress. Cryopreservation is known to impair acrosomal membranes through osmotic imbalance, lipid peroxidation and oxidative damage generated during freezing–thawing. In the present study, antioxidant supplementation improved acrosomal preservation compared with the control. Semen treated with α-tocopherol (T_1) maintained higher acrosomal integrity, which may be attributed to its role in protecting membrane phospholipids against peroxidative damage. Comparable improvements in acrosomal preservation with vitamin E supplementation have been reported in bovine semen (Kumar et al., 2018; Yuan et al., 2022). Quercetin treatment (T_2) also showed improved acrosomal status, likely due to its potent free-radical scavenging and membrane-stabilizing properties (Ahmed et al., 2019; Bang et al., 2022). The combined supplementation (T_3) consistently produced the highest proportion of intact acrosomes, suggesting a synergistic protective effect of α-tocopherol and quercetin during cryopreservation, as also reported in livestock semen by Reyes et al. (2022) and Luo et al. (2023).

3.4. Abnormal sperm percentage

The proportion of morphologically abnormal spermatozoa increased (p<0.05) progressively from PD to PT stage in all groups, indicating the detrimental effect of the freeze–thaw process on sperm structure. Cryopreservation is known to generate excessive reactive oxygen species and disturb membrane lipids, which can damage the head, midpiece, and tail regions of spermatozoa and thereby increase morphological defects (Agarwal et al., 2014). In the present investigation, antioxidant-treated groups showed a comparatively lower proportion of abnormal sperm than the control at all stages of processing. Supplementation with α-tocopherol (T_1) reduced structural defects, which may be related to its ability to stabilize membrane phospholipids and protect cells against oxidative injury. Comparable reductions in sperm abnormalities following vitamin E supplementation have been described in bovine semen by

Kumar et al. (2018) and Luo et al. (2023). The quercetin-treated group (T_2) also exhibited fewer abnormalities than the control, likely due to its capacity to neutralize reactive oxygen species and preserve membrane stability (Ahmed et al., 2019; Prajapati et al., 2022). The lowest abnormality levels were recorded in the combined treatment (T_3), suggesting that the simultaneous presence of α-tocopherol and quercetin provided stronger protection against cryo-induced structural damage to spermatozoa.

5. CONCLUSION

The antioxidant supplementation in semen extender improved the post-thaw quality of Murrah bull semen. Both α-tocopherol and quercetin individually enhanced motility, viability, acrosomal integrity and reduced abnormalities compared to control. Their combined use showed a superior protective effect, indicating synergistic action. The improvement was attributed to reduced oxidative stress and better membrane stabilization during cryopreservation.

6. ACKNOWLEDGEMENT

The authors are grateful to the Dean, Post-Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur, and the In-Charge, Frozen Semen Bank, Bassi, Jaipur, for providing the necessary facilities to conduct the study.

7. 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. DOI: https://doi.org/10.5534/wjmh.2014.32.1.1.

Ahmed, H., Jahan, S., Salman, M.M., Ullah, F., 2019. Stimulating effects of quercetin in Tris–citric acid extender on post-thaw quality and in vivo fertility of buffalo (*Bubalus bubalis*) bull spermatozoa. Theriogenology 134, 18–23. DOI: org/10.1016/J.THERIOGENOLOGY.2019.05.012.

Aitken, R.J., Drevet, J.R., 2020. The importance of oxidative stress in determining the functionality of mammalian spermatozoa: A two-edged sword. Antioxidants 9, 111. DOI: http://dx.doi.org/10.3390/antiox9020111.

Bang, S., Tanga, B.M., Fang, X., Seong, G., Saadeldin, I.M., Qamar, A.Y., Lee, S., Kim, K.J., Park, Y.J., Nabeel, A.H.T., Yu, I.J., 2022. Cryopreservation of pig semen using a quercetin-supplemented freezing extender. Life 12(8), 1155. DOI: doi.org/10.3390/life12081155.

Batool, I., Fayyaz, M.H., Hameed, A., Andrabi, S.M.H., Kausar, R., Shahzad, M., Mubashir, Y., Omur, A.D., Murtaza, G., Ditta, A., Hussain, T., 2024. Quercetin in semen extender improves frozen-thawed spermatozoa quality and in-vivo fertility in crossbred Kamori goats. Frontiers in Veterinary Science 11, 1–10. DOI: doi.org/10.3389/fvets.2024.1385642.

Jiménez-Aguilar, E., Quezada-Casasola, A., Prieto-Caraveo, M., Orozco-Lucero, E., Itzá-Ortiz, M., Carrera-Chávez, J., 2021. Evaluation of the addition of quercetin and vitamin E to the cryopreservation medium of ram semen on in vivo fertility. Abanico Veterinario 11. DOI: doi.org/10.21929/abavet2021.36.

Kanna, S., Shetty, A., 2023. Eosin-nigrosin staining technique in assessment of sperm vitality in medical laboratories – a snippet from our experience on implementing the staining, interpretation and quality control procedures. Indian Journal of Obstetrics and Gynecology Research 10(2), 227–229. DOI: https://doi.org/10.18231/j.ijogr.2023.048.

Kumar, N., Singh, A.K., Cheema, R.S., Kumar, A., Kaur, H., Brar, P.S., 2018. Effect of vitamin E supplementation during buffalo semen cryopreservation on sperm characteristics and oxidative stress. Journal of Animal Research 8(5), 797–805. DOI: 10.30954/2277-940X.10.2018.9.

Luo, X., Wu, D., Liang, M., Huang, S., Shi, D., Li, X., 2023. Effects of melatonin, glutathione and vitamin E on semen cryopreservation of Mediterranean buffalo. Theriogenology 197, 94–100. DOI: 10.1016/j.theriogenology.2022.11.035.

Manokaran, S., Patel, S.H., Nikhate, R.V., Chaudhari, N.N., Shukla, H.R., 2010. A modified Giemsa staining technique for evaluation of acrosomal integrity of frozen semen. Indian Journal of Animal Reproduction 31(2), 46. Available from: https://acspublisher.com/journals/index.php/ijar/article/view/4319.

Mittal, P.K., Madan, A.K., Anand, M., Yadav, S., Kumar, J., 2014. Effect of vitamin E, vitamin C and their combination on membrane integrity of Bhadawari bull cryopreserved spermatozoa. Indian Journal of Veterinary Research 23(2), 17–20. Available from: https://www.researchgate.net/publication/280345508.

Pardede, B.P., Kusumawati, A., Pangestu, M., Purwantara, B., 2023. Bovine sperm HSP-70 molecules: a potential cryo-tolerance marker associated with semen quality and fertility rate. Frontiers in Veterinary Science 10, 1–10. DOI: doi.org/10.3389/fvets.2023.1167594.

Prajapati, S., Vala, K., Solanki, G., Singh, V., Chavda, B., 2022. Effects of quercetin as an extender-additive on cryo-preservation of Gir bull semen. Indian Journal of Animal Reproduction 42, 42–47. Available from: https://issar.net.in/index.php/issar/article/view/653/246.

Prastiya, R.A., Rimayanti, Munir, M.M., Nugroho, A.P., 2021. The protective impacts of α-tocopherol supplementation on the semen quality of the Sapera goat preserved at 4°C. Tropical Animal Science Journal 44(3), 261–266. DOI: doi.org/10.5398/tasj.2021.44.3.261.

Reyes, A.E.I., Mosqueda, M.D.L.J., Pérez, O.G., Muñiz, A.R.O., Gonzalez, J.A.G., Mancera, A.E.V., Vazquez, A.G., Aparicio, P.S., Cedeno, C.J.B., Izquierdo, A.C., 2022. Effect of the addition of α-tocopherol and quercetin as antioxidants to the diluent, in the freezing of boar semen on sperm quality. European Journal of Biology and Biotechnology 3(4), 8–12. Available from: https://www.researchgate.net/publication/365399195.

Tironi, S.M.T., Martinez, A.C., Seixas, F.A.V., Stefanello, T.F., Nakamura, C.V., de Moraes, G.V., 2019. Effects of treatment with quercetin on the quality of cryopreserved bovine. Acta Scientiae Veterinariae 47, 1635. DOI: https://doi.org/10.22456/1679-9216.90279.

Yuan, C., Wang, H., Li, X., Liu, H., Zhao, J., Lu, W., Wang, J., 2022. Combined effect of flaxseed oil and vitamin E supplementation during bull semen cryopreservation on sperm characteristics. Bio Preservation and Biobanking 20(6), 520–528. DOI: 10.1089/bio.2021.0059.