The effect of lycopene and ascorbic acid on the post thaw Angora buck semen parameters

Research Article

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ABSTRACT

The main goal of this research was to examine the impact of antioxidants, specifically lycopene and ascorbic acid, on the viability of goat sperm following cryopreservation and thawing. Goat rearing plays a vital role worldwide by supplying animal protein, creating employment opportunities, and providing raw materials for various industries. Improving the reproductive efficiency of goats is crucial for genetic conservation, as well as for enhancing profitability and sustainability for breeders. This study illustrates that both lycopene and ascorbic acid, either alone or in combination, have a positive influence on motility, plasma and acrosome integrity, and mitochondrial activity after thawing. Semen samples were obtained from four mature goats and segregated into four groups: control (C), lycopene at 2 mM/mL (L2), ascorbic acid at 5 mM/mL (A5), and a combination of lycopene at 2 mM/mL + ascorbic acid at 5 mM/mL (L2A5). Following dilution in Tris/egg yolk diluent, the semen samples were cryopreserved in liquid nitrogen and then thawed for assessment. The L2A5 group displayed the highest values across all evaluated parameters (motility, plasma and acrosome integrity, and mitochondrial activity) when compared to the control group ($p<0.05$). These results indicate that the concurrent use of lycopene and ascorbic acid can significantly enhance the quality of cryopreserved goat semen, thereby contributing to improved reproductive outcomes and genetic preservation in goats.

Keywords: Ascorbic acid, buck, cryopreservation, lycopene, semen

INTRODUCTION

Due to its excessive hardiness and performance to harsh conditions outdoors, the goat is distributed in many geographical areas. Goat farming is a commercial enterprise that seeks to maximize sales, create capital, and provide employment. It also focuses on the availability of animal protein in the meat and dairy sector for human consumption and the provision of raw materials and garments to the leather and garment industries. The total number of goats raised worldwide is approximately 1.2 billion. Goat consumption has continuously increased, implying the influence of demands and the economic and environmental value of goats in different geographical locations. Goat meat is an essential food source throughout the world, especially in developing countries, which are mostly in the tropics. Despite its significance and consumption in developing countries, where over 90% of goat populations exist, with 56%, but 1.8% of goats are available in Europe. Goat meat is widely consumed in Asia, Africa, and the Caribbean. On the other hand, goat milk is ranked third globally in dairy production but consumed by more people than all other dairy products (Raheem, 2024; Hussein et al., 2023).

The main function of a semen extender is to provide the spermatozoa with nutrients, protect them from the harmful effects of cold shock, and

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keep the optimal conditions for sperm in the rice straw of the cryocenter. Cryopreservation remains one of the most challenging aspects of applications involving the preservation of goat origin sperm (Purdy, 2006). The buffer used to preserve the spermatozoa in goats includes Tris and citric acid, which is the same as that which is effective in preserving spermatozoa of a buck (Mishra et al., 2010, Narlicay and Uslu, 2022). In most cases, diluted semen of goats is done using the two most commonly utilized sex dampen, which are sex extender based on skim milk or egg yolk. Egg yolk contains lipids that are hydrolyzed by a specific lipase enzyme, which is an element of the bulbourethral gland of the goat. A lipase enzyme interacts with skim milk loin by hydrolyzing triglycerides and develops a compound that is detrimental to sperm vigor in males (Sias et al., 2005). The resultant interaction that happens in goat semen is not with bull sperm, boar sperm, or ram sperm, but it is exclusively observable in goat semen. The protein is the causal factor for the decrease in sperm lively and it is the protein fraction called SBU III originated from the parotid of the goat (Pellicer-Rubio et al., 1997). Cryopreservation may cause DNA fragmentation, acrosomal defects, membrane lipoid peroxidation (Atiken, 2020), mitochondrial potentialization (Shah et al., 2016) and apoptotic changes (Agarwal and Majzoub, 2017) of spermatozoa. Spermatozoan births are mainly induced by an increased manufacture of reactive oxygen (Klalique et al., 2023). The impact of ROS on spermatozoa is associated with a reduction in sperm vigorous and an increment in dead and slow spermatozoa, decreases in fertilization, implantation, and pregnancy rates, decreased cleavage, decreased quality of the embryo, and anoreduction in blastocyst initiation by Simon et al., (2017). The authority of ROS transactions within the physiological system must be understood in governance, which results in isotopic loss. Many studies have been conducted concerning providing the reactive balance of ROS and search sperm as out as a point of sperm during preservation by administering exogenous antioxidants namely lycopene and Vitamin C (ascorbic Acid) (Al-Mutary, 2021).

Lycopene, a naturally occurring carotenoid with strong antioxidant properties, can be commonly found in foods such as tomatoes, watermelon, papayas, and pink grapefruit. It plays a crucial role in protecting cells and tissues from lipid peroxidation by engaging in processes that involve quenching singlet molecular oxygen and scavenging peroxy radicals. The antioxidant activity of lycopene is primarily catalytic in nature (Velmurugan et al., 2004). Research indicates that lycopene supplementation can enhance sperm motility, cell membrane integrity, and DNA integrity by up to 70-73% (Bucak et al. 2015; Uysal and Bucak, 2007; Zini et al. 2010). In contrast, vitamin C (ascorbic acid) is an enzyme-free, water-soluble antioxidant known for its high efficacy in combating reactive oxygen species. Studies suggest that there might be saturation levels of vitamin C in certain tissues. Acting as a scavenger for reactive oxygen species, vitamin C has been shown to protect sperm by minimizing damage to sperm membranes. This protective effect can ultimately improve sperm motility, vitality, and potentially aid in preserving sperm health (Amini et al., 2015).

The primary goal of this study was to investigate the effect of antioxidants like lycopene and ascorbic acid on the properties of goat sperm after thawing their cryopreserved samples. With goats currently numbering into growing populations and circles worldwide, enhancing reproductive efficiency to save genetic capacity is of great importance. Improvements in sperm quality can result in significant gains for breeders regarding profitability and sustainability. Then, if cryopreservation techniques for goat semen improve development begins to occur, it undoubtedly will have a positive effect on population genetics. This study shows that when

used alone or in combination, lycopene and ascorbic acid will positively affect parameters such as motility, plasma and acrosome integrity, and mitochondrial activity after freezing and thawing of goat semen.

MATERIALS AND METHODS

Reagents

All chemicals and reagents used in study (Citric Acid C0706, Glycerol G2025, Fructose F2543, Lycopene L9879, L-Ascorbic Acid A4544, FITC-PNA L7381, Trisma Base T6066) were purchased from Sigma-Aldrich (St. Louis, MO, USA), Biological Industries (1 % Penicillin-streptomycin-amphotericin B mixture, 03- 033-1B) and Thermo Fisher Scientific, Waltham, MA, USA (Live/Dead™ Viability Kit L7011, JC-1 T3168). Lycopene dosage was prepared by modifying from Ren et al., (2018). Ascorbic acid dosage was prepared by modifying from Paudel et al., (2010).

Collection of semen

In the study, 4 adult goats (aged 2-5 years) were used. The care and feeding of the animals were carried out at Prof. Dr. Hümeyra Özgen Research and Application Farm. The research was conducted during the breeding season. Ejaculates were collected three times a week with the help of an electroejaculator. Mass and motility examinations were performed on the collected ejaculates. Ejaculates with a mass score of 3 or higher and a motility rate of over 80% were mixed and transferred to a water bath at 37°C. Tris extender (Fructose 82.66 mM, citric acid 96.32 mM, tris 297.8 mM, 1 egg yolk, 5% glycerol; pH: 7, 300 mosm) was used as the basic diluent for sperm. The mixed ejaculates were divided into four equal volumes as follows:

1. Control (C)
2. Lycopene 2 mM/mL (L2)
3. Ascorbic acid 5 mM/mL (A5)
4. Lycopene 2 mM/mL + Ascorbic acid 5 mM/mL (L2A5)

The samples were diluted in Tris/egg yolk diluent at 37°C to approximately 400 x 10^6 spermatozoa per milliliter. Following dilution, the semen samples were drawn into 0.25 mL French straws and equilibrated at +4°C for 3 hours. After equilibration, the semen samples were frozen in liquid nitrogen vapor (~-100°C) for 15 minutes and then stored in liquid nitrogen at -196°C. At the end of equilibration and after being stored in liquid nitrogen for at least 24 hours, the samples were thawed at 37°C for 25 seconds and evaluated for sperm quality parameters (motility, plasma and acrosome integrity, and mitochondrial activity).

Mass activity

According to Evans and Maxwell (1987), a drop of freshly obtained semen was placed on a slide and examined under a heated stage microscope with a 4x objective to assess the general movement of the sperm. The assessment was scored on a scale from 1 to 5.

Motility evaluation

Motility evaluation was conducted at 37°C using a heated stage phase-contrast microscope at 400x magnification. Semen sample (10 µL) was placed between a slide and coverslip, and motility was assessed by examining at least 7 different microscope fields. The average motility values from these fields were recorded as the motility rate (Evans and Maxwell, 1987).

Assessment of plasma membrane integrity

SYBR-14/PI was employed to evaluate spermatozoa viability (Garner and Johnson, 1995). Thawed sperm samples were diluted 1:3 with PBS (2 x 10^6 spermatozoa/mL). Subsequently, 30 µL of the sample was combined with 2.5 µL propidium iodide (PI) (2 mg PI in 1 ml distilled water) and 2.5 µL SYBR stock solution (diluted 1:5 with DMSO), followed by an incubation in darkness at 37°C for 15 minutes. The samples were then fixed with

10 µL Hancock solution. Evaluation was done using a fluorescent microscope (Leica DM 3000). Spermatozoa bearing green staining were deemed to possess intact plasma membranes, whereas those displaying red staining were interpreted as having compromised plasma membranes (Figure 1).

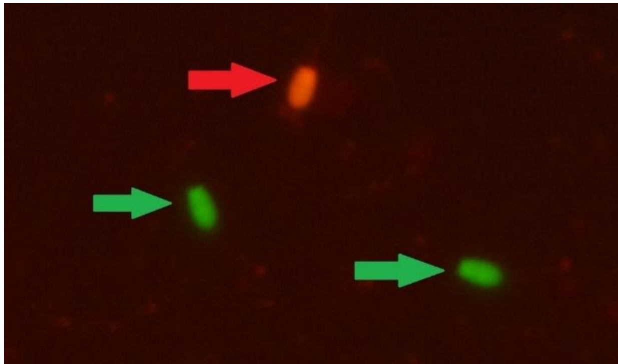

Figure 1. SYBR-14/PI staining for plasma membrane staining. Green spermatozoa heads represent intact plasma membrane (green arrows), red spermatozoa head represents damaged plasma membrane (red arrow).

Assessment of acrosome membrane integrity

A modified staining technique using Fluorescein isothiocyanate conjugated to Arachis hypogaea/PI (FITC-PNA/PI) was employed, as described by Nagy et al., (2003), to evaluate spermatozoa acrosome integrity. In this method, 5 µl of FITC-PNA stock solution (100 µg FITC-PNA/1 mL PBS) and 2.5 µl of PI solution were combined with 60 µl of diluted sperm sample at 37°C. The mixture was then dark-incubated at 37°C for 15 minutes followed by fixation with 10 µL Hancock solution. Acrosome integrity was assessed using a fluorescent phase-contrast microscope where spermatozoa exhibiting green, fluorescent acrosomes were classified as having damaged acrosomes, while those lacking green fluorescence were considered to have intact acrosomes (Figure 2).

Assessment of mitochondrial activity

Spermatozoon mitochondrial function was assessed using a modified JC-1/PI staining technique modified by Garner et al., (1997). A stock solution of JC-1 (1.53 mM) was prepared in DMSO. The cryopreserved spermatozoa were thawed in a 37°C water bath, diluted 1:3 with phosphate-buffered saline (PBS), and combined with 2.5 µL of JC-1 and 2.5 µL of PI in a 300 µL diluted sperm sample. Following a 15-minute incubation at 37°C in darkness, 10 µL of Hancock solution was added to stop reaction. Evaluation involved placing the sperm sample on a microscope slide, covering it with a coverslip, and examining 200 spermatozoa under a fluorescence microscope at 400× magnification. Mitochondrial activity was indicated by yellow-orange or bright green fluorescence in the midpiece, while pale dull green fluorescence signified the absence of mitochondrial activity (Figure 3).

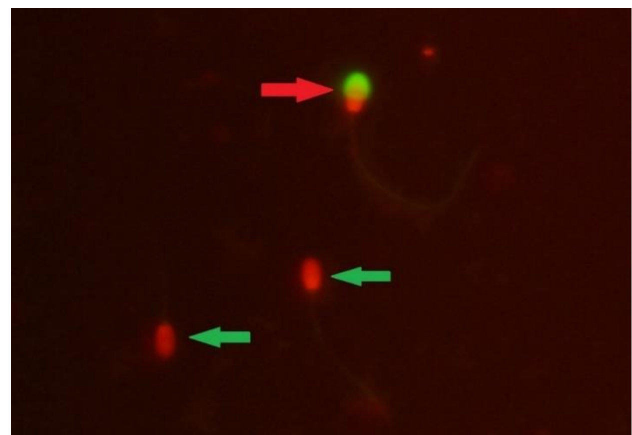

Figure 2. FITC-PNA/PI staining for acrosome membrane integrity. Spermatozoon with green hat represent damaged acrosome membrane (red arrow), spermatozoon without green hat represent intact acrosome membrane (green arrow).

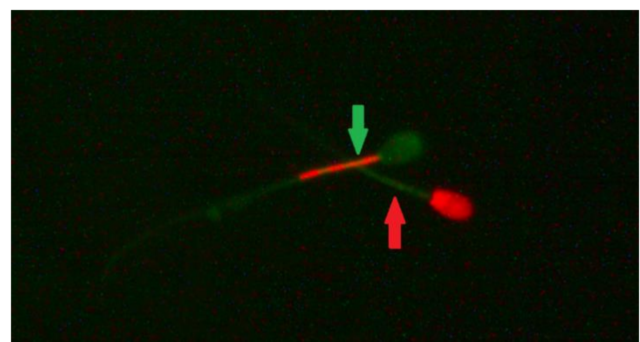

Figure 3. JC-1/PI staining for mitochondrial activity. Spermatozoa with orange midpiece represents the presence of mitochondrial activity (green arrow), spermatozoa with the pale green midpiece represents the absence of mitochondrial activity.

Statistical analysis

The research was replicated five times. Data was presented as the mean ± SEM. The average

values were assessed through ANOVA and Duncan's post-hoc test to identify notable variations in all parameters. These computations were carried out using SPSS/PC software version 25.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was denoted by p<0.05.

RESULTS

In the presented study, the values of motility, plasma and acrosome integrity, and mitochondrial activity in the K, L2, A5, and L2A5 groups are shown in Figure 4.

After freezing and thawing, the highest values for motility, plasma and acrosome integrity, and mitochondrial activity were obtained in the L2A5 group (63%, 72.96%, 69.91%, and 63.13%, respectively) compared to the control group (p<0.05).

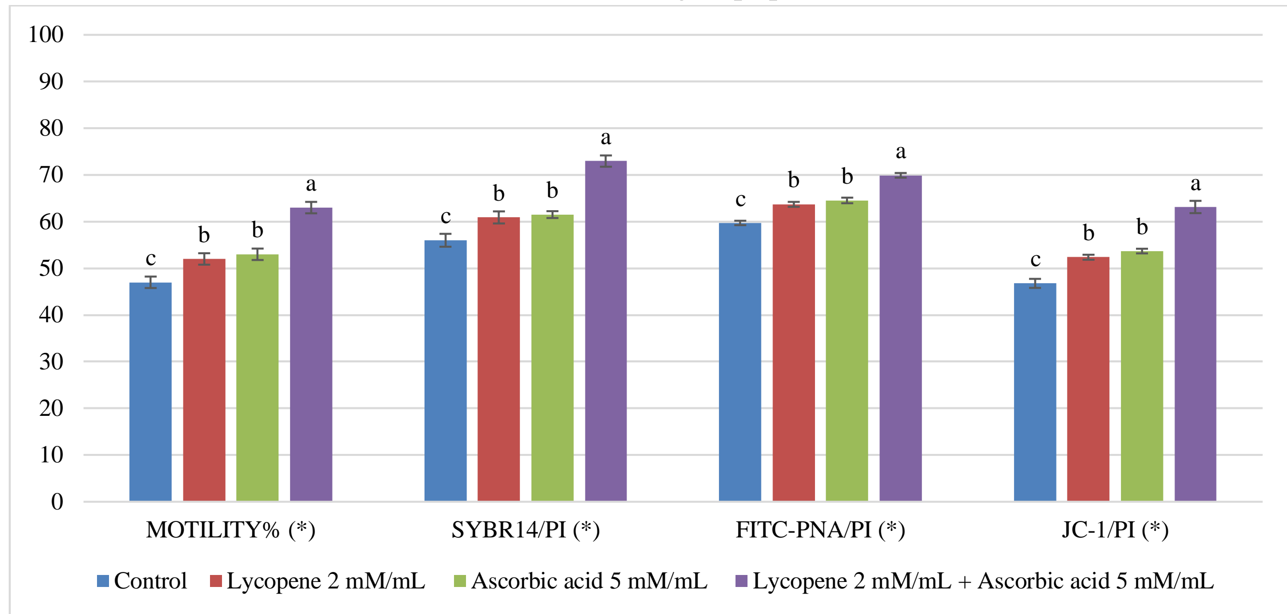

Figure 4. The effects of lycopene, ascorbic acid, and the combination of these two antioxidants on motility, plasma membrane integrity (SYBR14/PI), acrosomal membrane integrity (FITC-PNA/PI), and mitochondrial activity (JC-1/PI) in frozen-thawed Ankara buck semen. (*): Means with different letters (a,b,c) within each column indicate significant differences (p < 0.05).

DISCUSSION

The number of goats is increasing worldwide, necessitating the preservation of genetic capacity and the enhancement of reproductive efficiency. Improvements in sperm quality can result in significant gains for breeders regarding profitability and sustainability. In this context, advancements in cryopreservation techniques for buck semen will undoubtedly positively impact population genetics. Supplementing cryopreservation media with antioxidants enhanced semen quality by mitigating damage caused by free radicals (Bucak et al., 2019; Bucak et al., 2020; Bucak et al., 2024; Karaşör et al., 2022). The problem of antioxidants having different effects on sperm cryopreservation is still not solved, which is a big problem (Bucak et al., 2009). This study demonstrates that when used alone or in combination, lycopene and ascorbic acid positively affect parameters such as sperm motility, plasma and acrosome integrity, and mitochondrial activity after freezing and thawing processes. These findings significantly contribute to goat breeding and reproductive biotechnology, aiding in developing sustainable livestock practices.

Lycopene, present in tomatoes and red fruits, is recognized as the most powerful antioxidant

among various carotenoids. This polyene hydrocarbon features an unsaturated acyclic chain with 13 double bonds, 11 of which are conjugated and arranged in a linear fashion, providing its efficient antioxidant properties (Sen, 2019). Its antioxidant attributes include the ability to neutralize singlet oxygen and capture peroxyl radicals (Stahl et al., 1996). Ascorbic acid, also known as vitamin C, is a secure and water-soluble antioxidant. It has the capability to disrupt chain reactions and counteract the free radicals that initiate them, thus diminishing peroxidation. Ascorbic acid functions as a co-antioxidant, impeding the inclination toward lipid peroxidation. Acting as a robust free radical scavenger, ascorbic acid generates monodehydroascorbate radicals as end products—these are inert radicals that do not undergo reactions with other molecules or oxygen to produce highly reactive radicals (Bechara et al., 2022). This study illustrates the impact of lycopene, ascorbic acid, and their combined form on Ankara buck semen post freeze-thawing, as illustrated in Figure 1. The findings indicate that lycopene and ascorbic acid, both solo and in synergy, substantially safeguarded sperm motility, plasma and acrosome integrity, and mitochondrial activity compared to the control group post freeze-thawing ($p<0.05$). Notably, the combined dosage of 2 mM/mL lycopene and 5 mM/mL ascorbic acid exhibited superior protective effects on semen in comparison to all other groups ($p<0.05$).

In their study on Cashmere bucks, Ren et al. (2018) used lycopene doses of 0, 0.5, 1.0, 2.0, and 4.0 mg/mL. They obtained the highest motility, acrosomal and plasma membrane integrity, and mitochondrial activity rates with the 1 mg/mL lycopene dose. In our study, we used Ankara Bucks and added 2 mM lycopene. When calculated for molarity, 2 mM/mL is approximately equivalent to ~1 mg/mL. In our study, similar to the control group, the lycopene group showed higher motility and plasma and acrosomal membrane integrity, and mitochondrial activity integrity results. This suggests similar results can be obtained from sperm of the same species but different breeds. In their research on Sapudi rams, Bintara et al. (2023) used ascorbic acid and lycopene at concentrations of 1%, 2%, 3%, and 4%. They reported that the 3% lycopene and 3% ascorbic acid combination preserved semen quality after freeze-thawing. In another study on rams (Bucak et al., 2014), the 1×10^{-3} g/mL lycopene dose was reported to maintain mitochondrial activity and reduce DNA damage after freeze-thawing. Bucak and Uysal (2007) used an 800 µg/mL lycopene dose in Akkaraman rams and reported that it preserved sperm characteristics after freeze-thawing. In another study on bulls (Tuncer et al., 2014), the 500 µg dose of lycopene was reported to preserve semen parameters after freeze-thawing. Lycopene has also been reported to be effective under short-term storage conditions (Akalın et al., 2016). In their study on dogs, Sheikholeslami et al. (2020) used 500 and 750 µg of lycopene and incubated the semen at +4°C for 72 hours, reporting that both doses preserved progressive motility values. Besides bulls, rams, and dogs, studies have also reported the effectiveness of lycopene in mice (Babaei et al., 2021) and rabbits (Rosato et al., 2012). In their study on Boer buck semen, Memon et al. (2012) investigated ascorbic acid doses of 2.5, 4.5, 6.5, and 8.5 mg/mL. They observed that the 8.5 mg/mL dose preserved motility, viability, and acrosomal membrane integrity better than the other groups. In their research on Holstein Friesian x Haryana crossbred bulls, Paudel et al. (2010) tested the effects of ascorbic acid, chlorpromazine, and catalase at doses of 10 mM, 0.1 mM, and 200 IU, respectively, as well as their combination, and found that all antioxidant groups preserved sperm better than the control group. Ascorbic acid has also been effective in short-term studies. Akhter et al. (2023) tested the 1 mg/mL doses of Vit C and Vit E individually and in combination over a short term (72 hours) in Kail rams. They

reported that both individual and combined doses preserved sperm quality.

Research on the combined doses of lycopene and ascorbic acid in bucks is quite limited. The studies show that both lycopene and ascorbic acid preserve sperm parameters after freeze-thawing. In our study, the combined dose of lycopene and ascorbic acid provided the highest motility, plasma and acrosome integrity, and mitochondrial activity rates compared to all other groups ($p<0.05$). The lycopene used in our study belongs to the carotenoid class. The combination of two powerful antioxidants in our study worked synergistically to provide the highest sperm parameter values. Lycopene itself is a potent antioxidant (Stahl and Sies, 2003). As an antioxidant, ascorbic acid (Vitamin C) neutralizes free radicals and is oxidized to dehydroascorbic acid (DHA). Dehydroascorbic acid can be reduced back to ascorbic acid in the body through various enzymatic and non-enzymatic pathways. This redox cycle allows ascorbic acid to remain in an active form and maintain its antioxidant capacity (Buettner, 1993). Carotenoids, especially beta-carotene, effectively prevent lipid peroxidation (oxidation of lipids by free radicals). In this process, carotenoids trap free radicals and oxidize themselves, and these oxidized forms are reduced back by ascorbic acid, thus preserving the antioxidant efficacy of carotenoids (Sies et al., 1992).

This study reveals that combining lycopene and ascorbic acid significantly enhances the quality of cryopreserved goat semen, improving sperm motility, plasma and acrosome integrity, and mitochondrial activity. These findings suggest incorporating these antioxidants into semen extenders could benefit reproductive outcomes and genetic preservation, leading to increased profitability and sustainability in goat farming. Future research should optimize dosages for different breeds and explore long-term effects on fertility and offspring viability. Investigating other antioxidants and their potential synergistic effects could further enhance semen cryopreservation techniques.

CONCLUSION

In conclusion, it can be said that the quality of cryopreserved goat semen could efficiently be improved by using antioxidants such as lycopene and ascorbic acid. These antioxidants so help to enhance sperm motility, plasma membrane, acrosome membrane along with mitochondrial activity and reproductive outcomes after thawing. The most effective were extracts obtained from the combination of lycopene and ascorbic acid, since they gave that control group the highest values for all parameters tested. These results emphasize the ability of these antioxidants to reduce or overcome such damages due to cryopreservation that contribute towards maintaining the functional integrity of goat sperm. On the other hand, this can not only contribute to genetic conservation but also improve its profitability and sustainability.

On the basis of these promising results, we believe that there is a scope for research to optimize the cryopreservation protocols for buck sperm. Subsequent studies should also work in determining the optimal doses of lycopene and ascorbic acid which help to achieve maximum protective effect across various breeds under ambient stress. Furthermore, assessing the effects of these antioxidants on fertility and offspring viability in subsequent generations on a larger scale may provide greater evidence about their effectiveness. There is also room to explore other antioxidants combined with them which can be more protective against environmental stresses imparted by cryopreservation. These breakthroughs are vital in providing the livestock sector with sustainable breeding processes, meaning for thousands of

years to come farm animals are genetically safeguarded.

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Author Contributions: MB: Methodology, project administration, supervision, visualization, writing. AEÖ: Methodology and writing. ZK: Methodology, cryopreservation. ÖH: Methodology. MBA: Writing, software methodology. MNB: Methodology, supervision. MK: Software and writing.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Agarwal, A., & Majzoub, A. (2017). Laboratory tests for oxidative stress. *Indian Journal of Urology*, *33*(3), 199-206. https://doi.org/10.4103/iju.IJU_9_17

Aitken, R. J. (2020). Impact of oxidative stress on male and female germ cells: implications for fertility. *Reproduction*, *159*(4), R189-R201. https://doi.org/10.1530/REP-19-0452

Akalin, P. P., Bucak, M. N., Güngör, Ş., Başpinar, N., Coyan, K., Dursun, Ş., ... & Deniz, Y. E. N. İ. (2016). Influence of lycopene and cysteamine on sperm and oxidative stress parameters during liquid storage of ram semen at 5 C. *Small Ruminant Research*, *137*, 117-123. https://doi.org/10.1016/j.smallrumres.2016.03.017

Akhter, S., Zubair, M., Mahmood, M., Andrabi, S. M. H., Hameed, N., Ahmad, E., & Saleemi, M. K. (2023). Effects of vitamins C and E in tris citric acid glucose extender on chilled semen quality of Kail ram during different storage times. *Scientific Reports*, *13*(1), 18123. https://doi.org/10.1038/s41598-023-43831-2

Al-Mutary, M. G. (2021). Use of antioxidants to augment semen efficiency during liquid storage and cryopreservation in livestock animals: A review. *Journal of King Saud University-Science*, *33*(1), 101226. https://doi.org/10.1016/j.jksus.2020.10.023

Amini, M. R., Kohram, H., Zare Shahaneh, A., Zhandi, M., Sharideh, H., & Nabi, M. M. (2015). The effects of different levels of vitamin E and vitamin C in modified Beltsville extender on rooster post thawed sperm quality. *Cell and Tissue Banking*, *16*, 587-592. https://doi.org/10.1007/s10561-015-9506-9

Babaei, A., Asadpour, R., Mansouri, K., Sabrivand, A., & Kazemi-Darabadi, S. (2021). Lycopene protects sperm from oxidative stress in the experimental varicocele model. *Food Science & Nutrition*, *9*, 6806–6817. https://doi.org/10.1002/fsn3.2632

Bechara, N., Flood, V. M., & Gunton, J. E. (2022). A systematic review on the role of vitamin C in tissue healing. *Antioxidants*, *11*(8), 1605. https://doi.org/10.3390/antiox11081605

Bintara, S., Maharani, D., Tavares, L., & Sitaresmi, P. (2023). Comparison various level ascorbic acid and lycopene additions in semen diluent enhanced sperm quality of Sapudi ram. *Journal of Animal Science and Technology*. https://doi.org/10.5187/jast.2023.e54

Bucak, M. N., Ataman, M. B., Başpınar, N., Uysal, O., Taşpınar, M., Bilgili, A., ... & Akal, E. (2015). Lycopene and resveratrol improve post-thaw bull sperm parameters: sperm motility, mitochondrial activity and DNA integrity. *Andrologia*, *47*(5), 545-552. https://doi.org/10.1111/and.12301

Bucak, M. N., Bodu, M., Başpınar, N., Güngör, Ş., İli, P., Acibaeva, B., ... & Dursun, Ş. (2019). Influence of ellagic acid and ebselen on sperm and oxidative stress parameters during liquid preservation of ram semen. *Cell Journal (Yakhteh)*, *21*(1), 7. https://doi.org/10.22074/cellj.2019.5593

Bucak, M. N., Karaşör, Ö. F., Sarı, A., Bodu, M., Ili, P., Narlıçay, S., ... & Sari, F. (2024). Lipid mixtures (from a liposome kit) and melatonin improve post-thawed Angora goat sperm parameters. *Cryobiology*, *115*, 104897. https://doi.org/10.1016/j.cryobiol.2024.104897

Bucak, M. N., Keskin, N., Ili, P., Bodu, M., Akalın, P. P., Öztürk, A. E., ... & Dursun, Ş. (2020). Decreasing glycerol content by co-supplementation of trehalose and taxifolin hydrate in ram semen extender: Microscopic, oxidative stress, and gene expression analyses. *Cryobiology*, *96*, 19-29. https://doi.org/10.1016/j.cryobiol.2020.09.001

Bucak, M. N., Tuncer, P. B., Sarıözkan, S., & Ulutaş, P. A. (2009). Comparison of the effects of glutamine and an amino acid solution on post-thawed ram sperm parameters, lipid peroxidation and anti-oxidant activities. *Small Ruminant Research*, *81*(1), 13-17. https://doi.org/10.1016/j.smallrumres.2008.10.003

Bucak, M.N., Ataman, M.B., Başpınar, N., Uysal, O., Taşpınar, M., Bilgili, A., Öztürk, C., Güngör, Ş., İnanç, M.E. and Akal, E. (2015). Lycopene and resveratrol improve post-thaw bull sperm parameters: sperm motility, mitochondrial activity and DNA integrity. *Andrologia*, *47*, 545-552. https://doi.org/10.1111/and.12301

Buettner, G. R. (1993). The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Archives of Biochemistry and Biophysics*, 300(2), 535-543. https://doi.org/10.1006/abbi.1993.1074

Evans, G., & Maxwell, W. C. (1987). *Salamons' artificial insemination of sheep and goats* (No. Ed. 2, pp. xi+-194).

Garner, D. L., & Johnson, L. A. (1995). Viability assessment of mammalian sperm using SYBR-14 and propidium iodide. *Biology of Reproduction*, *53*(2), 276-284. https://doi.org/10.1095/biolreprod53.2.276

Garner, D. L., Thomas, C. A., Joerg, H. W., DeJarnette, J. M., & Marshall, C. E. (1997). Fluorometric assessments of mitochondrial function and viability in cryopreserved bovine spermatozoa. *Biology of Reproduction*, *57*(6), 1401-1406. https://doi.org/10.1095/biolreprod57.6.1401

Hussein, A., Aseffa, S., Kuraz, B., & Bedaso, B. (2023). Breeding and milking managements and Goat production constraints in Siltie Zone SNNPR, Ethiopia. *Heliyon*, *9*(12). https://doi.org/10.1016/j.heliyon.2023.e22573

Karaşör, Ö. F., Bucak, M. N., Cenariu, M., Bodu, M., Taşpınar, M., & Taşpınar, F. (2022). The effects of different doses of ROCK inhibitor, antifreeze protein III, and boron added to semen extender on semen freezeability of Ankara bucks. *Molecules*, *27*(22), 8070. https://doi.org/10.3390/molecules27228070

Khalique, M. A., Rehman, H., Andrabi, S. M. H., Majeed, K. A., Ahmad, N., Fayyaz, M. H., ... & Sulaiman, S. (2023). Antioxidant effects of zinc-oxide nanoparticles on post-thaw quality and in vivo fertility of Beetal buck spermatozoa. *Small Ruminant Research*, *225*, 107012. https://doi.org/10.1016/j.smallrumres.2023.107012

Memon, A., Wahid, H., Rosnina, Y., Goh, Y., Ebrahimi, M. and Nadia, F. (2013). Effect of Ascorbic Acid Concentrations, Methods of Cooling and Freezing on Boer Goat Semen Cryopreservation. *Reproduction in Domestic Animals*, *48*, 325-330. https://doi.org/10.1111/j.1439-0531.2012.02155.x

Mishra, B., Alam, M. G. S., Khandokar, M. A. M. Y., Mazumder, S., & Munsi, M. N. (2010). Qualities of goat semen in Tris-Citrate-Glucose extender containing glutathione. *The Bangladesh Veterinarian*, 27(2), 46 – 55. https://doi.org/10.3329/bvet.v27i2.7554

Nagy, S., Jansen, J., Topper, E. K., & Gadella, B. M. (2003). A triple-stain flow cytometric method to assess plasma-and acrosome-membrane integrity of cryopreserved bovine sperm immediately after thawing in presence of egg-yolk particles. *Biology of Reproduction*, *68*(5), 1828-1835. https://doi.org/10.1095/biolreprod.102.011445

Narliçay, S., & Uslu, B. A. (2022). Kangal Akkaraman koçu spermalarının seminal plazmasına farklı ısı uygulamalarının kısa süreli saklanmasında etkilerinin araştırılması. *Cumhuriyet Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi*, 7(2), 112-117. https://doi.org/10.51754/cusbed.1115552

Paudel, K., Kumar, S., Meur, S. and Kumaresan, A. (2010). Ascorbic Acid, Catalase and Chlorpromazine Reduce Cryopreservation-induced Damages to Crossbred Bull Spermatozoa†. *Reproduction in Domestic Animals*, *45*, 256-262. https://doi.org/10.1111/j.1439-0531.2008.01278.x

Pellicer-Rubio, M. T., Magallon, T., & Combarnous, Y. (1997). Deterioration of goat sperm viability in milk extenders is due to a bulbourethral 60-kilodalton glycoprotein with triglyceride lipase activity. *Biology of Reproduction*, *57*(5), 1023-1031. https://doi.org/10.1095/biolreprod57.5.1023

Purdy, P. H. (2006). A review on goat sperm cryopreservation. *Small Ruminant Research*, *63*(3), 215 225. https://doi.org/10.1016/j.smallrumres.2005.02.015

Raheem, K. A., Basiru, A., Raji, L. O., & Odetokun, I. A. (2024). Productive performance of goat. *Trends in Clinical Diseases, Production and Management of Goats*, 163-177. https://doi.org/10.1016/B978-0-443-23696-9.00001-8

Ren, F., Feng, T., Dai, G., Wang, Y., Zhu, H., & Hu, J. (2018). Lycopene and alpha-lipoic acid improve semen antioxidant enzymes activity and cashmere goat sperm function after cryopreservation. *Cryobiology*, *84*, 27-32. https://doi.org/10.1016/j.cryobiol.2018.08.006

Rosato, M. P., Di Iorio, M., Manchisi, A., Gambacorta, M., Petrosino, G., Centoducati, G., ... & Iaffaldano, N. (2012). In vitro survival and lipid peroxidation status of rabbit spermatozoa after both chilled and frozen storage in lycopene enriched extenders. *Livestock Science*, *146*(2-3), 199-202. https://doi.org/10.1016/j.livsci.2012.03.006

Sen, S. (2019). The chemistry and biology of lycopene: Antioxidant for human health. *International Journal of Advancement in Life Sciences Research*, 2(4), 08-14. https://doi.org/10.31632/ijalsr.2019v02i04.002

Shah, S. A. H., Andrabi, S. M. H., & Qureshi, I. Z. (2016). Effect of equilibration times, freezing, and thawing rates on post-thaw quality of buffalo (Bubalus bubalis) bull spermatozoa. *Andrology*, *4*(5), 972 976. https://doi.org/10.1111/andr.12214

Sheikholeslami, S. A., Soleimanzadeh, A., Rakhshanpour, A., & Shirani, D. (2020). The evaluation of lycopene and cysteamine supplementation effects on sperm and oxidative stress parameters during chilled storage of canine semen. *Reproduction in Domestic Animals*, *55*(9), 1229-1239. https://doi.org/10.1111/rda.13770

Sias, B., Ferrato, F., Pellicer-Rubio, M. T., Forgerit, Y., Guillouet, P., Leboeuf, B., & Carrière, F. (2005). Cloning and seasonal secretion of the pancreatic lipase-related protein 2 present in goat seminal plasma. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1686*(3), 169-180. https://doi.org/10.1016/j.bbalip.2004.09.008

Sies, H., Stahl, W., & Sundquist, A. R. (1992). Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. *Annals of the New York Academy of Sciences*, *669*, 7-20. https://doi.org/10.1111/j.1749-6632.1992.tb17085.x

Simon, L., Zini, A., Dyachenko, A., Ciampi, A., & Carrell, D. T. (2017). A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian Journal of Andrology*, *19*(1), 80-90. https://doi.org/10.4103/1008-682X.182822

Stahl, W., & Sies, H. (1996). Perspectives in biochemistry and biophysics. *Archives of Biochemistry and Biophysics*, *336*(1), 1-9.

Tuncer, P. B., Büyükleblebici, S., Eken, A., Taşdemir, U., Durmaz, E., Büyükleblebici, O., & Çoşkun, E. (2014). Comparison of cryoprotective effects of lycopene and cysteamine in different cryoprotectants on bull semen and fertility results. *Reproduction in Domestic Animals*, *49*(5), 746-752. https://doi.org/10.1111/rda.12359

Uysal, O., & Bucak, M. N. (2007). Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. *Acta Veterinaria Brno*, *76*(3), 383-390. https://doi.org/10.2754/avb200776030383

Velmurugan, B., Bhuvaneswari, V., Abraham, S. K., & Nagini, S. (2004). Protective effect of tomato against N-methyl-N′-nitro-N-nitrosoguanidine–induced in vivo clastogenicity and oxidative stress. *Nutrition*, *20*(9), 812-816. https://doi.org/10.1016/j.nut.2004.05.003

Zini, A., San Gabriel, M., & Libman, J. (2010). Lycopene supplementation in vitro can protect human sperm deoxyribonucleic acid from oxidative damage. *Fertility and Sterility*, *94*(3), 1033-1036. https://doi.org/10.1016/j.fertnstert.2009.04.004.