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The effect of adding different levels of lycopene on some semen characteristics and antioxidant enzymes of diluted and frozen semen of Arabian rams

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Abstract

The experiment was conducted in the animal field of the College of Agriculture / University of Basra / Karmat Ali site for a period of three months from 10/15/2023 to 1/15/2024. Four Arabian rams were purchased from local markets in Basra Governorate, aged 2.5-3 years and of similar weights. They were fed a ration consisting of a feed mixture of barley, bran, straw, and some mineral salts. The experiment was conducted in the animal field of the College of Agriculture / University of Basra / Karmat Ali site for a period of three months from 10/15/2023 to 1/15/2024. Four Arabian rams were purchased from local markets in Basra Governorate, aged 2.5-3 years and with similar weights. They were fed a ration consisting of a feed mixture consisting of barley, bran, straw and some mineral salts. Clean water was provided to them inside the pen. The rams were trained to collect semen using the artificial vagina for rams in the animal field for a period of two weeks. After completing their training, semen was collected from them three times a month. The semen was transferred to the laboratory using test tubes and protected from light. The tubes were placed in a water bath at a temperature of 37°C. After that, the semen was diluted with diluting agents (turs, citric acid, fructose, egg yolk, lycopene, gentamicin and glycerol). The diluted semen was placed in 1 ml of Papendrovat, tightly closed and placed in the refrigerator. After the temperature stabilized at 5°C, it was stored in liquid nitrogen bottles at a temperature of -196°C for 10, 20 and 30 days to test the effect of the diluted semen. Freezing in ram semen traits, adding lycopene and the duration of freezing storage in the integrity of the acrosome and plasma membrane of frozen semen, antioxidants and enzymatic oxidation indicators (SOD, TAC, GPX and MDA) in the seminal plasma of diluted and frozen ram semen. The results were: Treatment T4 recorded the highest values significantly ($P<0.05$)in the percentage of acrosome and plasma membrane integrity, and the 20-day period showed the highest percentages significantly ($P<0.05$)in acrosome integrity, while the 10-day period was significantly superior ($P<0.05$)in the percentage of plasma membrane of chilled and frozen semen. Treatment T4 was significantly superior ($P<0.05$)to the rest of the treatments in enzymatic antioxidants, SOD, TAC, GPX, while T4 recorded the highest values significantly ($P<0.05$)in the percentage of enzymatic oxidation indicator MDA. The 10-day freezing period significantly ($P<0.05$)outperformed the other periods in the percentage of enzymatic antioxidants SOD, TAC, and GPX. The 10-day freezing period also showed the lowest values ($P<0.05$) in the percentage of enzymatic antioxidant index MDA.

Keywords: Lycopene, acrosome integrity, enzymatic antioxidants, frozen semen, liquid nitrogen.

I. Introduction

It is essential that cryopreserved, frozen, and liquefied sperm remain intact in all their components, especially the acrosome, plasma membrane, and DNA. Furthermore, their individual progressive motility must be rapid and at a level that makes them effective and capable of reaching the female reproductive tract and penetrating the oocyte membranes (Hossain and Lee, 2019). The diluent type and composition also play an important role in maintaining the integrity of sperm during cryopreservation and freezing processes through its effect on the osmotic pressure of the sperm membrane and keeping the pH of the stored sperm within neutral limits, on the one hand. On the other hand, the diluent plays an important role in releasing enzyme antioxidants and

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reducing the rates of reactive oxygen species (ROS) as well as lipid peroxides (LOP) present in the seminal plasma and sperm cytoplasm, thus increasing the vitality of sperm and their ability to fertilize during artificial insemination processes (Zamiri et al., 2010). Lycopene is the red, yellow, or brown pigment found in some vegetables such as tomatoes and some fruits such as apricots, watermelons, and grapefruit, but it is found in high concentrations in red-colored plants such as tomatoes (Naviglio et al., 2008). Lycopene is a carotenoid derivative but does not carry vitamin A activity. It is considered a natural plant pigment produced by plants and microorganisms during photosynthesis to protect them from activity. Photosynthesis and increased photosensitivity (Choi and Seo, 2013). Many researchers have also confirmed the ability of lycopene to control and prevent the formation of free radicals, reduce oxidative stress rates, and prevent the formation of nitrogen dioxide and hydroxyl radicals in the living body, whether in humans or animals (Krishnamoorthy et al., 2011). Al-Sarray et al. (2019) also showed in their study on diluted semen of Awassi rams that adding lycopene with tartar and egg yolk improved individual motility and the percentage of live sperm, reduced the percentage of deformed sperm, and preserved sperm membranes and acrosome integrity in lycopene-diluted semen during refrigeration for 24, 48, and 72 hours at 5°C. In our current study, lycopene will be added to the diluted semen of Arabian rams with turmeric, egg yolk, fructose, and glycerol, and frozen in liquid nitrogen at -196°C for periods of 10, 20, and 30 days to maintain and increase the rate of individual sperm motility, progressive motility, and vitality during freezing storage processes to increase the efficiency and quality of semen stored in nitrogen for longer periods to achieve the highest rates of fertilization during artificial insemination in sheep. Therefore, the aim of this study was to investigate the role of lycopene in preserving the acrosome and plasma membrane of frozen semen, improve the function of natural antioxidant enzymes present in semen, reduce oxidation indicators, and study the relationship between lycopene and all semen characteristics, adopting it as a standard indicator for testing the quality of diluted and frozen semen.

II. -Materials and Methods

The experiment was conducted in the animal field of the College of Agriculture / University of Basra, Karma Ali site, for a period of three months (from 10/15/2023 to 1/15/2024). Four Arabian rams were purchased from local markets in Basra Governorate, with ages ranging from 2.5-3 years and close weights. They were fed a feed consisting of a feed mixture consisting of barley, bran, straw and some mineral salts. Clean water was provided to them inside the pen. The rams were trained to collect semen using the artificial vagina for rams in the animal field for a period of two weeks. After completing their training, semen was collected from them three times a month. The semen was transferred to the laboratory using test tubes and protected from light by wrapping it with a piece of aluminum foil. The tubes were placed in a water bath at a temperature of 37°C. All special physical examinations were performed (color, group and individual movement of sperm, sperm concentration and percentage of live, dead and deformed sperm) using a device Semen analyses, then the semen was diluted with diluents as shown in Table (1) below:

.Table (1): Solutions to be used in extending the semen of Arabi rams in the experiment

Diluents (volume 100 ml)				
the components	control (first)	Second diluent	Third diluent	Fourth diluent
Tris(gm)	3.07	3.07	3.07	3.07
Citric acid (gm)	1.64	1.64	1.64	1.64
Fructose(gm)	1.26	1.26	1.26	1.26
Egg yolk (ml)	2.5	2.5	2.5	2.5

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Gentamycin (ml)	0.5	0.5	0.5	0.5
Lycopene	----	0.5	1	1.5
Clycerol (ml)	8	8	8	8
Distilled water(ml)	Complete the volume to 100 ml			

The experiment was designed to study the characteristics of the semen of Arabian rams diluted with levels of lycopene (1.5, 1, 0.5, 0) g/100 ml. The diluted semen was placed in 1 ml of Papendrovat, tightly closed and placed in the refrigerator. After the temperature stabilized at (5°C) (to reach the equilibrium point), it was stored in liquid nitrogen bottles at a temperature of -196°C for 10, 20 and 30 days. The following tests were conducted:

Acrosome and plasma membrane integrity of sperm after freezing :

The integrity of the acrosome membrane of frozen sperm after thawing was tested according to the method (Hancock, 1946) using genshen violet and eosin solution. The percentage of sperm with intact acrosomes was calculated by counting 200 sperm from different areas of the slide, according to the following equation:

Number of sperm with normal acrosomes

$$\text{Percentage of sperm with normal acrosomes \%} = \frac{\text{Number of sperm with normal acrosomes}}{\text{Total sperm count}} \times 100$$

The integrity of the plasma membrane of frozen sperm was also tested according to the method of (Jeyendram et al., 1984), where 300 sperm were counted distributed in five directions on the slide, so that the sperm with a swollen head and a coiled tail were distinguished as having an intact plasma membrane (HOS+) and the sperm without a swollen head and a coiled tail (intact tail) were distinguished as having an unintact plasma membrane (HOS-) according to the following equation:

$$\text{Sperm with an intact plasma membrane \%} = \frac{\text{Number of sperm with a swollen head and a coiled tail}}{\text{Total sperm count}} \times 100$$

Measuring Antioxidants and Antioxidant Indicators in Seminal Plasma:

Each time semen is collected, some samples of the semen diluted with lycopene are isolated in 2 ml test tubes and placed in a liquid nitrogen tank (-196°C) and stored for 10, 20 and 30 days. After the freezing period is over, they are placed in a Centrfuge for 15 minutes at 4000 rpm. Then, the upper layer (plasma) is removed with a pipette, leaving the sediment. The samples are frozen at -18°C until all analyses are performed to measure antioxidants in seminal plasma, as shown below:

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Measurement of the antioxidant SOD (Super Oxide Dismutate)

The antioxidant SOD was measured in seminal plasma from diluted, frozen, and thawed semen according to the method of Peskin and Winterbourne, 2000, and using a kit from the Beyotime Institute of Biotechnology, China .

Measurement of the antioxidant TAC (Total antioxidant capacity)

The antioxidant TAC was measured in seminal plasma from diluted, frozen, and thawed semen according to the method of Apaketal, 2007, and using a US-made kit .

Measurement of the antioxidant GPX (Glutathione peroxidase)

The antioxidant GPX was measured in seminal plasma from diluted, frozen, and thawed semen according to the method of Apaketal, 2007 , and using a US-made kit.

Malondial dehyde (MDA) oxidation index measurement

The concentration of MDA in seminal plasma from diluted, frozen, and thawed semen was measured according to the method of Yagi (1998) and using a personal kit from the Beyotime Institute of Biotechnology, China.

III. Statistical analysis:

The data were statistically analyzed using SPSS (26) as a two-factor experiment. The first factor included different lycopene levels (1.5, 1, 0.5, and 0) grams, and the second factor included different freezing periods, according to the following mathematical equation:

$$TBij+eijk+Yijk=\mu+Ti+Bj$$

Where:

Yijk = the trait under study

μ = the overall mean

Ti = lycopene concentrations (0, 0.5, 1, and 1.5) grams

Bj = freezing periods (30, 20, and 10) days

TBij = the interaction between lycopene concentrations and freezing storage periods.

eijk = experimental error that is randomly and normally distributed with mean equal to zero and variance e2

IV. Discussion and Results:

Acrosome and plasma membrane integrity of sperm:

Table (2) shows that the treatment had a significant effect (P<0.05) on the integrity of the acrosome of sperm, as the T4 treatment outperformed the T2, T1 (control), and T3 treatments, with the averages being 60.18, 50.69, 47.97, and 45.97. It is also noted that the duration of freezing storage had a significant effect (P<0.05) on the integrity of the acrosome, as the 20-day period was significantly superior (P<0.05) to the 10- and 30-day storage periods, with the averages being 60.90, 56.81, and 49.43%, respectively. When studying the interaction between the effect of treatment and storage duration, it was found that T4 achieved the highest percentages significantly (P<0.05) compared to the rest of the treatments. For the rest of the storage periods,

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the marks were 63.68%, while the control treatment recorded the lowest percentages significantly (P<0.05) at 43.77% for the storage period. Table (3) shows that the treatment had a significant effect (P<0.05) on the integrity of the sperm plasma membrane, as the T4 treatment outperformed the T3, T2, and T1(control) treatments, with averages of 54.56, 50.99, 46.43, and 44.44%, respectively. It is also noted that the freezing storage period had a significant effect (P<0.05) on the integrity of the plasma membrane, as the 10-day period significantly outperformed the 20- and 30-day storage periods, with averages of 52.39, 49.45, and 45.50%, respectively. When studying the interaction between the effect of the treatment and the storage period, it was found that T4 at the 10-day period achieved the highest percentages significantly. (P<0.05) Compared to the rest of the treatments and for the rest of the storage periods, the integrity of the plasma membrane was 58.22%, while the control treatment recorded the lowest percentage (P<0.05) in sperm plasma membrane integrity at 41.15% for the 30-day storage period. The use of various protective materials plays an effective role in preserving the integrity of the acrosome and sperm plasma membrane from damage during the freezing process, by reducing membrane permeability and inhibiting the reactions that occur within the sperm plasma membrane. This contributes to reducing damage to the plasma membrane, acrosome, and acrosome (Al-Saiady et al., 2017). Prasadet et al., (2017) demonstrated that freezing affects semen quality through a significant decrease in sperm vitality and general motility, as well as in the plasma membrane and acrosome integrity. It was found that freezing causes many problems for both structures. Other studies have attributed this to... The damage was due to the formation of ice crystals during the freezing process, which causes damage to the sperm plasma membrane, rupture, and leakage of cellular components (Pavan and Bjorndahl, 2018; Sairam and Seshamma, 2018; Zhang and Liu, 2020).

The results of the current study were consistent with the findings of many researchers that lycopene has an effective effect in mitigating the effects of freezing and preserving the acrosome and plasma membrane of frozen sperm, in terms of its action with glycerol as a buffering substance with a synergistic effect during sperm freezing in rams, thus reducing the negative effects on the acrosome and plasma membrane and increasing the vitality and ability of sperm to fertilize (Zini et al., 2010; Rosata et al., 2012; Ahmed et al., 2015; Tvrda et al., 2017; AL-Sarray et al., 2019; Atefeh et al. al., 2021).

Table (2): Effect of treatments and freezing duration on acrosome integrity (%) of ram semen (mean ± SE)

Storage duration / Treatments	10	20	30	Treatments impact rate
T1	50. 47 ±0.48	46.93±0.14	43.77±0.29	47.06 ±2.87 C
T2	54.04 ±0.20	50.93 ±0.51	47.10±0.05	50.69 ± 2.98 B
T3	59.09±0.45	54.93 ±0.34	50.93±0.16	45.97 ± 3.48 C
T4	63.68 ±0.33	60.90 ±0.34	55.95 ± 0.23	60.18±3.35 A
Average effect of storage duration	56.81 ± 5.15 B	60.90 ± 5.42 A	49.43±4.68 C	LSD for interference 3.63

Means followed by different letter differ significantly at P=0.05.

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Table (3): Effect of treatments and freezing storage duration on plasma membrane integrity (%) of ram semen (mean ± SE)

Storage duration / Treatments	10	20	30	Treatments impact rate
T1	47.20±0.24	44.98±0.15	41.15±0.46	44.44 ± 2.63 D
T2	49.72 ± 0.45	46.36 ±0.52	43.24±0.06	46.43 ± 2.78 C
T3	54.41±0.41	52.14±0.12	46.43±0.37	50.99 ±3.52 B
T4	58.22 ± 0.20	54.32 ±0.50	51.14 ±0.15	54.56±3.04 A
Average effect of storage duration	52.39 ± 4.40 A	49.45 ± 4.03 B	45.50±3.90 C	LSD for interference 1.99

Means followed by different letter differ significantly at P=0.05.

Antioxidants and indicators: SOD, TAC, GPX, MDA

Table (4) shows that the treatment had a significant effect (P<0.05) on the antioxidant SOD of sperm, with the T4 treatment recording the highest percentages significantly (P<0.05) compared to the other treatments: T3, T2, and T1(control) , with averages reaching 6.33, 4.86, 2.84, and 1.70%, respectively. It is also noted that the duration of freezing storage had a significant effect (P<0.05), with the 10-day period recording the highest percentages significantly (P<0.05) in SOD compared to the 20- and 30-day storage periods, with averages reaching 5.14, 3.89, and 2.76%, respectively. When studying the interaction between the effect of treatment and storage period, it was found that T4 at 10 days recorded the highest percentages significantly (P<0.05) in SOD values compared to the rest of the treatments and the rest of the storage periods, as it was 7.79%, while the T1(control) treatment at 30 days recorded the lowest percentages significantly (P<0.05), as it was 0.95. It is also noted from Table (5) that the treatment had a significant effect (P<0.05) on the enzymatic antioxidant TAC, as the T4 treatment outperformed the T3, T2, and T1(control) treatments, as the averages reached 74.6, 29.5, 3.40, and 2.08%, respectively. It was also noted that the freezing storage period had a significant effect (P<0.05), as the 10-day period outperformed the 20- and 30-day storage periods, with averages reaching 6.21, 4.25, and 2.67%, respectively. When studying the interaction between the effect of treatment and storage period, it was found that T4 at the 10-day period achieved the highest significant percentages (P<0.05) compared to the rest of the treatments and the remaining storage periods, as it was 93.8%, while the T1(control) treatment at the 30-day storage period recorded the lowest significant percentages (P<0.05) in TAC percentages, as it was 1.15%. Table (6) shows that the treatment had a significant effect (P<0.05) on the enzymatic antioxidant GPX, as the T_4 treatment outperformed the T3, T2, and T1(control) treatments, with the averages reaching 94.17, 07.15, 93.12, and 60.8%, respectively. It is also noted that the freezing storage period had a significant effect (P<0.05), as the 10-day period outperformed the 20- and 30-day storage periods, with the averages reaching 16.30, 13.58, and 11.03%, respectively. When studying the interaction between the effect of treatment and storage period, it was found that T_4 at the 10-day period achieved the highest percentages significantly (P<0.05) compared to the rest of the treatments and the rest of the storage periods, as it was 20.84%, while the T1(control) treatment at the

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30-day storage period recorded the lowest percentages significantly (P<0.05) as it was 6.46%. It is also noted from Table (7) that the treatment had a significant effect (P<0.05) on the enzyme oxidation index MDA, as the T4 treatment showed a significant decrease (P<0.05) in the values of the percentages of the enzyme oxidation index MDA compared to the rest of the treatments T3, T2, T1(control), as the averages reached 50.4, 86.6, 8.87, 11.51%, respectively. It is also noted that the duration of freezing storage had a significant effect (P<0.05), as the 15-day period showed the lowest percentages significantly (P<0.05) compared to the 20- and 10-day storage periods, with averages reaching 6.43, 8.03, and 9.21%, respectively. When studying the interaction between the effect of treatment and storage period, it was found that T4 at 10 days showed the lowest significant percentages (P<0.05) in the enzyme oxidation index MDA, as it was 3.21%, while theT1(control) treatment at 30 days recorded the highest significant percentages (P<0.05) in MDA, as it was 13.70%. The prolonged freezing period may lead to an increase in the enzyme oxidation index MDA due to the high percentages of live and dead sperm, as well as the concentration of enzyme antioxidants SOD, TAC, GPX, and MDA being low during semen freezing. Many researchers have attributed this change in enzyme antioxidants to the reactions that occur within the sperm plasma membrane and the increased release of peroxides and free radicals during the freezing process (Al-Saiady et al., 2019 and Zhang et al., 2020). Hossain and Lee, 2019 and Kim et al., 2020). The importance of the additives to frozen and thawed semen is focused on their effectiveness in releasing enzymatic antioxidants, reducing the MDA enzyme oxidation index, and improving sperm efficiency and vitality and increasing their ability to fertilize (Tuncer et al., 2010, Bayejid et al., 2015, Castrol et al., 2016). Simoes et al., (2013), Abdulkareem , (2014) also explained. Freezing and thawing of male semen leads to structural damage to the frozen sperm membranes and increased release of the MDA enzyme oxidation indicator, which in turn works to integrate the sperm contents from the inside and form physiologically different sperm. The results of the study were consistent with what many researchers have explained that the addition of some substances with lycopene, glycerol, egg yolk, and turmeric has a high ability to raise the enzyme oxidation indicators SOD, TAC, CAT and reduce the MDA enzyme oxidation indicator by maintaining the osmotic balance of the sperm, preserving DNA, and ridding the sperm of free radicals and peroxides formed during the freezing and thawing processes. This is reflected in increased progressive motility of sperm, an increase in the percentage of live sperm, and a decrease in the percentage of dead and deformed sperm (Bayjed et al., 2015 and Tvrda et al., 2017).

Table (4): Effect of treatments and freezing period on the enzyme antioxidant (SOD%) of ram semen (mean ± SE)

Storage duration / Treatments	10	20	30	Treatments impact rate
T1	2.21±0.06	1.92±0.07	0.95±0.02	1.70 ±0.57 D
T2	4.40 ±0.18	2.60 ± 0.35	1.52±0.73	2.84 ±1.26 C
T3	6.17±0.08	5.00±0.25	3.40±0.62	4.86 ±1.20 B
T4	7.79 ±0.06	6.04 ±0.20	5.17 ±0.07	6.33±1.14 A
Average effect of storage duration	5.14 ± 2.14 A	3.89 ±1.76 B	2.76±1.72 B	LSD for interference 1.15

Means followed by different letter differ significantly at P=0.05.

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Table (5): Effect of treatments and freezing duration on the enzymatic antioxidant (TAC%) of ram semen (mean ± SE)

Storage duration / Treatments	10	20	30	Treatments impact rate
T1	2.96±0.12	2.13±0.33	1.15±0.24	2.08 ± 0.80 C
T2	5.21 ± 0.12	3.24 ±0.09	1.74±0.08	3.40 ±1.48 B
T3	7.73±0.29	4.89±0.23	3.24±0.06	5.29 ± 1.94 A
T4	8.93 ± 2.39	6.77 ±0.20	4.52 ± 0.35	6.74±1.90 A
Average effect of storage duration	6.21 ± 2.39 A	4.25 ±1.82 B	2.67±1.37 C	LSD for interference 1.32

Means followed by different letter differ significantly at P=0.05.

Table (6): Effect of treatments and freezing duration on the enzymatic antioxidant (GPX%) of ram semen (mean ± SE)

Storage duration / Treatments	10	20	30	Treatments impact rate
T1	11.12±0.07	8.23±0.07	6.46±0.06	8.60 ±2.00 D
T2	15.15 ± 0.12	13.00±0.13	10.64±0.09	12.93 ± 1.93 C
T3	18.09±0.23	15.06±0.16	12.06±0.16	15.07 ±2.58 B
T4	20.84 ± 0.44	18.03±0.12	14.95 ± 0.35	17.94±2.53 A
Average effect of storage duration	16.30 ± 3.37 A	13.58 ± 3.69 B	11.03±3.16 C	LSD for interference 2.14

Means followed by different letter differ significantly at P=0.05.

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Table (7): Effect of treatments and freezing period on the enzymatic oxidation index (MDA%) of ram semen (mean ± SE)

Storage duration / Treatments	10	20	30	Treatments impact rate
T1	9.20±0.14	11.63±0.33	13.70±0.06	11.51 ±1.93 A
T2	7.40 ±0.22	9.18 ± 0.07	10.02±0.11	8.87 ± 1.15 B
T3	5.90±0.19	6.88±0.35	7.27±0.07	6.68 ± 0.64 C
T4	3.21 ±0.03	4.44 ±0.40	5.84 ±0.22	4.50±1.15 C
Average effect of storage duration	6.43 ± 2.27 AB	8.03 ± 2.27 A	9.21±3.09 A	LSD for interference 2.19

Means followed by different letter differ significantly at P=0.05.

V. Conclusions:

Lycopene's ability to maintain the integrity of the acrosome and plasma membrane of sperm during cooling and freezing processes, and its synergistic role with glycerol in protecting sperm from cold shocks and preventing ice crystal formation. Lycopene also helped support and activate enzymatic antioxidants and reduce enzymatic oxidation indices in frozen ram semen for periods of 10, 20, and 30 days.

VI. References:

Abdulkareem,T. A. (2014). Influence of thawing period on some post- thaw semen characteristic of Holstein bulls following catalase addition to Tris extender. Al-anbar.J.Vet.Sci., 7:22- 82.

Ahmad, E., Naseer, Z., Aksoy, M., Küçük, N., Ucan, U., Serin, I., and Ceylan, A. (2015). Trehalose enhances osmotic tolerance and suppresses lysophosphatidylcholine-induced acrosome reaction in ram spermatozoon. Andrologia., 47:786-792.

Al-Saiady, F. A., Al-Mamun, M. A., and Hossain, M. M. (2019). Ultrastructural and functional alterations of frozen-thawed ram spermatozoa. Animal Reproduction Science, 205: 43-49.

Al-Sarray, M. A. R., Hobi, A. A. K. A. A. R., & Al-Ani, A. A. E. D. T. (2019). Effect of adding lycopene to the tris extender on Awassi rams semen quality preseveded at cooling and freezing storage. Plant Archives, 19(2), 3971–3976.

Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S. E., BektaÇoğlu, B., ... & Özyurt, D. (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules.,12: 1496-1547.

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 62-72

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/jr4xv363

Atefeh, B;Reza, A; Kamran, M; Adel, S; Siamak,K. (2022). Lycopene improves testicular damage and sperm quality in experimentally induced varicocele: Relationship with apoptosis, hypoxia, and hyperthermia. Food Sci Nutr. 2022;10:1469–1480.

Bayejid, H.; Rakibul, I.; Feroza, B.; Yearul, K.; and Zakri, H. H. (2015) Oxidative stress induced sperm DNA damage, apossible reason for male infertility.Iran J Repord Med Vol.13:525-532.

Castro,L.S.; Hamilton,T. R. S.; Mendes,C. M.; Nichi,M.; Barnabe,V. H.; Visintin,J. A.; and Assumpção, M. E. O. A. (2016) Sperm cryodamage occurs after rapid freezing phase: flow cytometry approach and antioxidant enzymes activity at different stages of cryopreservation. J.of Animal Sci and Biotec.7: 1- 9.

Choi, S.K. and J.S. Seo (2013). Lycopene supplementation suppresses oxidative stress induced by a high fat diet in gerbils. Nut. Res. & Prac., 7: 26–33.

Hancock,J.(1946). Morphology of bull spermatozoa.Nature.,157:474-454.

Hossain, M. M. A., & Lee, S. S. (2019). Effect of freezing duration on antioxidant activity of ram semen. Journal of Animal and Veterinary Science., 20: 193-199.

Jeyendran, R. S., Van der Ven, H. H., Perez-Pelaez, M., Crabo, B. G., & Zaneveld, L. J. D. (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. Reproduction., 70: 219-228.

Kim, J. S., Lee, S. H., Lee, S. J., & Kim, Y. H. (2020). Effects of cryoprotectants on antioxidant activity of ram semen during freezing. Animal R\production and Biotechnology., 23: 153-159.

Krishnamoorthy, G., K. Selvakumar, P. Elumalai, P. Venkataraman and J. Arunakaran (2011). Protective role of lycopene on polychlorinated biphenyls (aroclor 1254) induced adult rat sertoli cell dysfunction by increased oxidative stress and endocrine disruption. Biomed. Prev. Nutr., 1:116-125.

Naviglio, D., F. Pizzolongo, L. Ferrara, A. Aragon and A. Santini(2008). Extraction of pure lycopene from industrial tomato by-products in water using a new high-pressure process. J. Sci. Food Agric., 88(14): 2414–2420.

Pavan, S. S., & Björndahl, L. (2018). Cryopreservation of sheep semen: A review. Journal of Reproduction and Fertility., 154: 217-227.

Peskin, A. V., & Winterbourn, C. C. (2000). A microtiter plate assay for superoxide dismutase using a water-soluble tetrazolium salt (WST-1). Clinica chimica acta., 293: 157-166.

Prasad, R. B. M. M., & Sriranganathan, N. (2017). Cryopreservation of sheep sperm: A review. Journal of Reproduction and Fertility., 151: 35-44.

Rosato, M.P., Centoducati, G., Santacroce, M.P. & Iaffaldano, N., (2012). Effects of lycopene on in vitro quality and lipid peroxidation in refrigerated and cryopreserved turkey spermatozoa. British Poultry Sci. 53, 545-552. https://doi: 10.1080/00071668.2012.716508/.

Sairam, M. R. K., & Seshamma, G. (2018). Cryopreservation of buffalo semen: A review. Journal of Reproduction and Fertility., 154: 228-237.

Simões,R.; Feitosa,W. B.; Siqueira,A. F. P.; Nichi,M.; Paula-Lopes,F. F.; Marques, M. G.; Peres, M. A.; Barnabe, V. H.; Visintin, J. A.; and Assumpção, M. E. O. (2013) Influence of bovine sperm DNA fragmen- tation and oxidative stress on early embryo in vitro development out- come. Reproduction.146:433-441.

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https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/jr4xv363

SPSS, Statistical Package for the Social Sciences . (2026). Quantitative Data Analysis with IBM SPSS version 26: A Guide for Social Scientists. New York: Routledge. ISBN 978-0-415-57918-6.

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

Tvrda E, Lukac N, Jambor T, Lukacova J, Hashim F, Massanyi(2017). Antioxidant effects of lycopene on bovine sperm Veterinarni Medicina., 62(8): 429–436.

Yagi, K. (1998). Simple procedure for specific assay of lipid hydroperoxides in serum or plasma. Free radical and antioxidant protocols, 107-110.

Zamiri, M., Khalili, B., Jafaroghli, M., & Farshad, A. (2010). Seasonal variation in seminal parameters, testicular size, and plasma testosterone concentration in Iranian Moghani rams. Small Ruminant Research., 94: 132-136.

Zhang, Y., Zhang, J. and Liu, X. (2020). Effects of freezing and thawing on the acrosome and plasma membrane of sheep sperm. Journal of Reproductive Biology and Medicine., 28: 197-203.

Zini, A., Gabriel, M.S. & Libman, J. (2010). Lycopene supplementation in vitro can protect human sperm deoxyribonucleic acid from oxidative damage. Fertil. Steril. 94, 1033-1036. https://doi: 10.1016/j.fertnstert.2009.04.004.