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The effect of adding lycopene on improving the characteristics of diluted and cooled semen of Arabi rams

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Abstract

The experiment was conducted in the animal field of the College of Agriculture / University of Basrah / Karma Ali site for a period of three months from 10/15/2023 and in the forest 1/15/2024. Four Arabian rams were purchased from local markets in Basra Governorate, at ages ranging from 2.5 to 3 years and with similar weights. They were fed a diet consisting of a fodder mixture consisting of barley, bran, hay, and some mineral salts, clean water was provided to them inside the barn. The rams were trained in the process of collecting semen using the artificial vagina of rams in the animal field for a period of two weeks. After completing their training, semen was collected from them three times a month, and after the end of the training period, semen was collected three times a month. The study included adding different levels of lycopene (0,0.5,1,1.5) g/100 ml, and the tubes were closed tightly and placed in the refrigerator, and after the temperature stabilized at (5°C) for (0,24,48,72) hours, All characteristics of diluted and cooled semen were measured using a computer (CASA).

Key words: Lycopene , characteristics, semen, Arabi rams, Computer- assisted semen analysis(CASA).

The results of the study:

From the results, it is noted that T2 (lycopene 0.5 gm/100 ml) was significantly superior (P<0.05) to the rest of the parameters in individual motility and the percentage of live sperm from diluted and cooled semen. It also recorded the lowest values significantly (P<0.05) in the percentage of dead sperm. And the distorted ones, and the zero storage period (without refrigeration) showed significantly higher values (P<0.05) than the rest. Durations (24,48,72) hours. The T2 treatment was significantly (P<0.05) superior to the rest of the treatments in the values of straight line velocity (VSL) and the linearity of the sperm path (LIN) for diluted and cooled semen. Also, the zero period (without cooling) was significantly (P<0.05) superior to the rest of the periods (72, 48, 24). Hours in VSL ,LIN values.

I. Introduction

Fertility has many criteria, all of which depend mainly on the quality and quantity of semen obtained from the male. Likewise, the characteristics of the semen, especially the number of live sperm and their ability to fertilize, are among the most important characteristics of the semen used in artificial insemination (Vincent et al., 2012). Therefore, it is necessary that the sperm preserved by cooling, freezing, and liquefaction be intact with all their parts, especially the acrosome, its plasma membrane, and its sperm, in addition to that its progressive individual movement is rapid and at a level that makes it effective and able to reach the female reproductive tract and penetrate the egg membranes (Hossain and Lee, 2019). The diluted type and its composition also have an important role in maintaining the integrity of the sperm during cryogenic and freezing storage processes through its effect on the osmotic pressure of the sperm membrane and making the pH of the stored semen within neutral limits on the one hand. On the other hand, the diluted has an important role in releasing enzymatic antioxidants and reducing Reactive oxygen levels (ROS) As well as fatty peroxides (LOP), which are present in seminal plasma and sperm cytoplasm, thus increasing the vitality of sperm and their ability to fertilize during artificial insemination (Zamiri et al., 2010). In our current study, lycopene will be added to semen diluted

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with buckwheat, egg yolk, and fructose for godfather rams, according to the proportions suggested in the study. Lycopene is the red, yellow, or brown pigment that is found in some vegetables, such as tomatoes, and some fruits, such as apricots, watermelon, and grapefruit, but it is present in high concentrations. In red-colored plants such as tomatoes (Naviglio et al., 2008). Lycopene is a carotenoid derivative, but it does not carry the activity of vitamin A. It is considered a natural plant pigment produced by plants and microorganisms during the photosynthesis process to protect them from photosynthetic activity and increased sensitivity to light (Choi and Seo, 2013). Many researchers have also confirmed the ability of lycopene to control the formation of free radicals, reduce rates of oxidative stress, and prevent the formation of nitrogen dioxide and hydroxyl radicals inside the body, whether in humans or animals (Krishnamoorthy et al., 2011). Al-Sarray et al., (2019) stated in his study on diluted semen of Awassi rams that adding lycopene with gear and egg yolk led to improving individual motility and the percentage of live sperm, reducing the percentage of deformed sperm, and preserving the sperm membranes and acrosome integrity of the semen diluted with lycopene during Cooling operations for 72,48,24 hours at a temperature of 5 degrees Celsius, To maintain and increase the rate of individual sperm movement and the types of progressive movement and their vitality during cold storage operations to raise the efficiency and quality of the stored semen for longer periods to achieve the highest rates of fertilization during artificial insemination operations in sheep.

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 15/10/2023 to 15/1/2024 Four Arabi rams were purchased from local markets in Basrah Governorate with ages ranging from 2.5-3 years and with similar weights, and the study included:

Cooling by adding different concentrations of lycopene: Semen samples were collected from the four rams using the artificial vagina of the rams, the semen was transferred to the laboratory by test tubes and kept from light by wrapping with a piece of aluminum foil, the tubes were placed in the water bath at a temperature of 37 C° , and all special physical tests (color, collective and individual movement of all kinds) were carried out. For sperm, sperm concentration, and percentage of live, dead and deformed sperm) using Semen analyses device, then the semen was diluted with diluents and then the semen was diluted with thinners as shown in Table (1) and the experiment was designed to study the characteristics of the semen of the Arabi rams diluted with levels of lycopene (1.5,1,0.5,0) g / 100 ml, and closed The tubes are tightly placed in the refrigerator and after stabilizing the temperature at (5 C°) and for periods (zero, 24,48,72) hours. After the cooling period is completed, the readings of all kinds are taken using the Computer- assisted semen analysis(CASA), which included (individual movement of sperm, straight linear velocity of sperm VSL, linearity of sperm path LIN).) The percentage of live, dead and deformed sperm was also calculated by Chemineau et al.,(1991) so that a drop of diluted and cooled semen was taken and placed on a clean and warm glass slide (37 m) and a drop of eosin dye mixture (5%) and nchrocin (10%) was added and mixed with semen for 10 seconds and dried in air for 1-2 minutes and examined under a microscope with a strength of 400XIn the research, the dead sperm took the color of the dye (bluish-pink) while the live sperm remained transparent, and 200 sperm were calculated in different parts of the slide and towards the letter Z and according to the equations for calculating the percentages of live and dead sperm.

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

Statistical analysis

The data were analyzed statistically using the statistical program SPSS (26), as a two-factor experiment, the first factor includes different levels of lycopene (1.5, 1, 0.5, 0) grams, and the second factor includes periods of cooling and freezing for different durations, according to the following mathematical model equation:

Yijk=μ+Ti+Bj +TBij+eijk

Whereas:

Yijk = represents the studied adjective

μ = overall average

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

BJ = cooling durations (0,24,48,72) hours and freezing (10,20,30) days.

TBij = Interference between lycopene concentrations and cold storage and freezing durations.

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

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III. Results and discussion

Qualities of diluted and cooled semen for Orabi rams

Individual movement of sperm

Table (2) shows that the treatment had a significant effect (P<0.05) on individual sperm motility, as the T_2 treatment outperformed the T3, control, and 4T treatments, as the averages were 79.31, 75.20, 73.26, and 66.87%, respectively. It is noted that the duration of storage (one hour A significant effect (P<0.05) on individual sperm motility , The zero storage period outperformed the rest of the storage periods of 24, 48, and 72 hours, as the averages were 79.29, 76.42, 71.54, and 67.40%, respectively. When studying the interaction between the effect of the treatment and the storage duration, it was found that T_2 at the zero period achieved the best results, significantly (P<0.05) compared to the rest of the treatments and the rest of the storage periods, as the individual sperm movement was 85.67%. The T4 treatment, at a storage period of 72 hours, recorded the lowest percentage (P<0.05) in individual sperm motility, which was 57.99%. The results of the study were in agreement with the findings of (Rosato et al., 2012). That adding lycopene to diluted semen improved the individual motility of sperm during cold storage operations, and the results agreed with what Bintara et al., (2023) explained, that adding levels of lycopene 6, 4, 2, 0% to diluted semen cooled at 5°C for a period 24 hours of cooling has improved the individual motility of sperm, and researchers have attributed this to the role of lycopene and its effectiveness in maintaining the membranes. Sperm and acrosomes and prevent their deterioration during dilution and thawing processes, as it is characterized by its synergistic effect with glycerol in preserving sperm membranes by preventing oxidation processes and the formation of fatty peroxides, reducing reactive oxygen species (ROS) rates, preserving their membranes and thus improving their mobility and increasing their effectiveness during cooling and thawing processes.

Table (2): Effect of treatments and duration of cold storage on the individual motility percentage (%) of ram sperm (mean ± Std).

Storage duration / Treatments	Zero	24	48	72	Treatments impact rate
Control	78.49±0.77	75.66±0.46	70.94±0.68	67.95±0.21	4.2573.26 ± B
T2	±0.5085.67	83.06±0.14	75.50±0.48	73.00±0.23	±5.40 A79.31
T3	79.84±0.45	±0.4476.43	73.92±0.19	70.62±0.50	B75.20 ± 3.51
T4	73.81 ± 0.75	70.53 ± 0.43	65.81±0.40	±0.2557.99	66.87 ± 5.97 C
Average effect of storage duration	A79.29 ± 4.64	±4.6176.42 B	71.54 ± 3.84 C	67.40 ± 5.91 D	LSD for interference 2.86

Capital letters horizontally and vertically indicate significant differences at the level (P<0.05) for the effect of storage duration and treatments Control (without adding lycopene), T2 (adding 0.5 g lycopene), T3 (adding 1 g lycopene), T4 (adding 1.5 g lycopene) .

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Percentage of live, dead and deformed sperm

Table (3) shows that the treatment had a significant effect (P<0.05) on the percentage of live sperm, as the T_2 treatment outperformed the control treatments, T3, and T4, as the averages were 66.38, 73.21, 76.83, and 81.89%, respectively. It is also noted that the duration of cold storage (one hour) has a significant effect (P<0.05) on the percentage of live sperm, the zero storage period exceeded the rest of the period, 24, 48, and 72 hours, as the averages were 78.90, 76.76, 73.27, and 69.39%, respectively. When studying the interaction between the effect of the treatment and the storage period, it appears that T_2 at the zero period achieved the highest results significantly (P<0.05) compared to the rest of the treatments and for the rest of the storage periods, as the live sperm was 85.66%, while treatment 4T at the storage period of 72 hours recorded the lowest percentages significantly (P<0.05) in live sperm, which was 61.66%. Table (4) also shows that the treatment had a significant effect (P<0.05) on the percentage of dead sperm, as the T2 treatment recorded the lowest percentages significantly (P<0.05) in the percentage of dead sperm compared to the treatments T3, T_4, and control, as the averages were 13.01. 12.82, 11.35, and 9.54%, respectively. It is also noted that the duration of cold storage (one hour) has a significant effect (P<0.05) on the percentage of dead sperm, as the duration of zero storage recorded the lowest percentages significantly (P<0.05) over the remaining periods of 72, 48, and 24 hours, as the averages were 14.71, 12.63. 10.69 and 8.70%, respectively. When studying the interaction between the effect of the treatment and the storage period, it appears that T2 at the zero period recorded the lowest percentages significantly (P<0.05) compared to the rest of the treatments and for the rest of the storage periods, as the percentage of dead sperm was 7.34%, while treatment 4T at the storage period of 72 hours recorded the highest percentages. Significantly (P<0.05) in the percentage of dead sperm, which was 16.70%. Table (5) also shows that the treatment had a significant effect (P<0.05) on the percentage of deformed sperm, as the 2T treatment recorded the lowest percentages significantly (P<0.05) in the percentage of deformed sperm compared to the rest of the treatments T3, control, and T4, where the averages were 8.91. 8.53, 8.05, and 6.71%, respectively. It is also noted that the duration of cold storage (one hour) has an effect. Significantly (P<0.05) in the percentage of deformed sperm, as the storage period of 0 hours recorded the lowest percentages significantly (P<0.05) compared to the remaining periods of 24, 48, and 72 hours, as the averages were 8.40, 7.16, 6.37, and 10.24%, respectively. When examining the interaction between the effect of treatment and storage duration, it was found that T2 at the zero period recorded the lowest percentages significantly (P<0.05) compared to the rest of the treatments and for the remaining storage periods, as the percentage of deformed sperm was 5.32%, while treatment 4T at the storage period of 72 hours recorded the highest percentages. Significantly (P<0.05) in the percentage of deformed sperm, which was 12.06%. The reason for this discrepancy may be attributed to Percentages of live, dead, and deformed sperm It was 12.06%. The reason for this discrepancy in the percentages of live, dead, and deformed sperm may be attributed to the important role played by substances added to diluted semen, such as egg yolk, citric acid, and antibiotics. In our current study, it is noted that adding lycopene had a significant effect (P<0.05).) An increase in the percentage of live sperm and, correspondingly, a decrease in the percentage of dead and deformed spermThis is due to the effective role of lycopene in preserving the sperm acrosome and its membrane and preserving the osmotic pressure of the membrane on the one hand, and reducing effective oxygenation rates (ROS) on the other hand (Hyemin and Dong,2015 and Amidi et al., 2016, and He et al., 2016). The results of the study agreed with the findings of Al-Sarray et al., 2019 and Souza et al., 2019, that adding levels of lycopene to diluted and cooled semen improved the percentage of live sperm and reduced the percentage of dead and deformed sperm during different periods of cooling, and they attributed the reason to the role Who is playing lycopene helps maintain the sperm membranes through the balance that occurs between the fatty substances and proteins involved in the composition of the sperm membrane, thus reducing the permeability of the membrane and preventing the leakage of the sperm contents during the defoliation processes, which prolongs the period of its survival (Syuhriatin, 2021).

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Table (3): The effect of treatments and duration of cold storage on the percentage of live sperm (%) of ram semen (Mean ±Std).

Storage duration / **Treatments**	Zero	24	48	72	Treatments impact rate
Control	80.94±0.37	64±0.3178.	75.95±0.19	71.78±0.40	±3.5376.83 B
T2	±0.5485.66	±0.4683.59	±0.5481.30	0.1777.00 ±	A81.89 ± 3.35
T3	78.73±0.61	76.03 ± 0.32	70.96 ± 0.21	0.7767.11 ±	73.21±4.66 C
T4	±0.1170.21	±0.3768.78	±0.2864.86	61.66±0.35	3.4766.38 ± D
Average effect of storage duration	78.90±5.79 A	76.76 ± 5.53 B	±73.27 ± 0.28 C	±69.39 ± 5.87 D	LSD for interference 2.14

Capital letters horizontally and vertically indicate significant differences at the level (P<0.05) for the effect of storage duration and treatments Control (without adding lycopene), T2 (adding 0.5 g lycopene), T3 (adding 1 g lycopene), T4 (adding 1.5 g lycopene) .

Table (4): The effect of the type of diluent and the duration of cold storage on the percentage of dead sperm (%) of ram semen (Mean ±Std).

Storage duration / Treatments	Zero	24	48	72	Treatments impact rate
Control	±0.0810.23	±0.18212.02	14.13±0.16	15.69±0.50	13.01 ± 2.15 A
T2	±0.087.34	±0.059.15	10.25±0.35	11.39±0.27	9.54±1.55 C
T3	8.01±0.21	±0.0610.18	12.18±0.07	15.04±0.36	11.35 ± 2.68 B
T4	9.21±0.11	±0.2211.41	13.93±0.24	16.70±0.45	12.82 ± 2.91 A
Average effect of storage duration	±1.158.70 D	±1.1510.69 C	12.63 ± 1.63 B	14.71±2.10 A	LSD for interference 0.21

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Table (5): The effect of the type of diluent and the duration of cold storage on the percentage of abnormal sperm (%) of ram semen (Mean ±Std).

Storage duration / Treatments	Zero	24	48	72	Treatments impact rate
control	6.14±0.05	7.78±0.04	9.18±0.08	10.96±0.17	B8.53 ± 1.82
T2	5.32±0.08	±0.106.24	±0.077.15	8.06±0.14	D6.71 ± 1.05
T3	6.81±0.08	7.14±0.05	8.36±0.09	±0.449.87	±0.25C8.05
T4	±0.047.71	±0.087.41	±0.148.97	±0.1412.06	A8.91 ± 2.01
Average effect of storage duration	0.736.37 ± A	±0.596.16 B	±0.828.40 C	±1.5410.24 D	LSD for interference 0.38

Capital letters horizontally and vertically indicate significant differences at the level (P<0.05) for the effect of storage duration and treatments Control (without adding lycopene), T2 (adding 0.5 g lycopene), T3 (adding 1 g lycopene), T4 (adding 1.5 g lycopene).

Percentage of straight velocity VSL and linearity of sperm LIN path

Table (6) shows that the treatment has a significant effect (P<0.05) on the straight line speed (VSL) (Velocity Straight Line), as the T_2 treatment outperformed the rest of the control treatments, T3, and T4, as the averages were 65.55, 32.94, 30.07, and 28.24 micrometers/second, respectively. It is also noted that The duration of cold storage (one hour) had a significant effect (P<0.05) VSL, the zero storage period outperformed the other periods of 24, 48, and 72 hours, as the averages were 36.17, 38.16, 39.93, and 42.43 micrometers/second. When studying the interaction between the effect of treatment and the storage duration, it was found that T_2 at the zero period achieved the best results, significantly (P<0.05) in VSL was 70.42%, while treatment T4 was recorded at a period of Storage for 72 hours had a significantly lower percentage (P<0.05)in VSL, which was 25.78%. Table (7) shows that the treatment has a significant effect (P<0.05) on the linearity of the path LIN(Linearity(VCL\VSL), as the T_2 treatment outperformed the rest of the control treatments, T3, T4, as the averages were 54.71, 49.44, 35.98, 32.87 µm/, respectively. Again, it is also noted that the duration of cold storage (one hour) has an effect Significantly(P<0.05) in LIN, as the zero storage period outperformed the rest of the periods of 24, 48, and 72 hours, as the averages were 48.02, 44.81, 41.58, and 38.56 micrometers/second, respectively. When studying the interaction between the effect of the treatment and the storage period, it was found that T_2 at the zero period achieved the highest results significantly (P<0.05) compared to the rest of the treatments and the rest of the storage periods, as the LIN was 61.15 micrometers/second, while the T4 treatment at the storage period of 72 hours recorded the lowest percentages significantly(P<0.05) in LIN as it was 30.05 micrometers/second.

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Table (6): The effect of the type of diluent and the duration of cold storage on the Velocity Straight Line (VSL micrometers/second) of ram sperm (Mean ±Std).

Storage duration Treatments	Zero	24	48	72	Treatments impact rate
control	37.47±0.42	34.99±0.19	30.67 ± 0.94	28.64 ± 0.32	±3.6432.94 B
T2	70.42±0.49	65.93±0.15	63.75 ± 0.43	±0.1962.00	A65.55 ± 3.30
T3	31.87±0.49	30.02±0.17	±0.1830.09	±0.3628.05	1.4430.07 C
T4	30.32±0.59	±0.5628.71	0.2627.86 ±	0.3525.78 ±	±1.7328.24 D
Average effect of storage duration	16.90 42.43 ± A	±15.7339.93 B	B38.16 ± 15.36	36.17 ± 15.47 C	LSD for interference 1.79

Capital letters horizontally and vertically indicate significant differences at the level (P<0.05) for the effect of storage duration and treatments Control (without adding lycopene), T2 (adding 0.5 g lycopene), T3 (adding 1 g lycopene), T4 (adding 1.5 g lycopene).

Table (7): The effect of the type of diluent and the duration of cold storage on the linearity of the trajectory (LIN%) of ram sperm (Mean ±Std).

Storage duration / **Treatments**	Zero	24	48	72	Treatments impact rate
Control	54.84±0.34	±0.1851.12	±0.1947.03	44.71±0.38	49.44±4.03 B
T2	0.7961.15 ±	±0.6256.65	±0.2053.15	47.92±0.14	54.71 ± 5.02 A
T3	40.06±0.96	37.26 ± 0.94	±0.2735.01	0.4131.56 ±	35.98 ± 3.29 C
T4	36.03±0.81	0.6334.20 ±	31.16 ± 0.08	30.05 ± 0.17	32.87 ± 2.52 D
Average effect of storage duration	48.02 ± 10.67 A	B44.81 ± 9.67	41.58 ± 9.17 C	38.56±8.12 D	LSD for interference 3.11

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IV. .Conclusions:

Adding levels of lycopene improved the characteristics of refrigerated semen for long periods of cold storage, which included the individual motility of all types of sperm, which was measured by the CASA system, the percentage of live sperm, and a reduction in the percentage of dead and deformed sperm.

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