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The Effect of Adding Levels of the Organic Acid Taurine on Improving the Characteristics of Diluted and Cooled Semen of Arabi Rams

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Abstract: ***This investigation was place from 1/10/2022 to 31/1/2023 in the College of Agriculture / University of Basra / Karma Ali animal field. Five Orabih rams with similar weights and ages of 2.5 to 3 years were bred for the research. For two weeks, the animals were taught to collect semen using the Kabash's fake vagina and given a 2 kilogram diet with two meals each day. Semen was collected three times a month after training, and rams' sperm was added taurine organic acid (zero, 2, 4.6 mmol) and stored in a cryosafe at 5 º for up to 48 hours. A computer analyzed all diluted and chilled semen properties.***
Results of the study: T2 treatment significantly outperformed other coefficients in individual movement, sperm concentration, and percentage of live sperm in refrigerated semen (P<0.05). Cryostorage zero (hour) significantly outperformed other periods in pH values, individual movement, and sperm concentration, and exceeded 24 hours (P<0.05). T2 treatment significantly outperformed (P<0.05) other coefficients in pH, individual sperm motility, and live sperm percentage at zero hour, while T2 recorded the highest sperm concentration in refrigerated semen at 24 hours (P<0.05).
2: The control and T6 coefficients had the highest percentages of dead sperm (P<0.05) compared to other transactions, with the 72-hour cold storage period having the highest moral percentages (P<0.05). The T6 treatment and 72-hour storage period also had the highest moral percentages (P<0.05) in the percentage of dead sperm.
T2 transaction significantly outperformed (p<0.05) the straight linear velocity VSL and linearity of the path LIN of sperm, and the duration of cold storage zero (hour) significantly outperformed 72, 48, and 24 hours in VSL and LIN ratios for sperm. T2 transaction at cold storage zero (hour) significantly outperformed VSL and LIN ratios of spe

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1. INTRODUCTION

As a result of oxidative stress, effective oxygen release (ROS), and damage to the sperm membrane and its contents, the use of frozen semen for in vitro fertilization increases the rates of dead and malformed sperm (Pardede et al., 2020). Adding thinners to male sperm without first analyzing their effects reduces motility and the proportion of living sperm in the semen, while increasing blood flow to the sperm membrane as it dissolves. According to Farshad and Hosseini (2013) and Sangeeta et al. (2015), Common diluents include egg yolk and fructose. Yeah, Surerol In order to preserve sperm's viability and enhance its resistance to freezing and thawing (Hosen et al., 2015; Seify et al., 2019). So, researchers' thoughts came to fruition when they mixed amino and organic acids with diluted semen that had been treated with egg yolk, fructose, and everything else. Lycerin, better known as Yesrol, The organic acid taurine will be mixed with ram semen that has been diluted with egg yolk for our present investigation. Yeah, Surerol Based on the study's suggested ratios, taurine is an organic acid that plays several critical functions in the body's cells. It is a component of cell membranes and helps control the passage of nutrients across cell membranes while also making them more vulnerable. Its chemical formula is $C_2H_2NO_tS$ and its molecular weight is 125.15 g/mol; it is also an antioxidant and helps prevent toxins and remove free radicals. Taurine is an organic acid that the body can produce and also gets from outside sources like meat and fish. Several studies have shown that combining it with cysteine and other amino acids makes bull semen thinners, which protect the acrosome during thawing and storage, and keeps sperm active and vital (Aly & Khafagy, 2014; Chhillar et al., 2012; Sarıözkan et al., 2009). A number of recent studies have shown that adding taurine as a diluent to egg yolk and fructose improves the fertility of male agricultural animals' sperm. This is because taurine keeps the osmotic pressure inside the sperm from breaking down and its components from exuding during thawing after freezing, making for more effective sperm during artificial insemination and higher rates of fertilization and pregnancy (Seify et al., 2019; Zhang et al., 2021).

2. RELATED WORK

Individual movement, concentration, living sperm, dead sperm, and straight linear velocity (VSL) are some of the sperm quality metrics that are investigated in this study. The research investigates the influence of various treatments (T1, T2, and T3) and storage durations (zero, 24, 48, and 72 hours) on sperm quality parameters. Findings that are congruent with those of prior research are shown by comparative analysis. The research conducted by Sarıozkan et al. in 2009 and Lambert et al. in 2014 shed light on the beneficial impact that taurine, an organic acid, has on the mobility and features of individuals, specifically with regard to their sperm. While the research conducted by Zhang et al. (2021) highlights the antioxidant properties of taurine, the findings of Atte et al. (2022) highlight the effectiveness of taurine in protecting sperm membranes. In addition, a number of studies have emphasized the significance of amino and organic acids, egg yolk, fructose, and glycerol in the process of preserving sperm membranes and density. Research conducted by Atessahin et al. (2008), Bucak & Tekin (2007), UYSAL et al. (2005), and Chhillar et al. (2012) highlights the relevance of chemical additives in the reduction of oxidative stress and the preservation of sperm membranes throughout the

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processes of cooling and freezing. Research conducted by Yang et al. (2010) and Zhang et al. (2021) provides more insights into the influence that taurine has on the movement of sperm, highlighting the function that taurine plays in preserving motility after a variety of activities. A full knowledge of the relationships between treatments, storage durations, and sperm quality measures may be obtained via the accumulation of information from these investigations.

3. MATERIALS AND METHODS

This study was conducted in the animal field of the College of Agriculture / University of Basra/ Karma Ali Complex, for the period from 1/10/2022 to 31/1/2022 (four months), the study included (5) adult rams with ages ranging from 2.5-3 years and with similar weights, and the study included:

Dilution of Semen with Different Concentrations of Taurine and Cooled

The five rams' artificial vaginas were used to collect semen three times a month, which was pooled and transported by test tubes to the laboratory. The tubes were placed in the water bath at 37 ° C and diluted with the materials in Table (1). All tests for semen were conducted after diluting it (and was considered a control group), after which different levels of taurine acid (2,4,6) mmol were added, the tubes were tightly closed, and the tubes were refrigerated until the neutralization blister was reached and for periods (zero, 72,48,24) hours after dilution and cooling. So a drop of diluted and cooled semen was placed on a clean and warm glass slide (37 m), a drop of eosin dye mixture (5%) and nacrosin (10%) was added, mixed for 10 seconds, dried in air for 1-2 minutes, and examined under a 400X microscope, research. The dye turned dead sperm purplish pink, while living sperm remained clear. 200 sperm were estimated in various places of the slide and toward the letter Z using the formulae for live and dead sperm percentages.

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
Taurine (mmol)	----	2	4	6
Clycerol (ml)	8	8	8	8
Distilled water(ml)	Complete the volume to 100 ml			

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Statistical Analysis

The data were analyzed statistically using the statistical program SPSS, 26), as an experiment of two factors, the first factor includes different levels of taurine (0,2,4,6) mmol, and the second factor, which includes cooling periods and different periods according to the following mathematical model equation:

TBij+eijk+Yijk=μ+Ti+Bj

Whereas:

Yijk = represents the studied adjective

μ = overall average

Ti = concentrations of organic acid (0,2,4,6) mmol

BJ = cooling durations (0,24,48,72) hours.

TBij = Interaction between amino acid concentrations and cold storage times

eijk = randomly and naturally distributed experimental error with an average equal to zero and e2 variance.

4. RESULTS AND DISCUSSION

Qualities of Diluted and Cooled Semen for Orabi Rams

Individual Movement of Sperm

As it is noted from Table (2) that the transaction has a significant effect (0.05(P< in the individual movement, as the T_2 transaction outperformed the T_4 coefficients , control, T_6, as the averages were 79.28, 75.19, 73.13, 66.81% respectively. It is also noted that the storage period (hour) has a significant effect (0.05(P< In the individual movement of sperm, as the storage period exceeded zero over the rest of the storage periods 24, 48, 72 hours, as the averages were 79.25, 76.26, 71.50, 67.39% respectively, and when studying the interaction between the effect of the transaction and the duration of storage, it was found that (T_2 at the zero period) achieved the best results significantly (0.05(P< compared to the rest of the transactions and the rest of the storage periods, as the individual movement of sperm was 85.66% and the treatment T_6 was recorded at the storage period of 72 hours, the lowest percentages significantly (0.05(P< In the individual movement of sperm, which was 54.38%, the results of the study were consistent with the findings of (Sarıözkan et al., 2009) that the addition of organic acid taurine to the diluted and cooled semen of bulls improved the individual movement of sperm during dissolving processes. As shown (Lambert et al., 2014) that the addition of organic acid taurine to diluted semen has improved the individual movement of sperm by maintaining the osmotic pressure of the sperm and neutralizing the pH and preventing fructose decomposition processes, and taurine acts as an antioxidant and rid the sperm of free radicals and peroxides of ram semen during cooling processes and improve all semen characteristics, including individual movement of sperm (Banday et al., Zhang et al.,(2021) confirmed in his study of diluted ram semen with different levels of taurine that the individual movement of sperm was 74.41% higher when stored for three hours at room temperature while starting to decrease when the storage period increased from 7-5 hours.The researchers explained that taurine acted as an antioxidant and rid semen of free radicals and peroxides during dissolving processes, and it also kept the sperm membranes and sperm acrosome from deterioration, as it was shown (Atte et al., 2022) in his study on African dwarf

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rams that the individual movement of sperm recorded the highest levels of 86.23, 74.60, 62.35%, which were fed on (1, 0.5, zero) % Taurine respectivelyThe researchers attributed this to the role of taurine and its effectiveness in preserving sperm membranes and preventing their deterioration during collection, dilution and cooling.

Table (2): Effect of Treatments and Duration of Cold Storage on the Individual Motility Percentage (%) Of Ram Sperm (Mean ± Standard Error)

Storage duration / Treatments	Zero	24	48	72	Treatments Impact Rate
control	78.34 ± 0.54	75.44 ± 0.40	70.80 ± 0.47	67.94 ± 0.21	73.13 ± 4.17 C
T2	85.66 ± 0.48	82.96 ± 0.15	75.49 ± 0.49	72.99 ± 0.24	79.28 ± 5.38 A
T4	79.85 ± 0.45	76.40 ± 0.43	73.91 ± 0.18	70.61 ± 0.49	75.19 ± 3.51 B
T6	73.15 ± 0.75	70.26 ± 0.08	65.81 ± 0.40	54.38 ± 0.31	66.81 ±5.90 D
Average effect of storage duration	79.25 ± 4.63 A	76.26 ± 4.67 B	71.50 ± 3.83 C	67.39 ± 5.89 D	LSD for interference 5.04

Large letters horizontally and vertically mean that there are significant differences at the level ($p<0.05$) of the effect of storage period and treatments. Control (no taurine added), T2 (2 mmol taurine added), T4 (4 mmol taurine added), T6 (6 mmol taurine added).

Sperm Concentration

Table (3) shows that the treatment has a significant effect ($p<0.05$) on sperm concentration, as the T_2 treatment outperformed the control coefficients, T_4, T_6 as the averages were 61.75 , 57.20, 57.02, and 41.59% respectively. It is also noted that the storage period (hour) has a significant effect ($p<0.05$) in the concentration of sperm, as the storage period exceeded 24 hours on the rest of the periods zero, 48, 72 hours as the averages were 58.25, 55.68, 51.93 and 51.69% respectively. When studying the interaction between the effect of the transaction and the duration of storage, it is shown that (T_2 at the period 24) achieved the best results significantly ($p<0.05$) compared to the rest of the transactions and for the rest of the storage periods, as the concentration of sperm was 65.32%, and T_6 was recorded at the storage period of 72 hours, the lowest percentages significantly ($p<0.05$) in the concentration of sperm, as it was 36.68%. The results of the current study agreed with the findings of many researchers that the addition of amino and organic acids with egg yolk, fructose and glycerol has an

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effective role in maintaining sperm density by reducing oxidative stress and defense mechanism in maintaining sperm membranes and preventing their decomposition . *et al., 2009* and 2009Bucak et al and Dziekońska et al., 2010 (*Zamiri* et al*., 2010*; Brunet *et al*., 2011) their study on rams and males of goats that the process of generating (Spermatogenesis) inside the testicle is affected by the variation of temperature, humidity and health status of rams by affecting the sperm generators and hindering their work and thus the low concentration and density of sperm and that their storage by cooling increases their deterioration and that adding many substances, especially organic or amino acids or some substances to them. The ability to reduce the osmotic pressure inside the sperm and the effective effect in affecting the concentration of sperm by preserving the sperm membranes and preventing the decomposition of their membranes and thus obtaining balanced sperm with high efficiency on fertilization during artificial insemination. Atte et al*., 2022)* also confirmed in their study on African dwarf males that feeding vegetable powders containing taurine in proportions of (0, 0.5, 1, 1.5) achieved a significant difference in sperm concentration by (2.26, 3.50, 4.17, 5.17%) respectively The researchers attributed this improvement in sperm concentration to the efficiency of taurine in maintaining the contents of sperm and controlling osmotic pressure during semen dissolution during cooling processes.

Table (3): The Effect of the Type of Diluent and the Duration of Cold Storage on the Concentration of Ram Sperm ($\times 10^6$) (Mean ±Std)

Storage duration / Treatments	**Zero**	**24**	**48**	**72**	**Treatments Impact Rate**
control	35.35 ± 1.02	66.24 ± 0.92	64.91 ± 0.45	61.59 ± 0.39	57.20 ± 3.41B
T2	65.32 ± 0.82	63.45 ± 0.52	61.63 ± 0.61	56.59 ± 0.65	61.75 ± 3.14A
T4	61.24 ± 0.79	58.96 ± 0.12	55.97 ± 0.24	51.92 ± 0.19	57.02 ±3.62 C
T6	45.82 ± 1.85	43.67 ± 0.98	40.19 ± 0.75	36.68 ± 0.66	41.59 ± 3.74D
Average effect of storage duration	61.93 ± 12.46A	58.25 ± 9.20 B	55.68 ± 9.82B	51.69 ± 9.63C	LSD for interference 7.12

Percentage of Live and Dead Sperm

Table (4) shows that the treatment has a significant effect ($p<0.05$) in live sperm, as the T_2 treatment outperformed the control coefficients, T_4, T_6 as the averages were 81.90, 76.81, 73.21, and 66.37% respectively. It is also noted that the storage period (hour) has a significant effect ($p<0.05$) in live sperm, as the storage period exceeded zero on the rest of the extended 24, 72, 48 hours if it was, The averages are 78.90, 76.73, 69.37, and 37.30% respectively. When studying the interaction between the effect of the transaction and the duration of storage,

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it is shown that (T_2 at the zero period) achieved the best results morally ($p<0.05$) compared to the rest of the transactions and for the rest of the storage periods as the live sperm was 85.67%, and the treatment T_6 was recorded at the storage period of 72 hours with the lowest percentages significantly ($p<0.05$) in live sperm as it was 61.66%, as well as Table (5) shows that the treatment has a significant effect ($p<0.05$) in dead sperm, The transaction control of the transactions T_6, T_4, T_2 outperformed the averages of 13.02, 12.81, 11.31 , and 9.50 respectively. It is also noted that the duration of storage (hour) has a significant effect ($p<0.05$) in dead sperm, as the storage period of 72 exceeded the rest of the periods 48, 24, zero hours, as the averages were 14.64, 12.62, 10.68, 8.70% respectively. When studying the interaction between the effect of the transaction and the duration of storage, it is shown that (T_6 At the period 72) achieved the best results morally ($p<0.05$) compared to the rest of the transactions and the rest of the storage periods as the dead sperm 16.71% and the treatment T_2 was recorded at the storage period zero hours the lowest percentages significantly ($p<0.05$) in dead sperm as it was 7.36%, and the reason for this discrepancy in the proportions of live and dead sperm may be attributed to the process of generating sperm and secreting sex hormones, and that additives such as glycerol, which is usually added at rates of 8% to diluted semen It has an important role in preserving sperm membranes during freezing processes through its penetration into the sperm membrane and not allowing the formation of ice crystals, and that the release of active oxygen types (ROS) leads to a decrease in antioxidants present in the seminal plasma and negatively affects the percentage of live sperm and an increase in the percentage of dead and deformed sperm (Atessahin *et al*., 2008; Bucak & Tekin, 2007; UYSAL *et al*., 2005), as well as many researchers confirmed that chemical additives represented by some amino acids and organic acids have an active role in maintaining sperm membranes during cooling, freezing and thawing processes by preventing the rupture of these membranes and reducing the concentration of free oxygen types (ROS) and levels of peroxides and lactic acid formation, which have an active role in reducing the percentages of live sperm, Chhillar *et al*., 2012) indicated in his study on diluted and cooled semen for rams containing organic acid taurine at levels of 50,100 mmol that organic acid has an effective effect on preserving sperm membranes during freezing and thawing processes and reducing reactive oxygen levels (ROS) and thus improving the ability, movement and activity of sperm, increasing mobility and live sperm and reducing the percentage of dead sperm.

Table (4): The Effect of Treatments and Duration of Cold Storage on the Percentage of Live Sperm (%) of Ram Semen (Mean ± Standard Error).

Storage duration / Treatments	**Zero**	**24**	**48**	**72**	**Treatments Impact Rate**
control	80.97 ± 0.39	78.58 ± 0.36	75.96 ± 0.19	71.73 ± 0.46	76.81 ± 3.56 B
T2	85.67 ± 0.53	83.57 ± 0.42	81.38 ± 0.56	76.99 ± 0.18	81.90 ± 3.34A

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T4	78.74 ± 0.61	76.01 ± 0.31	71.02 ± 0.26	67.09 ± 0.77	73.21 ± 4.67 C
T6	70.22 ± 0.11	68.77 ± 0.36	64.85 ± 0.28	61.66 ± 0.35	66.37 ± 3.48D
Average effect of storage duration	78.90 ± 5.80 A	76.73 ± 5.53 B	64.30 ± 6.31 D	69.37 ± 5.87 C	LSD for interference 5.75

Table (5): The Effect of the Type of Diluent and the Duration of Cold Storage on the Percentage of Dead Sperm (%) Of Ram Semen (Mean ± Standard Error)

Storage duration / Treatments	**Zero**	**24**	**48**	**72**	**Treatments impact rate**
control	10.25 ± 0.06	12.01 ± 0.18	14.13 ± 0.16	15.70 ± 0.50	13.02 ± 2.15 A
T2	7.36 ± 0.09	9.17 ± 0.05	10.24 ± 0.33	11.90 ± 0.46	9.50 ± 1.49 D
T4	8.0 ± 0.21	10.16 ± 0.05	12.18 ± 0.07	14.90 ± 0.46	11.31 ± 2.64 C
T6	9.21 ± 0.11	11.40 ± 0.21	13.94 ± 0.24	16.71 ± 0.46	12.81 ± 2.91 B
Average effect of storage duration	8.70 ± 1.15 D	10.68 ± 1.14 C	12.62 ± 1.63 B	14.64 ± 2.16 A	LSD for interference 2.15

Percentage of Straight Velocity VSL and Linearity of Sperm LIN Path

Table (6) shows that the transaction has a significant effect on the straight linear velocity VSL, as the T_2 transaction outperformed the rest of the control coefficients, T_4, T_6, as the averages were 65.59, 32.95, 29.99, 28.21% respectively, as well as it is noted that the storage period (hour) has a significant effect ($p<0.05$)(pin VSL), as the storage period exceeded zero over the rest of the periods 24, 48, 72 hours, and when studying the interaction between the effect of the transaction and the duration of storage, it was found that (T_2 At the zero period) achieved the best results morally ($p<0.05$)in VSL, as it was 25.83%, and The results of this study agreed

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with the findings of (Yang et al., 2010), as it found a clear improvement in the types of sperm movement of rams that were fed on organic acid taurine as a result of the use of taurine, as it has a major role in maintaining sperm motility and stimulating them even after cooling, freezing and thawing processes, and the results of this study were confirmed by (Zhang et al., 2021) in their study of ram semen diluted with taurine and cooled that storing semen for about 71 days by cooling and using taurine by 100 mmol reduced the progressive movement (LIN) of sperm while using 20 mmol reached progressive motion (LIN).) to the highest level and the reason for this change is because the high concentration of taurine changes the osmotic pressure of the dilution solution used and affects the permeability of the sperm membrane on the one hand and on the other hand may cause sperm toxicity, destroy the structure of sperm, and thus reduce the overall mobility in sperm (Zhang et al., 2017).

Table (6): The Effect of Parameters and Duration of Cold Storage on the Velocity Straight Line (VSL) of Arabi Ram Sperm (Mean ± Standard Error)

Storage duration / Treatments	**Zero**	**24**	**48**	**72**	**Treatments Impact Rate**
control	37.53 ± 0.42	34.93 ± 0.26	30.72 ± 0.94	28.63 ± 0.32	32.95± 3.63 B
T2	70.53 ± 0.49	65.99 ± 0.16	63.81 ± 0.43	62.02 ± 0.20	65.59± 3.30 A
T4	31.90 ± 0.49	30.00 ± 0.15	30.04 ± 0.18	28.04 ± 0.36	29.99± 1.44 C
T6	30.36 ± 0.59	28.74 ± 0.57	27.92 ± 0.26	25.83 ± 0.35	28.21± 1.74 D
Average effect of storage duration	42.58 ±16.90 A	39.92 ± 15.74B	38.12 ± 15.36 C	36.13± 15.48 D	LSD for interference 3.57

5. CONCLUSION

Adding levels of the organic acid taurine during cooling periods improved the characteristics of diluted semen, represented by the individual and linear movement of sperm, the percentage of live sperm, the concentration of sperm, and a reduction in the percentage of dead and deformed sperm. Thus, it is concluded that the organic acid taurine has the ability to maintain the integrity of the acrosome and the plasma membrane of sperm during cooling processes by supporting and activating enzymatic antioxidants and reducing enzymatic oxidation indicators of ram semen, thus improving the characteristics of cooled semen.

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