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EFFECT OF ADDING QUERCETIN TO TRIS EXTENDER IN SOME SEMEN CHARACTERISTICS OF AWASSI RAMS AFTER DIFFERENT PERIODS OF CRYOPRESERVATION

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ABSTRACT

The study evaluated the effect of adding quercetin to some characteristics of the sperm of the ram. This study was conducted in the animal field, Department of Animal Production, College of Agricultural Engineering Science, University of Baghdad for the period 5/12/2021 to 20/2/2022. In this experiment, 3 rams were used at the age of 2-2.5 years and weighed 50-55 kg. The semen was collected early in the morning and once a week and the semen was pooled to remove the individual differences. The treatments were divided: quercetin-free control group, treatment T1 (3 µL/mL quercetin), T2 treatment (6 µL/mL quercetin), T3 treatment (9 µL/mL quercetin). The result of the study showed a significant increased ($p<0.01$) of T2 treatment in the percentage of individual sperm motility during the cooling period (2 and 24 h). T3 treatment showed a significant increase ($p<0.05$) at (48 and 72 h) of individual motility. On the other hand, T2 treatment showed a significant increased ($p<0.01$) in the viability of sperm at (2 and 24 h) of cooling preservation, while T3 treatment showed a significant increase at (48 and 72 h) cooling preservation for the viability of sperm. The result indicated that adding quercetin does not effect of total abnormality of sperm for all treatments. The result presented significant increased ($p<0.05$) percentage of HOST for T2 and T3 treatments. The results showed a significant increase at ($p<0.01$) for T2 treatment at (2 and 48 h) on the other hand T1 showed a significant increase at 72 h. for the same character.

keywords: quercetin, semen, awassirams.

تأثير إضافة الكيرستين الى مخفف Tris في بعض صفات السائل المنوي للكباش العواسي بعد مدد مختلفة من الحفظ بالتبريد

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الخلاصة

أجريت هذه الدراسة في الحقل الحيواني التابع لقسم الانتاج الحيواني/ كلية علوم الهندسة الزراعية/ جامعة بغداد، للمدة من ٢٠٢١/١٢/٥ ولغاية ٢٠٢٢/٢/٢٠، بهدف بيان تأثير إضافة ألكيرستين لمخفف Tris للكباش العواسي في بعض الصفات الفيزيائية والكيمياحيوية للسائل المنوي، اذ استعمل في هذه التجربة ٣ كباش عواسي بعمر ٢-٢.٥ سنة وبوزن ٥٠-٥٥ كغم، وتم جمع السائل المنوي في الصباح الباكر مرة كل اسبوع ولمدة ٨ اسابيع وتم تجميع السائل المنوي Pooled semen لأزالة الفروق الفردية، وقسمت الى المجاميع التالية: مجموعة السيطرة (C) مخفف Tris، المعاملة الأولى (T1) أضيف الكيرستين بنسبة 3 مايكرولتر/مللتر، المعاملة الثانية (T2) أضيف الكيرستين بنسبة 6 مايكرولتر/مللتر، المعاملة الثالثة (T3) أضيف الكيرستين بنسبة 9 مايكرولتر/مللتر، وبينت الدراسة الحالية بأن اضافة 6 مايكرولتر/مللتر أدى الى زيادة معنوية ($P<0.01$) في النسبة المئوية لحركة النطف الفردية في اثناء مده الحفظ ٢ و٢٤ ساعة وادى اضافة 9 مايكرولتر/مللتر الى زيادة معنوية ($P<0.05$) اثناء مده الحفظ ٤٨ و٧٢ ساعة من الحفظ بالتبريد لنفس الصفة، وأضهرت نتائج الدراسة تفوق معنوي ($P<0.01$) للمعاملة T2 عن مدد الحفظ ٢ و٢٤ ساعة في صفة نسبة النطف الحية وزيادة معنوية للمعاملة T3 للمدد ٤٨ و٧٢ ساعة لنفس الصفة، في حين لم يكن هناك تفوق معنوي لأي معاملة في نسبة تشوهات النطف الكلية، نسبة تشوهات الرأس، نسبة تشوهات القطعة الوسطية، نسبة تشوهات الذيل، واتضح من نتائج الدراسة الحالية زيادة معنوية ($P<0.05$) للمعاملة T2 وT3 في النسبة المئوية لسلامة الغشاء البلازمي مقارنة ببقية المعاملات، كما ازدادت نسبة سلامة الاكروسوم معنويا ($P<0.01$) لدى المعاملة T2 لنطف الكباش العواسي للمدد ٢ و٤٨ ساعة، كما اظهرت المعامله T1 تفوق معنوي ($P<0.01$) عند الوقت ٧٢ ساعة لنفس الصفة.

الكلمات المفتاحية: الكيرستين، السائل المنوي، الكباش العواسي.

INTRODUOTION

Quercetin is part of the flavonoids and is commonly found in foods such as fruits and vegetables, It has many biological activities including being an antioxidant **(Gibb *et al.*, 2013)**. Ram sperm contain a high percentage of unsaturated fatty acid in the plasma membrane, so they are more sensitive to oxidative stress and free radical formation during cooling periods **(Diaz *et al.*, 2016)**. Studies have indicated that quercetin has positive effects on fresh sperm and after thawing in different types of agricultural animals **(Gibb *et al.*, 2013)** adding quercetin has a significant effect on the improvement of the viability of sperm by reducing the damage of oxidative stress and reactive oxygen species **(Talwar & Hayatnagarkar, 2015)**. quercetin was prevent lipid peroxidation by inhibiting the production of free radicals with Alpha-tocopherol to delay the oxidation and stimulate gene expression of enzymes such as glutathione s-transferase and glucuronosyl transferase, it's an antioxidant capable of eliminatin reactive oxygen spcies and hydroxyl radicals, its more effective against oxidation and ROS than vitamin E or vitamin C, studies have also indicated the necessity of using quercetin in semen diluents for many animals, including rams **(Silva *et al.*, 2016)** Therefore, the research aims to explain the effect of adding quercetin to the Tris in some semen characteristics of the ram.

MATERIAlS AND METHODS

This study was conducted in the animal farm of the department of animal production, collage of agricultural engineering sciences, university of Baghdad for the period from 5/12/2021 to 20/2/2022. The process of collecting sperm from 3 rams by artificial vagina once a week. The samples were folded and diluted 1:10. The strain dilator was prepared by **Salamon & Maxwell (2000)** and three concentrations of Quercetin, the first treatment 3 µL/mL, the second 6 µL/mL and the third 9 µL/mL for diluted semen plus control group. Attributes studied the researchers calculated the concentration according to the method of researchers **Guidet & Shah (1989)**. Effectiveness of ALT and AST: Effectiveness was estimated by **Reitman & Frankel (1957)** based on the kit prepared by French biomerieux. The statistical analysis system **(SAS, 2012)** was used for data analysis, and the differences between the averages were compared with the Duncan (1955).

RESULTS AND DISCUSSION

The results of the study showed a significant increased ($p< 0.01$) in individual motility of sperm for T2 treatment was (87.37±1.01, 80.50±1.50%) at (2 and 24 h) of cooling preservation respectively compared with the other treatments. On the other hand, T3 treatment showed a significant increased ($p<0.05$) at 48 h. (72.25±1.62%) compared with the other treatments. Also, T3 treatment showed a significant increase ($p< 0.01$) (65.50±2.23%) at 72 h for the same character (Table 1).

Table (1): effect of adding different concentrations of quercetin to Tris extender on the individual motility of sperm (%) of awassi rams with different cooling preservation (mean ± standard error).

Treatments	Time (h)				Level of Significance
	0	24	48	27	
C	79.75±1.48 Ab	71.75±1.75 Bb	63.25±1.83 Cb	51.87±0.19 Db	**
T1	83.87±1.72 Ab	76.12±1.16 Bab	68.62±1.56 Cab	60.50±1.36 Da	**
T2	87.37±1.01 Aa	80.50±1.50 Aa	69.62±2.98 Ba	62.62±3.66 Ba	**
T3	84.00±1.37 Aa	78.75±1.73 Ba	72.25±1.62 Ca	65.50±2.23 Da	**
Level of Significance	**	**	*	*	-----

Different superscripts within column are significantly different (P<0.01) (p<0.05)***

The results of this study showed a significant effect of quercetin on individual motility of sperm at T3 treatment compared with the other groups, Because may be due to the adding quercetin to the semen extender to preserve sperm motility and protect mitochondria against reactive oxygen species. Or, the reason may be due to the interaction of quercetin with a Ca^{+2}-enzyme that regulates sperm motility **(Tvrda *et al.*, 2016)**. The concentration of Ca^{+2} inside the cells is significant for sperm motility through the production of cAMP that leads reduce ATP production for sperm, mitochondria have an important role in the fertility of sperm because of its relationship with the energy for its movement as it is the main site for the production of the reactive oxygen species **(Kasai *et al.*, 2002)**. The importance of adding quercetin to the semen extender is in protecting the mitochondria and producing energy, contributing to reducing oxidative damages through its ability to inter cells and collect inside the mitochondria and thus control the production of reactive oxygen species **(Carrasco-Pozo *et al.*, 2012)**.

The results of the study indicated a significant effect ($p<0.01$) of T2 treatment on the viability of sperm at 2 and 24 h (90.75±1.08, 84.12±1.54%) respectively, compared with the other treatments. At 48 and 72 h of cooling preservation T3 showed a significant increase ($p<0.05$) (75.00±1.64, 67.00±2.56%) respectively, compared with the other treatments (Table 2).

Table (2): effect of adding different concentrations of quercetin to Tris extender on the viability of sperm (%) of awassi rams with different cooling preservation (mean±standard error).

Treatments	Time (h)				Level of Significance
	0	24	48	27	
C	83.37±1.48 Ac	75.62±1.63 Bb	66.62±1.83 Cb	58.87±0.91 Db	**
T1	87.87±1.17 Aab	79.62±1.45 Bab	72.62±1.66 Ca	60.00±1.83 Dab	**
T2	90.75±1.08 Aa	84.12±1.54 Ba	74.37±2.47 Ca	66.12±3.10 Bab	**
T3	86.37±1.73 Abc	82.50±1.66 Abc	72.00±1.64 Ba	67.00±2.56 Ca	**
Level of Significance	**	**	*	*	-----

Different superscripts within column are significantly different (P<0.01) (p<0.05)***

The current study showed a significant effect of adding quercetin on the viability of sperm in Awassi rams this may be because it is improvement the semen characteristics such as the viability through reducing oxidation damages and reducing the level of ROS **(Talwar & Hayatnagarkar, 2015)**. The occurrence of oxidation during the cooling period of sperm especially polyunsaturated fatty acids increases the production of peroxides that is leading to many changes in the membrane structure of sperm especially in the head this lead to reduces the viability of sperm **(Aitken *et al.*, 2010)**. The results showed no significant effect of quercetin on abnormalities of sperm between all treatments. Adding quercetin to the Tris extender led to a significant increase in the percentage of plasma membrane integrity because it is important to succeeding the fertility **(Esterbauer *et al.*, 1991)**. Moreover, the adding quercetin can scavenge free radicals and then reduce the reactive oxygen species, therefore, maintaining the plasma membrane **(Ardeshirnia *et al.*, 2017)**.

The results of the study showed no significant effects of adding quercetin to Tris extender for abnormality of Awassi rams semen between all treatments (Table 3).

Table (3): Effect of adding different concentrations of quercetin to Tris extender on the abnormalities of sperm (%) of Awassi rams with different cooling preservation (mean±standard error).

Treatments	Time (h)	Level of Significance

	0	24	48	27	
C	7.50±1.13 Ca	10.50±1.16 BCa	12.50±1.05 Ba	16.00±1.03 Aa	**
T1	6.62±1.06 Ca	9.12±1.30 BCa	11.25±1.03 Ba	14.50±0.84 Aa	**
T2	6.50±1.19 Ca	9.62±1.32 BCa	12.00±1.21 Ba	15.50±1.06 Aa	**
T3	6.37±1.03 Ca	9.25±1.27 BCa	12.00±1.08 Aba	14.50±0.90 Aa	**
Level of Significance	N.S	N.S	N.S	N.S	-----

N.S: Non-significant.

The results of the study indicated a significant effect ($p< 0.05$) of T2 treatment for HOST of sperm at 2 and 24 h (86.75±1.35, 78.75±1.44%) respectively, compared with the other treatments. T1 treatment showed a significant increased ($p< 0.05$) of HOST (69.37± 1.29%) compared with control treatment. At 72 h of cooling preservation T1 showed a significant effect ($p<0.05$) (65.00±2.36%) compared with the other treatment (Table 4).

Table (4): Effect of adding different concentration of quercetin to Tris extender on the HOST of sperm (%) of Awassi rams with different cooling preservation (mean±standard error).

Treatments	Time (h)				Level of Significance
	0	24	48	27	
C	78.12±2.34 Ac	73.25±2.80 Bb	62.62±1.17 Cb	54.87±1.61 Db	**
T1	83.75±0.95 Aab	75.87±1.45 Bab	69.37±1.29 Ca	65.00±1.83 Dab	**
T2	86.75±1.35 Aa	78.75±1.44 Ba	70.75±1.58 Ca	63.62±2.67 Bab	**
T3	83.00±1.99 Abc	77.87±1.69 Abc	71.00±2.04 Ba	62.87±2.36 Ca	**
Level of Significance	**	**	*	*	-----

Different superscripts within column are significantly different (P<0.01) (p<0.05)***

The integrity of the Plasma membrane of the sperm is very important for the success of the fertilization process, and since the sperm membrane contains a high percentage of polyunsaturated fatty acide and is sensitive to free radicals, which leads to a decrease in the quality of semsn **(Eesterbauer *et al.*, 1991)**. Therefore, the addition of Quercetin works remove to radicals and reduce reactive Oxygen Species, and then works to perpetuate the plasma membrane by reducing the damage caused by oxidative stress in ram sperm **(Ardeshirnia *et al.*, 2017)**.

The result of the study showed a significant effect ($p<0.05$) of T2 treatment after 2 h of cryopreservation of acrosome integrity (88.00±1.06%) compared with the control group, at 24 h there were no significant effects of adding quercetin to extender in acrosome integrity for all treatments. T2 treatment showed a significant effect ($p<0.01$) at 48 h of acrosome integrity (76.25±1.56%) compared with the control group (68.25±1.76%). T1 treatment showed a significant effect ($p<0.01$) for the same character it was (71.12±1.92%) compared with the other treatments (Table 5).

Table (5): Effect of adding different concentration of quercetin to Tris extender on the acrosome integrity of sperm (%) of Awassi rams with different cooling preservation (mean ± standard error).

Treatments	Time (h)				Level of Significance
	0	24	48	27	
C	78.12±2.34 Ab	73.25±2.80 Ab	62.62±1.17 Bb	54.87±1.61 Cb	**
T1	83.75±0.95 Aa	75.87±1.51 Bab	69.37±1.29 Ca	65.00±1.83 Da	**
T2	86.75±1.35 Aa	78.75±1.44 Ba	70.75±1.58 Ca	63.62±2.67 Da	**
T3	83.00±1.99 Aab	77.87±1.69 Aab	71.00±2.02 Ba	62.87±2.36 Ca	**
Level of Significance	**	**	*	*	-----

Different superscripts within column are significantly different (P<0.01) (p<0.05)***

Studies have indicated that quercetin has the ability to reduce the formation of reactive oxygen species, which leads to a defect in the function of mitochondria **(Tvrda *et al*, 2016)**. Studies have also shown that quercetin has a significant effect in reducing the high levels of hydrogen peroxide H_2O_2, which are associated with high concentrations of reactive oxygen

species, and then reduce the damage caused to the integrity of the sperm's terminal particlesb **(Goss *et al,* 2016)**. The high concentration of hydrogen peroxide H_2O_2 is associated with the permeability of the sperm membrane, which contributes to damage to the structure of the sperm membranes, which contributes to damage to the structure of the sperm membranes. Therefore, quercetin contributed to reducing the levels of H_2O_2 and thus maintaining the integrity of the terminal particles **(Sahin *et al,* 2017)**.

CONCLUSIONS

1. The addition of quercetin at a concentration of 3 µL/mL to Tris extender improvement in some characteristics of cryopreserved semen in Awassi rams (the percentage of live sperm, integrity of the plasma membrane and acrosome integrity).
2. The addition of quercetin at a concentration 6 µL/mL to Tris extender improvement in some characteristics of cryopreserved semen in Awassi rams, namely (motility, viability of sperm, integrity of the plasma membrane and acrosome integrity).
3. The addition of quercetin at a concentration of 9 µL/mL resulted in an improvement in some of the cooled semen pages in Awassi rams, which are (motility, viability of sperm, integrity of the plasma membrane and acrosome integrity).
4. The addition of quercetin had no significant effect on the percentage of abnormalities in the semen of Awassi rams during cryopreservation.

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