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Effect of Adding Quercetin in Dimitropoulos Extender on the Sperm Characteristics and Biochemical Parameters of Arabian Stallion Semen stored at 5 °C

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Abstract

THE present study aimed to determine the effect of adding Quercetin in Dimitropoulos (DM) extender on the Sperm characteristics and biochemical parameters of equine semen at 5 °C. Three fertile Arabian stallions were used in this study continued for eight consecutive weeks (from August to September 2021). Semen collected twice weekly (n= 48) by Missouri artificial vagina was aliquoted and diluted with DM extenders containing different concentrations of Quercetin (0 µM (control), 25 µM, 50 µM, and 100 µM) to a final concentration of 25×10^6 sperm/ml. All groups were kept at 5 ºC and evaluated daily (every 12 hours) for three days for motility, livability, total abnormality, metabolic enzymes (ACP, ALP, AST), lactate dehydrogenase (LDH), acrosine activity, antioxidants (CAT, SOD, GPx), and lipid peroxidation (MDA). The results revealed that Quercetin at a concentration of 50 µM has an improving effect on sperm motility at 36 and 48 hrs post-chilling. In addition, it lowered extracellular ACP and enhanced catalase enzyme activities, and lowered MDA accumulation throughout the experiment. In conclusion, adding quercetin at a concentration of 50 µM has a potential protective effect on sperm motility and decreases oxidation-reduction during cold storage of stallion semen.

Keywords: Arabian Stallion, Chilled semen, Dimitropoulos, Quercetin, Spermiogram.

Introduction

The Arabian horse is one of the oldest and most influential breeds, bred for centuries for intelligence, endurance, and refined beauty. It developed an outstanding adaptation to the harsh desert climate of the Middle East and became invaluable for warfare, transport, and the preservation of culture. High concentration of polyunsaturated fatty acids in spermatozoa plasma membrane increases its susceptibility to oxidative stress and lipid peroxidation, which decreases sperm motility and livability, and damages DNA [1,2]. Moreover, semen dilution reduces the antioxidant concentration of seminal plasma [3]. Therefore, supplementation of extenders intended for semen preservation with antioxidants is extremely desirable [4].

Quercetin is a flavonoid antioxidant present in fruits and vegetables, capable of scavenging hydroxyl radicals and reactive species [5], and providing beneficial effects including antimicrobial, anticarcinogenic, and anti-inflammatory properties [6,7]. Quercetin is a potent antioxidant with more intense reactive oxygen species (ROS) scavenger activity than vitamin E or C due to the existence and location of the hydroxyl (OH) substitutions and the catechol-type B ring [8,9]. The beneficial effects of Quercetin on frozen-thawed semen have been reported in stallions [10], bulls [11], buffalo [12], rams [13], and bucks [14].

Many extenders have been developed for semen preserved by cooling over time [15]. The extender preserves sperm motility through its buffering capacity and optimal osmotic pressure [2]. Egg yolk in the extender protects spermatozoa against temperature fluctuation during cold storage [16]. The egg yolk in the extender reduces the sperm membrane lipid removal and improves sperm preservation through interacting with the L-binding

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proteins in the sperm plasma membrane via its low-density lipoprotein [16, 18].

To our knowledge, no reports are available about the effect of adding Quercetin on stallion semen stored at 5 °c. So, the present study aimed to investigate the effect of adding different concentrations of Quercetin in Dimitropoulos (egg yolk-based) extender on spermatozoa characteristics and biochemical properties of stallion chilled semen

Material and Methods

Animals

Three Arabian stallions of proven fertility, belonging to the Armed Forces Equestrian Cub, aged 4 - 8 years, body condition score 4-5, were used in this study. Stallions were kept individually in stalls (L×W, 5×3 meters) and fed Barseem (4 kg/head) or Drees (1 kg/head), and concentrates mix 9% protein (6 kg/head). Mineral salts and water were available *ad libitum*.

Dimitropoulos extender (DM extender) preparation [17]

Dimitropoulos extender (DM extender) composed of 3 parts; part A 30% (consisted of glucose 2.00 g, fructose 2.00 g, and milli-Q water 100 ml), part B 50% (consisted of sodium citrate 2.00 g, glycine 0.94 g, sulfanilamide 0.35 g, and milli-Q water 100 ml), and egg yolk 20%. On the day of use, the components of DM extender were mixed and centrifuged at 1200 ×g for 20 min. The supernatant was separated and supplemented with 1000 IU/ml Penicillin and 1000 µg/ml Streptomycin per milliliter.

Semen collection and chilling

Semen was collected twice weekly for eight weeks (n=48 ejaculates), during the period August to September 2021, using a pre-warmed (45–50 °C) stallion artificial vagina (Missouri model, Nasco, Ft. Atkinson, WI, USA) with a filter to separate the gel fraction of the ejaculates [18]. Semen was evaluated after the collection for motility, viability, morphology, and concentration. Each stallion semen sample was extended to a final concentration of 25×10^6 sperm/ml using Dimitropoulos extender implemented with Quercetin (Sigma-Aldrich, USA, Code 117-39-5) at 0 (control), 25, 50 and 100 µM concentrations based on a pilot study prior to the present study (unpublished data). Semen groups were stored at 5 ºC and evaluated twice daily (at 12 hours interval) for 72 hours.

Assessment of chilled semen quality

Before each evaluation, half ml of extended semen was maintained at 35 ºC for 10 minutes

Spermatozoa characteristics

Spermatozoa progressive motility was assessed using computer-assisted sperm analysis (CASA, CEROS II sperm analysis for animal semen, France, Ref. 024905) according to company instructions. Sperm livability was assessed by 5% eosin and four parts of 10% nigrosine aqueous solutions prepared according to Łącka et al. [19]. The total sperm abnormalities were evaluated in semen-stained film according to Dowsett et al. [20].

Assessment of sperm metabolic activity and lipid peroxidation

Extended semen samples were centrifuged for 5 minutes at 1800 ×g, and the supernatant was collected and analyzed for biochemical parameters which included Acid phosphatase (Catalog # AC1011, Randox, Spain), Alkaline phosphatase (Catalog # AP3802, Randox, Spain), Aspartate aminotransferase (Catalog # AS3804, Randox, Spain), and Lactate dehydrogenase L-P (LDH) (NAD) kit (Catalog # LD3842, Randox, Spain), Catalase (Catalog #K773-100, BioVision, USA). Superoxide dismutase (Catalog #K335-100, BioVision, USA), Glutathione peroxidase (Catalog #K762-100, BioVision, USA) (Cayman Chemical Co., Ann Arbor, MI, USA), and Malondialdehyde (Catalog # K739, BioVision, USA) spectrophotometrically (Jenway 6300, USA) using appropriate commercial kits. The N-α-benzoyl-DL-arginine-p-nitroanilide (BNPNA) method was used to assess the activity of sperm acrosome enzyme activity according to Cui et al. [21].

Statistical analysis

Using SPSS (Ver. 25), the data, tested for normal distribution with the Shapiro-Wilk test, were presented as the mean ± SE and analyzed using one-way analysis of variance (ANOVA) and Tukey post-hoc test. The statistical significance was set at $P < 0.05$.

Results

Changes in spermatozoa characters and biochemical parameters on the 1st day post-storage at chilling temperature

Table 1 showed that at 12 and 24 hrs (the 1st day post-storage), the extracellular acid phosphatase activity was significantly ($P< 0.05$ and 0.01) decreased at 50 µM Quercetin in DM extender when compared to the control. Catalase enzyme activity substantially ($P< 0.001$) increased in all Quercetin-supplemented groups. Quercetin at concentrations of 25 and 50 µM significantly ($P= 0.07$ and 0.05) decreased MDA compared to the control.

Changes in spermatozoa characters and biochemical parameters at the 2nd day post-storage at chilling temperature

At 36 and 48 hrs (the 2nd day post-storage), spermatozoa progressive motility at 50 µM Quercetin was markedly ($P= 0.04$ and 0.07) higher than at 100 µM Quercetin. Acid phosphatase activity

significantly (P= 0.01 and 0.009) decreased at 25 and 50 µM Quercetin compared to the control. All concentrations of Quercetin improved CAT (P< 0.001) activity after 36- and 48-hour post-chilling. Quercetin at concentrations of 50 µM at 36 hrs, and 25 and 50 µM significantly (P= 0.07 and 0.0001) decreased MDA compared to the control (Table 2).

Changes in spermatozoa characters and biochemical parameters at the 3rd day post-storage at chilling temperature

At 72 hrs (the 3rd day post-storage), spermatozoa progressive motility significantly improved (P< 0.05) with the concentration of 50 µM compared to 100 µM Quercetin. Acid phosphatase activity significantly (P= 0.013 and 0.008) decreased at 25 and 50 µM Quercetin compared to control after 60- and 72-hours post-chilling. Catalase enzyme activity substantially (P< 0.001) increased in all Quercetin groups compared to control at 60 and 72 hrs. Quercetin at concentration 25 and 50 µM at 60 hrs significantly (P< 0.05) decreased MDA compared to control and 100 µM Quercetin (Table 3).

Discussion

The cooled-shipped semen has been used routinely in horse breeding since the late 1980s [22]. However, during low-temperature storage, the stallion sperm motility, viability, and fertility are seriously affected [12, 23]. This is perhaps associated with oxidative stress and lipid peroxidation during cooling [1,2]. In the present study, Quercetin supplementation in extended stallion semen maintained a relatively high sperm progressive motility, reduced the extracellular acid phosphatase, increased catalase activity, and reduced lipid peroxidation during storage at low temperature.

In the present study, Quercetin at 50 µM in DM extender sustained high sperm motility in the 2nd day of storage, but viability and morphology changes did not reach statistical significance between all groups. The protective effect of Quercetin on sperm motility is due to its interactions with calcium-ATPase, an important enzyme that controls sperm motility [24]. The low intracellular Ca2+ levels are essential for maintaining sperm motility through their role in the synthesis of intracellular cyclic adenosine monophosphate (cAMP) [25]. The present results contrast with the previous findings of Seifi-Jamadi et al. [10], who recorded a positive stimulating effect of Quercetin at a concentration of 0.1 mM on frozen-thawed stallion sperm motility. In the same manner, Quercetin at a concentration of 0.25 mM significantly improved the frozen–thawed boar sperm progressive motility compared to the higher concentrations (0.50 and 0.75 mM) [26]. On the other hand, Quercetin improved the buffalo frozen-thawed sperm motility with the lower concentration (2.5-20 µM) while the higher concentrations (40 and 80 µM) adversely affected the sperm motility [12]. In the same respect, Quercetin at a concentration of 10 µg/ml the total sperm motility of frozen-thawed ram sperm, while the higher concentration (50 µg/ml) significantly decreased the motility [27]. The difference in the effect of concentrations may be attributed to the storage conditions of semen, which were chilling in the present study and freezing in the other studies.

Cold shock changes the normal configuration of sperm membrane and results in membrane damage due to ROS production [18, 28]. Biochemical analysis here reveals the reduction of sperm membrane damage as indicated by the low extracellular acid phosphatase, improvement in the oxidation-reduction potential by increasing the activity of catalase enzyme, and lowering of lipid peroxidation by reducing MDA levels. Quercetin has the structural components of an antioxidant through its hydroxylation pattern of 3, 5, 7, 30, and 40 and a catechol B-ring [13,29]. Mitochondrial status is associated with the sperm's energetic status and motility. Therefore, it plays an important role in sperm fertility [30]. A negative relationship has been reported between the level of ROS and the sperm mitochondrial membrane potential [31]. Through its antioxidant activity, Quercetin controls the production of ROS as it can enter cells, accumulate inside mitochondria, and prevent sperm mitochondrial dysfunction [32,33]. Moreover, Tvrdá et al. [11] reported that Quercetin with concentrations between 50 and 100 µM/L protected bull sperm motility and mitochondrial activity against injury brought by lipid peroxidation and ROS. In contrast to our result, El-Khawagah et al. [12] reported a significantly decreased level of leaked AST and ALT in the semen-free extender with a 10 µM Quercetin. Such differences might be species-based histophysiology variances of sperm plasma membrane (stallion in our study vs. bull [12], or Quercetin concentration 50 µM in our study vs. 10 µM [12].

Conclusion

Dimitropoulos extender supplemented with Quercetin at a concentration of 50 µM is potentially a perfect combination to preserve stallion sperm and improve antioxidant defenses during chilling. In addition, further assessments including DNA integrity, ultrastructure changes and ROS levels are required to provide deep understanding of the role of quercetin on chilled stallion sperm. Additional studies also are required to verify the relation between improved semen quality parameters and actual reproductive performance e.g., fertility potential and/or conception rate of equine chilled sperm.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

In accordance with the ARRIVE guidelines and relevant national regulations for the care and use of animals in research, the experiments of the present study were conducted after approval by the Animal Welfare and Ethics Committees of Benha University and had an ethical approval number (BUFVTM 02-02–23).

TABLE 1. Effect of Quercetin incorporation in Dimitropoulos extender on the quality of stallion chilled semen on the 1st day post-storage (n= 16 replicates)

Time post-storage	Item	Abbrev.	Unit	Control	Q 25 µM	Q 50 µM	Q 100 µM	P value
12 hrs	**Sperm progressive motility**		%	72.03±1.79	72.73±1.76	72.57±3.93	68.33±1.73	0.58
	Sperm livability		%	76.50±5.03	72.33±4.91	81.33±3.18	74.33±3.84	0.53
	Sperm total abnormalities		%	15.83±2.03	14.67±2.19	12.67±2.33	16.00±1.15	0.63
	Acid phosphatase	ACP	U/L	28.22±.81a	25.60±.61ab	25.33±.64b	26.50±.44ab	0.047
	Alkaline phosphatase	ALP	U/L	16.86±0.80	16.85±0.80	16.89±0.84	17.40±0.71	0.94
	Aspartate aminotransferase	AST	U/L	202.60±0.23	188.47±8.01	188.07±8.01	203.57±10.87	0.37
	Acrosine	Acr.	U/L	285.33±9.53	274.67±5.36	269.33±6.69	268.00±5.77	0.35
	Lactate dehydrogenase	LDH	U/L	333.33±4.33	330.67±5.21	330.67±6.49	337.33±5.04	0.79
	Catalase	Cat	U/L	102.10±0.23b	188.47±8.01a	188.07±8.01a	203.57±10.87a	0.001
	Superoxide dismutase	SOD	U/L	105.20±2.81	104.47±2.56	109.83±1.89	103.57±2.64	0.34
	Glutathione peroxidase	GPx	U/L	329.00±5.20	332.33±4.33	329.67±4.33	337.67±4.91	0.57
	Malondialdehyde level	MDA	nmol/mg	48.17±0.81a	45.60±0.61b	45.33±0.64b	46.50±0.44ab	0.07
24 hrs	**Sperm progressive motility**		%	59.43±4.38	60.40±4.54	62.50±4.41	55.97±4.19	0.77
	Sperm livability		%	71.50±5.03	74.00±5.03	79.00±3.51	72.00±5.13	0.68
	Sperm total abnormalities		%	18.17±3.18	15.67±2.73	13.67±2.73	17.67±2.33	0.66
	Acid phosphatase	ACP	U/L	30.72±1.56	27.00±.59	26.90±.67	28.17±.75	0.078
	Alkaline phosphatase	ALP	U/L	16.87±0.79	16.86±0.79	16.94±0.87	17.43±0.70	0.94
	Aspartate aminotransferase	AST	U/L	204.13±0.35	199.57±9.44	199.17±9.39	215.27±8.60	0.48
	Acrosine	Acr.	U/L	265.67±2.91ab	272.33±2.96a	262.67±1.20b	269.00±2.08ab	0.09
	Lactate dehydrogenase	LDH	U/L	337.00±4.36	335.67±5.46	333.67±5.46	343.33±4.18	0.56
	Catalase	Cat	U/L	103.63±0.35b	199.57±9.44a	199.17±9.39a	215.27±8.60a	0.001
	Superoxide dismutase	SOD	U/L	105.67±2.80	105.37±3.11	108.47±1.76	104.80±2.80	0.76
	Glutathione peroxidase	GPx	U/L	333.00±4.93	336.00±4.36	332.00±4.36	341.67±4.26	0.46
	Malondialdehyde level	MDA	nmol/mg	47.00±0.59	48.17±0.75	44.00±2.24	46.90±0.67	0.16

Values with different letters at the same row were significantly different

TABLE 2. Effect of Quercetin incorporation in Dimitropoulos extender on the quality of stallion chilled semen on the 2nd day post-storage (n= 16 replicates)

Time post-storage	Item	Abbrev.	Unit	Control	Q 25 µM	Q 50 µM	Q 100 µM	P value
36 hrs	**Sperm progressive motility**		%	52.80±1.29ab	53.33±1.17ab	55.80±1.35a	49.33±1.18b	0.04
	Sperm livability		%	62.50±7.02	67.67±6.36	71.67±6.36	62.67±6.36	0.72
	Sperm total abnormalities		%	18.83±2.33	16.67±2.73	14.67±2.91	18.67±2.91	0.68
	Acid phosphatase	ACP	U/L	32.85±1.37a	28.57±.42b	28.40±.40b	30.67±.78ab	0.017
	Alkaline phosphatase	ALP	U/L	16.88±0.79	16.87±0.79	16.95±0.87	17.45±0.70	0.94
	Aspartate aminotransferase	AST	U/L	205.57±0.30	205.13±11.29	204.47±11.18	221.90±8.26	0.48
	Acrosine	Acr.	U/L	263.67±2.91ab	270.00±2.52a	260.33±1.45b	266.33±1.86ab	0.07
	Lactate dehydrogenase	LDH	U/L	346.67±2.96	344.00±4.93	342.33±4.98	348.67±5.55	0.78
	Catalase	Cat	U/L	105.07±0.30b	205.13±11.29a	204.47±11.18a	221.90±8.26a	0.001
	Superoxide dismutase	SOD	U/L	106.80±2.15	106.67±2.10	109.47±2.80	105.03±2.72	0.65
	Glutathione peroxidase	GPx	U/L	338.67±5.81	345.67±2.96	343.33±3.28	352.00±3.51	0.21
	Malondialdehyde level	MDA	nmol/mg	48.57±0.42a	44.00±2.91ab	42.80±1.37b	48.40±0.40a	0.07
48 hrs	**Sperm progressive motility**		%	44.90±1.46ab	44.13±0.46ab	47.53±1.55a	41.47±1.49b	0.07
	Sperm livability		%	57.83±6.77	65.67±6.33	68.33±6.23	59.33±6.23	0.62
	Sperm total abnormalities		%	23.17±3.48	18.00±3.06	16.00±2.65	22.33±4.10	0.42
	Acid phosphatase	ACP	U/L	33.75±1.46a	29.20±.32b	29.10±.30b	30.63±.24ab	0.009
	Alkaline phosphatase	ALP	U/L	16.89±0.79	16.88±0.79	16.95±0.87	17.45±0.69	0.94
	Aspartate aminotransferase	AST	U/L	205.33±0.32	203.93±10.84	203.40±10.91	219.20±8.70	0.56
	Acrosine	Acr.	U/L	262.00±2.89	267.00±2.52	257.33±1.86	263.67±2.19	0.11
	Lactate dehydrogenase	LDH	U/L	348.67±5.46	349.67±5.84	348.00±6.03	355.33±3.71	0.76
	Catalase	Cat	U/L	104.83±0.32b	203.93±10.84a	203.40±10.91a	219.20±8.70a	0.001
	Superoxide dismutase	SOD	U/L	107.07±2.19	107.00±1.99	111.03±2.57	106.03±2.02	0.42
	Glutathione peroxidase	GPx	U/L	342.33±5.21	347.67±5.46	345.67±5.36	354.00±4.04	0.46
	Malondialdehyde level	MDA	nmol/mg	49.20±0.32a	40.63±0.24b	43.70±1.46b	49.10±0.30a	0.00001

TABLE 3. Effect of Quercetin incorporation in Dimitropoulos extender on the quality of stallion chilled semen on the 3rd day post-storage (n= 16 replicates)

Time post-storage	Item	Abbrev.	Unit	Control	Q 25 µM	Q 50 µM	Q 100 µM	P value
60 hrs	**Sperm progressive motility**		%	42.73±8.16	36.37±1.68	38.97±1.56	31.27±0.74	0.35
	Sperm livability		%	55.17±6.89	61.67±5.90	66.67±5.84	57.00±6.66	0.60
	Sperm total abnormalities		%	24.50±4.04	19.33±3.38	16.67±3.28	23.67±4.06	0.44
	Acid phosphatase	ACP	U/L	33.52±1.42a	28.97±.32b	28.87±.43b	31.03±.71ab	0.013
	Alkaline phosphatase	ALP	U/L	16.89±0.79	16.87±0.79	16.95±0.87	17.45±0.69	0.94
	Aspartate aminotransferase	AST	U/L	204.97±0.27	202.80±10.55	202.17±10.72	218.50±8.91	0.54
	Acrosine	Acr.	U/L	260.00±2.89	264.00±3.06	254.00±2.52	261.33±2.40	0.14
	Lactate dehydrogenase	LDH	U/L	348.00±4.36	344.67±3.84	342.67±3.84	352.67±3.33	0.34
	Catalase	Cat	U/L	104.47±0.27b	202.80±10.55a	202.17±10.72a	218.50±8.91a	0.001
	Superoxide dismutase	SOD	U/L	107.40±2.27	107.37±2.40	110.30±2.51	105.70±2.19	0.59
	Glutathione peroxidase	GPx	U/L	340.33±5.21	347.00±4.36	343.67±5.04	353.33±5.24	0.35
	Malondialdehyde level	MDA	nmol/mg	48.97±0.32a	44.37±2.68b	43.47±1.42b	48.87±0.43a	0.05
72 hrs	**Sperm progressive motility**		%	26.17±1.23ab	26.27±1.24ab	28.80±1.21a	22.77±1.28b	0.05
	Sperm livability		%	53.17±6.84	60.33±6.17	63.67±4.84	54.00±6.00	0.57
	Sperm total abnormalities		%	26.50±4.62	21.33±4.37	17.33±3.53	25.33±5.24	0.50
	Acid phosphatase	ACP	U/L	33.25±1.42a	28.77±.32b	28.47±.32b	30.00±.40ab	0.008
	Alkaline phosphatase	ALP	U/L	16.88±0.79	16.87±0.79	16.94±0.87	17.44±0.69	0.94
	Aspartate aminotransferase	AST	U/L	204.43±0.34	201.60±10.95	201.23±10.93	217.73±8.88	0.54
	Acrosine	Acr.	U/L	257.67±2.60	262.00±3.06	251.67±2.73	258.00±3.06	0.16
	Lactate dehydrogenase	LDH	U/L	345.00±5.86	343.67±5.46	341.00±5.57	350.33±3.67	0.65
	Catalase	Cat	U/L	103.93±0.34b	201.60±10.95a	201.23±10.93a	217.73±8.88a	0.001
	Superoxide dismutase	SOD	U/L	107.37±2.13	106.93±2.12	111.00±2.51	105.70±2.33	0.43
	Glutathione peroxidase	GPx	U/L	338.33±5.21	344.00±5.86	340.67±5.81	351.33±6.69	0.47
	Malondialdehyde level	MDA	nmol/mg	47.43±1.35	43.33±2.99	43.20±1.42	48.47±0.32	0.14

Values with different letters at the same row were significantly different

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تأثير الكيرسيتين فى مخفف داى ديميتروبولوس على خصائص السائل المنوى والمعايير الكيموحيوية للسائل المنوي المبرد للخيول العربية

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الملخص

هدفت هذه الدراسة إلى تحديد تأثير الكيرسيتين في مخفف ديميتروبولوس على خصائص السائل المنوي والخصائص الكيميائية الحيوية للسائل المنوي المُبرَّد للخيول. استُخدمت ثلاثة فحول عربية خصبة في هذه الدراسة واستمرت لمدة ثمانية أسابيع متتالية. جُمِعَ السائل المنوي مرتين أسبوعيًا (عدد الجمعات 48) بواسطة المهبل الاصطناعي، ثم خُفِّفَ باستخدام مخفف ديميتروبولوس تحتوي على تركيزات مختلفة من الكيرسيتين (0 ميكرومولار (مجموعة ضابطة)، 25 ميكرومولار، 50 ميكرومولار، و100 ميكرومولار) حتى وصل التركيز النهائي إلى 25 مليون حيوان منوي لكل مللى. تم حفظ جميع المجموعات عند درجة حرارة 5 درجات مئوية و تقييمها يوميًا (كل 12 ساعة) لمدة ثلاثة أيام من حيث الحركة والحيوية وانسبة التشوهات الكلية. هذا بالاضافة الى انزيمات الأيضي (**ACP** و**ALP** و**AST**) وديهيدروجين اللاكتات (**LDH**) ونشاط الأكروسين ومضادات الأكسدة (**CAT** و**SOD** و**GPx**) ومالوندهيد (**MDA**). أظهرت النتائج أن الكيرسيتين بتركيز 50 ميكرومولار له تأثير محسن على حركة الحيوانات المنوية بعد 36 و48 ساعة من التبريد. بالإضافة إلى ذلك، فقد خفض انزيم الفوسفاتيز الحمضى خارج الخلية وعزز أنشطة إنزيم الكاتاليز، وخفض تراكم مالوندهيد طوال التجربة. من هنا نستخلص ان السائل المنوي للخيول المخفف بالديميتروبولوس المضاف إليه الكيرسيتين بتركيز 50 ميكرومولار له تأثير وقائي يحافظ على حركة الحيامن وإمكانية الأكسدة والاختزال أثناء التخزين البارد للسائل المنوي.

الكلمات الدالة: حصان عربي، سائل منوي مبرد، ديميتروبولوس، كيرسيتين، تحليل السائل المنوي.