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Preservative Impact of Curcumin on Sperm Variables and Seminal Plasma Antioxidant Activity in Summer and Winter Goat Semen after Cryopreservation

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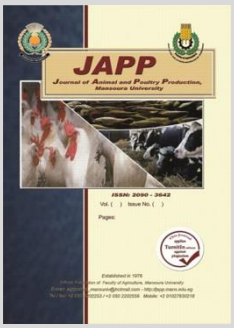

ABSTRACT

During semen cryopreservation, oxidative stress (OS) leads to marked reduction in structure and function of sperm cells. Curcumin (CM) acts as antioxidant for scavenging free radical induced by OS during cryopreservation. The current work aimed to evaluate the CM impacts on sperm variables of cryopreserved goat semen collected in summer and winter season in Egypt. Semen of five Damascus goat bucks was pooled, diluted (1:20), equilibrated (5ºC for 2h), divided into three aliquots supplemented with CM at 0, 10, and 20 µg/ml in Tris extender, representing E0, E10, and E20, respectively. Post-thawed semen was incubated and evaluated at 0, 1, 2, 5, and 7h post-incubation at 37ºC. Results showed that all sperm variables were significantly better in winter than in summer. Supplementation of CM (20 µg/ml) resulted in beneficial effects on maintaining sperm progressive motility, vitality, acrosome integrity of summer and winter goat post-thawed semen incubated for 7h. Both CM levels showed positive impact on increasing total antioxidant capacity and reducing malondialdehyde level in sperm medium of post-thawed summer and winter season. The action of CM on improving sperm variables, lipid peroxidation, and antioxidant status was observed to be more obvious on summer than on winter semen.In conclusion, adding curcumin to goat semen extenders can facilitate and enhance semen processes and storage protocols to improve fertility of cryopreserved goat spermatozoa in semen collected in summer or winter season.

Keywords: Goat; semen, *Curcuma longa*; cryodamage; sperm variables, incubation, antioxidative action.

INTRODUCTION

Hot environmental temperature leads to heat stress (HS) which is an important reason for animal sub-fertility (Hansen, 2009). Seasonal variation in semen quality of bovine with conflicted results have been studied (Malama et al., 2017; Sabes-Alsina et al., 2017). In Egypt, several studies have highlighted the negative impacts of high temperature in summer on bovine semen quality, reporting negative effects on semen quality in comparing with cold temperature in winter. In mammalians, exposure to HS has been disrupts spermatogenesis, reduces sperm motility, count of live spermatozoa, and testosterone levels (Alves et al., 2016).

Spermatozoa in mammals are highly sensitive to OS because their antioxidant defenses system is weak. OS causes sperm damage due to polyunsaturated fatty acids in sperm membrane that readily undergo peroxidation, alter redox signaling (Agarwal *et al*., 2014; Mostek *et al*., 2017). In normal semen (Kapadiya *et al*, 2018) and reproductive fluids of males (Bansal and Bilaspuri 2011), different antioxidant enzymes are present for the protection of spermatozoa from reactive oxygen species (ROS). Nevertheless, the antioxidant imbalance by increasing OS causes dysfunction of sperm cells via lipid peroxidation and this system is prone to fail to prevent OS in post-thawed semen (Bansal and Bilaspuri, 2011; Bucak *et al.* 2010, 2012).

Semen cryopreservation causes reduced sperm motility and livability and alters the acrosome and membrane integrities of sperm cells (Royere *et al*., 1996). Cold shock, formation of ice crystals, osmotic change, and OS are the probable mechanisms of these alterations and damages (Thomson *et al*., 2009; Lu *et al*., 2018). The generation of ROS is detrimental to sperm function, leading to a reduction in sperm characteristics (Bilodeau *et al*., 2000). Increasing OS leads to high ROS production, causing negative impacts on the membrane of mitochondria and plasma membrane with lipid peroxidation, impairing chromatin condensation and DNA integrity, then sperm motility and livability were reduced (Taylor *et al*., 2009).

Many studies reported that addition of different levels of antioxidants in semen extender improved quality and fertility of cryopreserved semen (Amidi *et al*., 2016; Mustapha *et al*, 2017; Yadav *et al*, 2018; Kaur *et al*, 2020; Virmani *et al*, 2020;). In various species, enrichment of cryopreserved semen extenders with curcumin improved sperm quality and fertility of semen in bovine bull (Bucak *et al*, 2012; Tvrda *et al*, 2018), buffalo bull (Shah *et al*, 2016), Angora buck (Bucak *et al*, 2010), and rabbi buck (Abdelnour *et al*., 2020) semen.

Curcumin (CM) is a bioactive compound and phyto-polyphenol pigment isolated from the plant Curcuma longa L., Zingiberaceae family known as turmeric. It is the main active ingredient in turmeric having antioxidant properties beside several pharmacological actions against inflammation, bacteria, viruses, fungi, and cancer (Thomson *et al*., 2009). The free radical scavenging activity of CM increases eight folds more than that of vitamin E (Jayaprakasha *et al*., 2006). The CM has strong free radical

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scavenging activity to prevent lipid peroxidation by giving hydrogen atoms to lipid peroxyl radicals from the b-diketone moiety in its structure (Slobodan *et al*., 2001) and able to protect sperm cells from thermal shock and oxidative damage during freezing process (Bucak *et al*., 2010). The CM is a strong ROS scavenger and an effective inhibitor of lipid peroxidation and has important role in male fertility, in vivo (Salahshoor *et al*., 2012) and in vitro (Bucak *et al*. 2012; Soleimanzadeh and Saberivand 2013; Tvrda *et al*. 2016). The CM promotes the activity of antioxidant enzymes such as glutathione peroxidase (GPx), catalase, and super oxide dismutase (SOD) that neutralize free radicals, and inhibits enzymes like lipoxygenase, cyclooxygenase, xanthine oxidase that generate ROS (Lin et aal., 2000).

Determining frozen semen quality 3 h after thawing can help to understand the quality and survival of sperm cells after artificial insemination (Llamas-Luceño *et al*., 2019). Furthermore, to our knowledge, no studies have been published on the influence of antioxidant supplementation to extender of goat semen collected in different seasons.

The current field of study is addition of antioxidant substances to the cryopreserved semen to minimize sperm cell damage and maximize sperm quality. The effects of cryopreservation are in association with semen quality. Based on previous reports about the possible impacts of CM on spermatozoa, we proposed the supplementation of the extender with curcumin may improve the quality and fertility of goat semen after cryopreservation to overcome the reported reduction in sperm characteristics undergoing challenges and complications of the summer season. In addition, we evaluated the impact of curcumin in the extender to improve sperm variables in winter goat semen post-thawing.

MATERIALS AND METHODS

This study was conducted at Animal Production Research Institute (APRI), Agriculture Research Center (ARC) in cooperation with Animal Production Department, Faculty of Agriculture, Minia University, Egypt. This study was approved by the Scientific Research Ethics Committee of APRI in accordance with Animal Research: Reporting of In-vivo Experimental guidelines.

Semen donors:

Total of 5 adult bucks (Damascus goats) having 50-60 kg LBW and 30–36-month-old kept at Sakha Experimental Station, Kafrelsheikh governorate, belonging to APRI were used in this experiment, as semen donors. Bucks were reared at the same environmental, feeding system, and managerial conditions. They were fed a diet including 40% concentrate feed mixture (14.5% CP) and 60% roughage, being fresh berseem in winter and berseem hay in summer. Trace-mineralized salt lick blocks and fresh drinking water were available at all times.

Collection of the semen:

Fresh semen:

Immediately after the collection of semen, individual fresh samples during each season were evaluated for ejaculate volume, sperm concentration (hemocytometer), progressive motility, vitality, abnormality, acrosome integrity, and then sperm output/ejaculate, as motile (MSOP), live (LSOP), normal (NSOP), and total (TSOP) was calculated as the following:

TSOP/ejaculate = Ejaculate volume x sperm concentration
MSOP/ejaculate = TSOP x progressive motility percentage
LSOP/ejaculate = TSOP x live sperm percentage
NSOP/ejaculate = TSOP x (abnormality percentage-100)

Semen cryopreservation:

On day of the semen collection, semen was taken from all semen donors by using a conventional artificial vagina for semen ejaculation from goat bucks. Semen was collected early morning before feeding (7-8 a.m.) twice/week for 4 successive weeks (40 ejaculates) per season, during December month in winter and august month in summer season. Semen was individually collected and maintained at 37ºC in a water bath, then transported to the laboratory. In the laboratory, semen of all donors was pooled, diluted, equilibrated, frozen, thawed, and evaluated.

Freezing processes:

The pooled semen of all donor's bucks was diluted at a ratio of 1: 20 (semen/extender) by Tris-citric-egg yolk extender (TCEY). In this experiment, TCEY extender contained 30.28 g Tris, 16.75 g citric acid monohydrate, and 12.5 g glucose per liter plus 15% fresh egg yolk, 6% glycerol, and antibiotics (100 µg penicillin/ml) and 100 µg streptomycin /ml, Sigma, Chemical Co., St. Louis, MO, USA). The extender was gently shaken, filtered, and warmed up to 37°C in a water bath.

Osmolarity level of TCEY was assessed at 280-300 mOsmol/l using an osmometer (Micro-Osmometer, Loser Type 6; Loser Messtechnik, Berlin, Germany). Also, pH value of TCEY extender was assessed by pH meter (pH/mV Temperature Meter, Jenway 3510; Jenway, Staffordshire, UK) for value of 6.9-7. As designed in this study, TCEY was divided into 3 types of extenders (E0, E10, and E20) supplemented with curcumin (Nano Gate Company, Nasr City, 11765, Cairo, Egypt) at levels of 0, 10, and 20 µg/ml before dilution, respectively.

After dilution, the semen extended by TCEY supplemented with different curcumin levels was shaken gently, equilibrated at 5ºC for 2 h, loaded into French straws (0.25 mL), exposed over 4 cm of liquid nitrogen, and then deeply dropped in liquid nitrogen for 2 wk.

Evaluation of the semen:

The cryopreserved semen was thawed at a temperature of 37ºC in water bath for 30 seconds and evaluated for progressive motility, vitality, abnormality and acrosome integrity. All evaluations were performed by a professional investigator. Semen was evaluated immediately after thawing (0h) and 1, 2, 5, 7h post incubation at 37ºC and 5% CO2. All evaluations were conducted in 5 microscopic fields.

Progressive motility:

The percentage of progressive motility was measured by a phase-contrast microscope (DM 500; Leica, Switzerland), adjusted with warmed stage, in fresh semen and thawed semen samples (10 µL aliquot) on a warm slide (37°C). Count of sperm cells showing forward movement in a long semiarch pattern relative to about 200 sperm cells, then percentage of progressive motility was calculated.

Sperm vitality:

Both vitality and abnormality were conducted (Moskovtsev and Librach, 2013). Semen samples were mixed with 5% eosin and 10% nigrosine, then smear of the mixture was made on a glass slide, and examined in 5 microscopic fields (Leica DM 500; Leica Mikrosysteme

Vertrieb GmbH, Wetzlar, Germany) at magnification ×400. Stained and unstained sperm cells were counted as dead and live cells, respectively, then vitality percentage was calculated relative to the total sperm count.

Morphological abnormal forms of spermatozoa:

On the slides of sperm vitality, we counted all morphological abnormalities in head, middle piece, and main piece as well as cytoplasmic droplets according to Menon *et al*. (2011), then total sperm abnormality percentage was calculated.

Acrosome integrity:

Semen samples were smeared on a pre-warmed slide (37°C), dried, fixed for 15 min (10% buffered formal saline), washed by tape water, air dried, and stained by Giemsa (3.8 g Giemsa, 375 ml of 100% methanol, and 125 ml glycerol), and examined by a light microscope at 1000x (oil immersion lens). Count of spermatozoa with intact and attached acrosome was determined relative to total sperm count/field, then acrosome integrity was calculated.

Total antioxidant and enzyme activities in post-thawed sperm medium:

Post-thawed sperm medium was obtained by centrifugation of post-thawed semen incubated for 2h. The level of total antioxidant capacity (Koracevic *et al*., 2001) and malondialdehyde (Ohkawa *et al*., 1979) were assayed in post-thawed sperm medium. The activity of aspartate (AST) and alanine (ALT) transaminase was also determined sperm medium (Reitman and Frankel, 1957). A spectrophotometer (Spectro UV-Vis Auto, UV-2602; Labomed, Los Angeles, CA, USA) and commercial kits (Biodiagnostic, Giza, Egypt) were used to achieve All assays according to the manufacturer's instructions.

Statistical analysis:

The homogeneity of variance of the obtained data were checked by Levene's test, while the normal distribution was tested by the Shapiro-Wilk test. The effect of supplements (1–3) was analyzed by one-way ANOVA using SAS (2008). The significant effect of supplementation was tested at $P < 0.05$ by Tukey's test of SAS program.

RESULTS AND DISCUSSION

Results

Effect of season on physical characteristics of goat semen:

Results in Table (1) revealed that all physical semen characteristics were significantly (P<0.05) higher in winter than in summer season. In comparable with this trend in Damascus goats, Abdullah *et al*. (2019) found insignificant differences between ejaculate volume in summer and winter. The observed seasonal changes in ejaculate volume may be in relation with pronounced changes in environmental air temperature, relative humidity, and lighting period (day length) in each season.

The accessory sex glands are the main source of the seminal plasma (Hafez, 1986), and level of testosterone responsible for its secretion (Abdel-Khalek *et al*., 2000). A negative relationship was reported between semen volume of Korean goat bucks and day length, being higher with short day length (winter) than long day in summer (0.9 vs 0.5 ml).

Table 1. Physical semen characteristics and sperm output of goat bucks in winter and summer.

Parameter	Season		SEM	P-value
	Winter	Summer		
Physical semen characteristics:				
Semen volume (ml)	0.910	0.570	0.582	0.009**
Progressive motility (%)	79.31	58.42	2.718	0.005*
Sperm vitality (%)	84.60	54.12	3.421	0.004**
Sperm abnormality (%)	12.92	23.45	0.884	0.000***
Intact acrosome (%)	83.31	74.42	2.928	0.095
Sperm count ($x10^9$/ml)	2.660	2.210	0.172	0.042*
Sperm count ($x10^9$)/ejaculate:				
Total	2.4206	1.2597	1.542	0.000 ***
Motile	1.9198	0.7359	1.614	0.000 ***
Live	2.0478	0.6817	1.547	0.000 ***
Normal	1.8881	0.9826	1.415	0.000***

*** Significant season differences at P<0.05. ** Significant season differences at P<0.01.*** Significant season differences at P<0.001.**

Sperm variables including progressive motility, vitality, abnormality, and sperm count in buck semen significantly (P<0.05) increased in winter than in summer season (Table 1). Abdullah *et al*. (2019) found that progressive motility, vitality, and intact acrosome were significantly (P<0.05) higher in winter than summer, while no differences were found in abnormality and count of spermatozoa. In contrast to our results, sperm abnormality percentage in Damascus semen were significantly higher in winter than in summer (El-Wishy *et al*., 1971). Several authors (Moussa, 1987; El-Saidy, 1988) agreed with the present results that sperm count was the lowest in summer season.

Abdullah *et al*. (2019) found that all sperm counts/ejaculate (total, motile, live and normal) were the lowest in summer season. This result is in association with increasing semen volume, sperm count/ml, and percentage of sperm variables. The rate of spermatogenesis is in relation with testis volume, seminiferous tubule diameter (Abdel-Khalek *et al*., 2000), and may in association with the impact of testosterone on sperm production (Massoud *et al*., 1991).

Effect of curcumin on sperm variables in incubated post-thawed goat semen:

Progressive motility:

The percentage of sperm progressive motility in summer and winter post-thawed goat semen incubated for 7h is shown in Table 2.

Results of summer semen revealed that CM in E10 and E20 maintained the percentage of sperm progressive motility to be significantly (P<0.05) higher than in E0 up to 2h after incubation. However, CM in E20 significantly (P<0.05) maintained sperm motility percentage up to 7h after incubation as compared to E0 and E10 (Table 2).

Results of winter semen showed significant (P<0.05) improvement of sperm motility percentage by E20 as compared to E0 and E10 at all incubation times (Table 2).

It is well known that a proper sperm motility is important for the ability of sperm cells to transport the

genital tract through the cervix, and to penetrate cumulus cells and zona pellucida of the oocytes. Our results indicated beneficial effects of CM at a level of 20µg/ml on maintaining sperm progressive motility of summer and winter goat post-thawed semen incubated for 7h. In this context, Bucak *et al.* (2010) found that CM (2.5 mM) increased progressive sperm motility of in Angora bucks. In bulls, Chaudhary *et al.* (2022) found that advantages of CM (25 and 50 µM) on progressive sperm motility in post-thawed semen compared with control or CM at a level of 75 µM. The CM at a level of 10 mM (Karakus *et al*., 2021) or 20 mM (Santonastaso *et al*., 2021) in cryopreserved human sperm medium was reported to prevent the decrease of sperm motility in post thawed semen. Tvrdá *et al*. (2018) reported maintaining the sperm motility significantly by CM after cryopreservation of bovine semen. In rats, similar trend was observed in sperm motility following feeding protein-deficient diets (Ahmed-Farid *et al*., 2017). Also, Salahshoor *et al*. (2012) reported that CM in extender of mic semen improved dose-dependently sperm motility.

Table 2. Effect of CM in extender on sperm progressive motility (%) in winter and summer post-thawed goat semen incubated for different times.

Item	Sperm progressive motility (%)			SEM	P-value
	E0	E10	E20		
		Summer season:			
0 h	52.42	55.14	57.74	2.74	0.091
1h	42.15^{b}	55.34^{a}	56.26^{a}	2.23	0.004
2h	32.44^{c}	42.50^{b}	56.08^{a}	3.16	0.010
5h	22.33^{b}	30.41^{b}	44.84^{a}	3.45	0.047
7h	14.46^{b}	17.50^{b}	24.22^{a}	1.93	0.050
		Winter season:			
0 h	70.33	78.74	76.21	2.47	0.058
1h	65.15^{b}	68.23^{ab}	76.54^{a}	3.89	0.042
2h	60.14^{b}	62.33^{ab}	66.45^{a}	2.09	0.044
5h	34.22^{b}	36.44^{b}	54.55^{a}	4.46	0.039
7h	20.74^{b}	23.61^{ab}	30.29^{a}	2.99	0.048

a, b,c: Significant differences for means in the same row at P<0.05.

Improving progressive motility in post-thawed buck semen may be due to the ability of CM to inhibit protein kinase C. This enzyme plays essential role to modulate the sperm flagellar motion (Reddy and Aggarwal, 1994) and this action is in association with possible detrimental effects of CM on the sperm movement (Rithaporn *et al.* 2003).

It clarifies that lower levels of CM may protect and augment the activity of male sex cells, whereas increasing its level may impair sperm function. Several in vivo toxicological reports suggested that motility and functions of sperm cells were protected by CM against the impaired effects of toxic agents (Dev *et al*., 2013). Despite of this finding, Naz (2014) observed negative impact of CM (≥50 mM) on sperm motility via a reduction in intracellular pH value, leading to membrane hyperpolarization during co-incubation of sperm cell of human or mouse. In another study of Naz (2011), CM at 125 µmol/l CUR negatively affects sperm motility, and CM (250 µmol/l) suppressed motility of sperm cells.

Sperm vitality:

Effect of CM on sperm vitality percentage in summer and winter post-thawed goat semen incubated for 7h is presented in Table 3.

Tris-extender supplemented with CM in E10 and E20 showed significantly (P<0.05) increased sperm vitality percentage in summer semen compared with E0, being higher (P<0.05) in E20 at all incubation times. However, the significant (P<0.05) impact of CM levels in E10 and E20 as compared to E0 appeared only after 7h of incubation of winter semen (Table 3).

These results indicated positive impacts of both CM levels on sperm survival in summer post-thawed semen, while lower impacts were exhibited on sperm survival in winter post-thawed semen incubated for 7h.

Table 3. Effect of CM in extender on sperm vitality (%) in winter and summer post-thawed goat semen incubated for different times.

Item	Sperm vitality (%)			SEM	P-value
	E0	E10	E20		
		Summer season:			
0h	53.72	57.15	58.18	2.53	0.0641
1h	49.06^{c}	51.32^{b}	62.27^{a}	2.31	0.0412
2h	44.16^{c}	50.20^{b}	60.05^{a}	2.08	0.0487
5h	35.88^{c}	39.60^{b}	49.35^{a}	1.83	0.0381
7h	24.03^{c}	32.54^{b}	43.20^{a}	1.60	0.0071
		Winter season:			
0h	70.24	74.18	78.71	5.26	0.109
1h	64.15	68.27	72.36	4.80	0.089
2h	57.74	66.05	69.63	4.32	0.094
5h	50.84	59.35	61.31	3.81	0.088
7h	44.5^{b}	53.2^{a}	53.67^{a}	2.33	0.042

a, b,c: Significant differences for means in the same row at P<0.05.

In other reports, CM supplemented to Tris-yolk fructose extender improved sperm viability in cryopreserved bovine semen (Pinto *et al*., 2020; Zaki *et al*., 2020). Also, live sperm count was increased by CM semen of rats fed diet with deficient CP (Ahmed-Farid *et al*., 2017). In cryopreserved rabbit semen extended with Tris-extender supplemented with CM, sperm vitality percentage was improved as compared to control (Abdelnour *et al*., 2020).

Sperm abnormality:

The percentage of sperm abnormality in summer and winter post-thawed goat semen incubated for 7h is illustrated in Table 4.

Percentage of sperm abnormality was significantly (P<0.05) lower in Tris-extender supplemented with CM in E20 than in E0 and E10 after 2, 5, and 7h of incubation of

post-thawed summer semen. Both CM levels (E10 and E20) significantly (P<0.05) improved sperm abnormality percentage as compared to E0 only 5h after incubation of post thawed winter semen (Table 4).

Table 4. Effect of CM in extender on sperm abnormality (%) in winter and summer post-thawed goat semen incubated for different times.

Item	Sperm abnormality (%)			SEM	P-value
	E0	E10	E20		
		Summer season:			
0h	25.42	21.78	20.73	2.92	0.241
1h	24.99	20.24	21.25	2.66	0.094
2h	29.72^{a}	24.15^{ab}	23.26^{b}	1.32	0.038
5h	30.52^{a}	27.21^{ab}	25.35^{b}	1.98	0.032
7h	34.38^{a}	30.42^{ab}	29.79^{b}	2.56	0.043
		Winter season:			
0h	12.03	13.63	15.73	2.34	0.174
1h	12.81	11.67	11.52	1.49	0.248
2h	16.14	14.29	13.25	2.99	0.189
5h	21.66^{a}	17.48^{b}	17.26^{b}	1.13	0.048
7h	24.14	20.76	20.35	2.06	0.124

a and b: Significant differences for means in the same row at P<0.05.

The positive effect of CM (20µg/ml) on improving sperm abnormality was more obvious on summer than winter semen. In agreement with our results, CM (2.5, 5, or 10 mmol/l) reduced the abnormality percentage of sperm cells in Angora goats (Bucak *et al*. 2010). Also, CM (100 µg/ml) reduced the abnormality of sperm cells in cryopreserved semen of goat buck (Ismail *et al*., 2020). The effectiveness of CM on decreasing the total abnormalities of spermatozoa was noticed in cryopreserved ram semen extended with CM at levels of 1 and 2 mmol/l (Soleimanzadeh and Saberivand 2013; Omur and Coyan 2016). Similar results were reported on bovine semen by Chaudhary *et al*. (2022). In accordance with the obtained results, Also, CM improved sperm abnormalities in bovine (Bucak *et al*., 2012), mice (Jalili *et al*., 2014), and rats fed protein-deficient diets (Ahmed-Farid *et al*., 2017).

Acrosome integrity:

The percentage of acrosome integrity in summer and winter post-thawed goat semen incubated for 7h is shown in Table 5.

Results of summer semen revealed that CM in E20 maintained the percentage of acrosome integrity to be significantly (P<0.05) higher than in E0 after all incubation times of summer and winter semen. However, acrosome integrity percentage was significantly (P<0.05) improved by CM in E1 only 7h in summer semen and 2, 5, and 7h after incubation of winter semen (Table 5).

Table 5. Effect of CM in extender on acrosome integrity in winter and summer post-thawed goat semen incubated for different times.

Item	Acrosome integrity (%)			SEM	P-value
	E0	E10	E20		
		Summer season:			
0h	72.31	71.63	75.82	1.45	0.215
1h	70.56^{b}	70.48^{b}	74.40^{a}	1.12	0.035
2h	69.85^{b}	70.83^{b}	74.01^{a}	0.49	0.009
5h	67.42^{b}	69.11^{ab}	72.25^{a}	0.84	0.042
7h	62.34^{b}	65.01^{a}	67.33^{a}	0.84	0.031
		Winter season:			
0h	81.61	83.80	82.44	1.21	0.145
1h	80.03^{b}	82.12^{ab}	82.33^{a}	1.35	0.042
2h	75.44^{b}	80.22^{a}	80.48^{a}	0.58	0.034
5h	71.65^{b}	75.55^{a}	78.10^{a}	1.21	0.031
7h	68.33^{b}	72.11^{a}	73.54^{a}	0.91	0.048

a and b: Significant differences for means in the same row at P<0.05.

Oocyte penetration of sperm gets affected by the integrity of the sperm plasma membrane and acrosome (Esteves *et al*, 2007). The obtained results indicated beneficial effects of CM at a level of 20µg/ml on maintaining sperm acrosome status in summer and winter goat post-thawed semen incubated for 7h. Other results indicated the beneficial impact of CM on acrosome status of sperm cells in semen of small ruminants, including semen of goats (Ismail *et al*., 2020) and rams (Omur and Coyan, 2016). Also, CM in semen extender decreased the percentage of abnormality in the acrosomal structure in Angora goats (Bucak *et al*. 2010). In bovine (Tvrda *et al*., 2018; Chaudhary *et al*., 2022), buffalo (Shah *et al*., 2016), and boar (Chanapiwat and Kaeoket, 2015) semen the CM showed positive impacts on intact acrosome percentage. Abdelnour *et al*. (2020) used the transmission electron microscopic (TEM) to assess the acrosome status of rabbit sperm cells cryopreserved with CM at levels 1 or 1.5 µg/ml. They found higher disturbance in acrosome membrane structure of in control group than in CM supplemented group.

On the other hand, Bucak *et al*. (2012) found no significant effects on morphological alterations to the acrosome as affected by CM supplementation in bull semen. However, Naz (2011) has reported negative effects CM

doses ≥250 µmol/l in semen extender on acrosome status of human sperm cells.

Effect of curcumin on conditions of sperm medium of post-thawed goat semen incubated for 2h:

Enzyme activity (AST and ALT):

Effect of CM in extender on the activity of AST and ALT in sperm medium of winter and summer post-thawed goat semen incubated for two hours is illustrated in Fig. 1.

Activity of AST in sperm medium of summer and winter semen was significantly (P<0.05) reduced only by E20 as compared to E0. However, ALT activity was significantly (P<0.05) decreased by E10 and E20 in summer and only by E20 in winter semen as compared to E0. Generally, E20 showed significantly (P<0.05) the lowest activity of AST and ALT in summer and winter semen (Fig. 1).

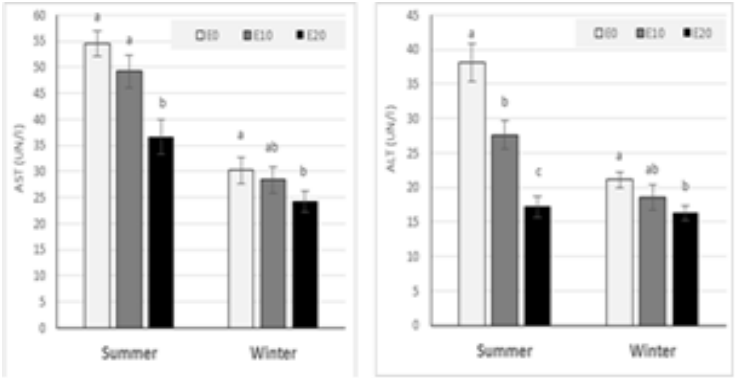

Fig. 1. Effect of CM in extender on the activity of AST and ALT in sperm medium of winter and summer post-thawed goat semen incubated for two hours.(a and b: Significant differences for column means in the same season at P<0.05).

These results revealed that adding CM (20µg/ml) in Tris-extender had pronounced improvement in sperm membrane integrity during incubation of cryopreserved goat semen. The present results agreed with those reported by Chaudhary et al. (2022), who found that CM in extender (25 µM) of bull semen decreased the activity of AST after thawing. They added that AST activities showed significantly a positive correlation with antioxidant enzymes activity. Such enzymatic alteration and correlation using different semen additives other than curcumin have been reported using Melatonin (Kapadiya et al., 2018); EDTA, and Caffeine (Patel and Siddiquee, 2013).

Antioxidant status

Effect of CM in extender on the level of TAC and MDA in sperm medium of winter and summer post-thawed goat semen incubated for two hours is illustrated in Fig. 2.

Both CM levels in E10 and E20 exhibited significant (P<0.05) increase in TAC and significant (P<0.05) reduction in MDA in sperm medium of summer and winter semen compared with E0. In general, E20 showed significantly (P<0.05) the highest TAC level and the lowest MDA level in sperm medium of post-thawed goat summer and winter semen during the incubation (Fig.2).

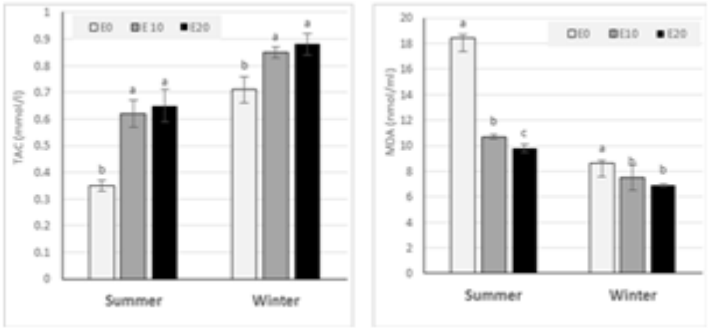

Fig. 2. Level of total antioxidant capacity (TAC) and malondialdehyde (MDA) in sperm medium of winter and summer post-thawed goat semen incubated for two hours.(a and b: Significant differences for column means in the same season at P<0.05).

In vitro incubation and cryopreservation of mammalian semen may alter the oxidative milieu either by uncontrolled ROS production or by aberrations in the antioxidant mechanisms of sperm cells (Tvrdá *et al*., 2018). In our study, marked enhancement in antioxidant status of sperm medium in post-thawed goat semen occurred by increasing TAC level, as an antioxidant marker, while decreasing level of MDA, as a lipid peroxidation marker, leading to elevating the antioxidant capacity and balancing OS. Bucak *et al.* (2010) indicated the antioxidant properties of CM in Angora goat and also in bulls (Bucak *et al*, 2012; Shah *et al*., 2016; Tvrda *et al*., 2016, 2018).

Our results are in consistent with various reports showing response in the antioxidant activity of male reproductive structures by CM (Karbalay-Doust and Noorafshan 2011; Soleimanzadeh and Saberivand 2013; Tvrda *et al.* 2016). A proper oxidative balance has a relationship with male fertility and is as a complex antioxidant network assures beneficial conditions for spermatozoa activity (Aitken 1995). Similarly, Chaudhary *et al*. (2022) found that CM supplemented to bovine semen

extender decreased MDA levels after cryopreservation. Abdelnour *et al*. (2020) showed an increase in enzyme activity (SOD and GPx) and a reduction in the level in freezing medium of rabbit semen supplemented with CM (1 or 1.5 µg/ml) compared with the control, reflecting an elevation of antioxidant capacity in sperm medium. Raza *et al*. (2008) revealed that CM has the ability to improve many defense enzyme activities in the seminal plasma by sustained the mitochondrial redox signaling and respiratory functions.

It is of interest to observe that all sperm variables studied were improved in association with increasing antioxidant status (increasing TAC and reducing MDA) by CM supplementation in Tris-extender of goat buck semen. These improvements are due to that CM can reduce the quantity of lipid peroxidation and oxidized proteins in sperm cells because CM has direct ROS-trapping properties and ability to prevent the residual ROS to alter the structure or function of plasma membrane and the protein molecules found in the sperm cells. Also, the beneficial impacts of CM on the vitality of spermatozoa may be its ability for stimulating the internal ROS-quenching and detoxification system of sperm cells (Tvrdá *et al*., 2018). Based on the obtained results and previous findings, CM has a strong antioxidant activity and effectiveness against cryodamage, which was reported to be eight folds of that of vitamin E, and its effectiveness against cryodamage has been studied in many studies (Santonastaso *et al*., 2021).

CONCLUSION

The effects of cryopreservation on are depending on fresh semen quality. The future studies can be planned on semen collected with various quality in different seasons focusing on the antioxidant action of curcumin. Based on the foregoing results, curcumin shows promise for increasing quality of cryopreserved semen collected under summer conditions or improving semen quality of semen collected in winter by reducing goat sperm cryodamage to identify the impacts on semen incubated with curcumin *in vitro*. In our study, curcumin supplemented to Tris-extender showed beneficial and protective impacts against the damage to goat sperm cells. The curcumin supplementation can protect the structure, function, and oxidative status of spermatozoa during cryopreservation.

In conclusion, adding curcumin to goat semen extenders can facilitate and enhance semen processes and storage protocols to improve fertility of cryopreserved goat spermatozoa in semen collected in summer or winter season.

REFERENCES

Abdel-Khalek, A.E.; S.A. Darwish; A.F. Mehrez and M.B. Aboul-Ela (2000). Relationships between sexual desire and testicular, scrotal and penile morpho-histometrics in Egyptian buffalo-bulls. J. Agric. Sci., Mansoura Univ., 25(8): 4903-4913.

Abdelnour, S.A., Hassan, M.A.E., Mohammed, A.K., Alhimaidi, A.R., Al-Gabri, N., Al-Khaldi, K.O., and Swelum, A.A. (2020). The effect of adding different levels of curcumin and its nanoparticles to extender on post-thaw quality of cryopreserved rabbit sperm. Animals, 10, 1508; doi:10.3390/ani10091508.

Abdullah, N.B.A. (2019). Effect of humic acid on productive and Reproductive performance of small ruminants. Ph. D., Faculty of Agriculture, Tanta University, Egypt.

Agarwal, A., Virk, G., Ong, C., and du Plessis, S.S. (2014). Effect of oxidative stress on male reproduction. World J. Mens Health, 32, 1–17. [CrossRef]

Ahmed-Farid, O.A.H.; Nasr, M.; Ahmed, R.F.; Bakeer, R.M. (2017). Beneficial effects of curcumin nano-emulsion on spermatogenesis and reproductive performance in male rats under protein deficient diet model: Enhancement of sperm motility, conservancy of testicular tissue integrity, cell energy and seminal plasma amino acids content. J. Biomed., Sci., 24, 66.

Aitken, R.J. (1995): Free radicals, lipid peroxidation, sperm function. Reproduction, Fertility and Development, 7, 659–668.

Alves, M. B., A. F. Andrade, R. P. Arruda, L. Batissaco, S. A. Florez-Rodriguez, B. M. Oliveira, M. A. Torres, R. Lanconi, G. M. Ravagnani, R. R. Prado Filho, V. S. Vellone, J. D. Losano, C. R. Franci, M. Nichi, and E. C. Celeghini. (2016). Recovery of normal testicular temperature after scrotal heat stress in rams assessed by infrared thermography and its effects on seminal characteristics and testosterone blood serum concentration. Theriogenology, 86:795–805.e2. https: doi .org/ 10 .1016/ j. theriogenology .2016 .02 .034.

Amidi, F, Pazhohan A, Shabani Nashtaei M, Khodarahmian M, Nekoonam S. (2016). The role of antioxidants in sperm freezing: a review. Cell Tissue Bank, 17 (4):745–56.

Bansal, A.K., Bilaspuri G.S. (2011). Impacts of oxidative stress and antioxidants on semen functions. Veterinary Medicine International, 2011, 23–32.

Bilodeau, JF, Chatterjee S, Sirard MA and Gagnon C (2000). Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Molecular Reproduction and Development: Incorporating Gamete Research 55(3):282-288.

Bucak, M.N., Basspinar N., Tuncer B.P., Coyan K., Sari-ozkan S., Akalin P.P., Buyukleblebici S., Kucukgunay S. (2012): Effects of curcumin and dithioerythritol on frozen-thawed bovine semen. Andrologia, 44, 102–109.

Bucak, M.N., Sariozkan S., Tuncer P.B., Sakin F., Atessahin A., Kulaksiz R., Cevik M. (2010): The effect of antioxidants on post-thawed Angora goat (Capra hircus ancryrensis) sperm parameters, lipid peroxidation and antioxidant activities. Small Ruminant Research, 89, 24–30.

Chanapiwat, P and Kaeoket K (2015). The effect of Curcuma longa extracted (curcumin) on the quality of cryopreserved boar semen. Animal Science Journal 86(9):863-868.

Chaudhary, K.F., Suthar, B.N., Nakhashi1, H.C., and Mohapatra, S.K. (2022). Effect of curcumin as a semen additive on seminal attributes and biochemical parameters of kankrej bull spermatozoa. Ruminant Science, *Vol 11 No 1, p 57-62.*

de Lamirande, E, O'Flaherty C. Sperm activation: Role of reactive oxygen species and kinases. C. 1784, Biochimica et Biophysica Acta - Proteins and Proteomics.Elsevier; 2008. s. 106–15.

Dev, K.R., Yadamma K., Reddy K.D. (2013): Protective effects of curcumin in cyclophosphamide induced sperm head abnormalities in male mice. International Journal of Pharmacy and Biological Sciences, 4, 1131–1137.

El-Saidy, B.E.I. (1988). Studies on reproductive physiology in male goats. M.Sc. Thesis, Fac. Agric., Mansoura Univ., Egypt.

El-Wishy, A.B.; El-Sawaf, S.A.; El-Mikkawi, F. and Omar, A.A. (1971). Monthly and seasonal variation in sexual activity of male Damascus goats. Ind. J. Anim. Sci., 41: 562-569

Esteves, SC, Sharma RK, Thomas AJ Jr and Agarwal A (2007). Evaluation of acrosomal status and sperm viability in fresh and cryopreserved specimens by the use of fluorescent peanut agglutinin lectin in conjunction with hypo-osmotic swelling test. International Brazilian Journal of Urology 33:364-374.

Ismail, AA, Abdel-Khalek AKE, Khalil WA, Yousif AI, Saadeldin IM, Abomughaid MM and El-Harairy MA (2020). Effects of mint, thyme, and curcumin extract nanoformulations on the sperm quality, apoptosis, chromatin decondensation, enzyme activity, and oxidative status of cryopreserved goat semen. Cryobiology 97:144-152.

Jalili, C, Khani F, Salahshoor MR and Roshankhah SH (2014). Protective effect of curcumin against nicotine-induced damage on reproductive parameters in male mice. International Journal of Morphology 32(3):844-849.

Jayaprakasha, GK, Jaganmohan Rao L, Sakariah KK. Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Food Chem 2006;98(4):720–4.

Kapadiya, PS, Nakhashi HC, Chauhan PM, Sutaria TV and Suthar BN (2018). Efficacy of melatonin as an antioxidant for cryopreservation of Kankrej bull semen. Ruminant Science 7(1):89-92.

Karbalay-Doust, S., Noorafshan A. (2011): Ameliorative effects of curcumin on the spermatozoon tail length, count, motility and testosterone serum level in metronidazole-treated mice. Prague Medical Report, 112, 288–297.

Kashou, A.H., Sharma R., Agarwal A. (2013): Assessment of oxidative stress in sperm and semen. Methods in Molecular Biology, 927, 351–361.

Kaur, N., Sharma, M., Lonare, M.K., and Singh, D. (2020). Cytoprotective potential of curcumin and resveratrol during freeze-thaw cycle of buffalo bone marrow derived mesenchymal stem cells. Ruminant Science, 9(2):215-222.

Koracevic, v., Koracevic, G., Djordjevic, V., Andrejevic, S., and Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. Journal of Clinical Pathology, vol. 54, no. 5, pp. 356–361.

Lin, J-K, Pan M-H, Lin-Shiau S-Y. Recent studies on the biofunctions and biotransformations of curcumin. BioFactors 2000;13(1-4):153–8.

Llamas-Luceño, N., Hostens, M., Mullaart, E., Broekhuijse, M. Lonergan, P., and Van, S. (2019). High temperature-humidity index compromises sperm quality and fertility of Holstein bulls in temperate climates. J. Dairy Sci. 103:9502–9514(2019)

Lu, X, Zhang Y, Bai H, Liu J, Li J, Wu B. Mitochondria-targeted antioxidant MitoTEMPO improves the post-thaw sperm quality. Cryobiology 2018;80 (105):26–9.

Malama, E., Y. Zeron, F. Janett, M. Siuda, Z. Roth, and H. Bollwein. 2017. Use of computer-assisted sperm analysis and flow cytometry to detect seasonal variations of bovine semen quality. Theriogenology 87:79–90. https: / / doi .org/ 10 .1016/ j. theriogenology .2016 .08.002.

Menon, A. G., J. C. Thundathil, R. Wilde, J. P. Kastelic, and H. W. Barkema, "Validating the assessment of bull sperm morphology by veterinary practitioners," The Canadian Veterinary Journal, vol. 52, no. 4, pp. 407-408, 2011.

Moskovtsev, S.I., Librach C.L. (2013): Methods of sperm vitality assessment. In: Carrell D.T., Aston K.I. (eds): Spermatogenesis: Methods and Protocols. Springer Science and Business Media, LLC, New York, USA, 13–19.

Mostek, A.; Dietrich, M.A.; Słowi ´ nska, M.; Ciereszko, A. Cryopreservation of bull semen is associated with carbonylation of sperm proteins. Theriogenology **2017**, 92, 95–102. [CrossRef]

Moussa, I.A. (1987). Semen characteristics and freezing of male goats. M.Sc. Thesis, Fac. Agric., Tanta Univ., Egypt.

Massoud, F.I.; El.S.M. Fattoh and M.S. Abdou (1991). Reparation a new herbal therapy for an oestrum. In: Buffaloes a Preliminary Report. Egypt. Soc. Anim. Reprod. Fert., 3rd Ann. Cong. January 12-14, Cairo.

Mustapha, AR, Beigh YA, Abhishek K, Rahul K, Omer D, Kumar A, Prasad JK, Bhure S, Srivastava N and Ghosh SK (2017). Effects of partial deoxygenation of extender on plasma membrane integrity and enzyme activity in frozen-thawed crossbred bull semen. Ruminant Science 6(2):327-331.

Naz, R.J. (2011): Can curcumin provide an ideal contraceptive? Molecular Reproduction and Development, 78, 116–123.

Naz, RK. The effect of curcumin on intracellular pH (pHi), membrane hyperpolarization and sperm motility. J Reprod Infertil 2014;15(2):62–70.

Ohkawa, H., Ohishi, N., and Yagi, K. "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," Analytical Biochemistry, vol. 95, no. 2, pp. 351–358, 1979.

Omur, A.D., Coyan K. (2016): Protective effects of the antioxidants curcumin, ellagic acid and methionine on motility, mitochondrial transmembrane potential, plasma membrane and acrosome integrity in freeze-thawed Merino ram sperm. Veterinarni Medicina, 61, 10–16.

Patel, BR and Siddiquee GM (2013). Effect of semen additives on leakage of certain enzymes during cryopreservation of Kankrej semen. Ruminant Science 2(1):63-66.

Pinto, S.C.C.; Almeida, D.S.; Alves, M.B.R.; Florez-Rodriguez, S.A.; Abreu Júnior, G.S.; Alves, N.; Celeghini, E.C.C.; Laskoki, L.M.; Souza, F.A. Does supplementation of vitamin C, reduced glutathione or their association in semen extender reduce oxidative stress in bovine frozen semen? Arquivo Brasileiro de Medicina Veterinária e Zootecnia **2020**, 72, 9–17. [CrossRef]

Raza, H.; John, A.; Brown, E.M.; Benedict, S.; Kambal, A. Alterations in mitochondrial respiratory functions, redox metabolism and apoptosis by oxidant 4-hydroxynonenal and antioxidants curcumin and melatonin in PC12 cells. Toxicol. Appl. Pharmacol. 2008, 226, 161–168. [CrossRef] [PubMed]

Reitman, S. and S. Frankel, "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases," American Journal of Clinical Pathology, vol. 28, no. 1, pp. 56–63, 1957.

Rithaporn, T., Monga M., Rajasekaran M. (2003): Curcumin: a potential vaginal contraceptive. Contraception, 68, 219–223.

Royere, D, Barthelemy C, Hamamah S, Lansac J. Cryopreservation of spermatozoa: a 1996 review. C. 2, Human Reproduction Update; 1996.

Sabes-Alsina, M., A. Johannisson, N. Lundeheim, M. Lopez-Bejar, and J. M. Morrell. 2017. Effects of season on bull sperm quality in thawed samples in northern Spain. Vet. Rec. 180:251. https: / / doi.org/ 10 .1136/ vr .103897.

Salahshoor, M.R., Jalili C., Khazaei M., Khani F. (2012): Effects of curcumin on reproductive parameters in male mice. Journal of Clinical Research in Paramedical Sciences, 1, 1–3.

Santonastaso, M, Mottola F, Iovine C, Colacurci N, Rocco L. Protective Effects of Curcumin on the Outcome of Cryopreservation in Human Sperm. Reprod Sci 2021;28(10):2895–905. Salahshoor MR. Effects of curcumin on reproductive parameters in male mice. J Clin Res Paramed Sci 2012; 1:31–7.

Shah, S.A.H., Andrabi, S.M.H., and Qureshi, I.Z. (2016). Freezability of water buffalo bull (*Bubalus bubalis*) spermatozoa is improved with the addition of curcumin (diferuoyl methane) in semen extender. Andrologia, 49(8): e12713.

Soleimanzadeh, A, Saberivand A. Effect of curcumin on rat sperm morphology after the freeze-thawing process. Artic Vet Res Forum 2013;4(3):185–9.

Thomson, LK, Fleming SD, Aitken RJ, De Iuliis GN, Zieschang J-A, Clark AM. Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis. Hum Reprod 2009;24 (9):2061–70.

Tvrda, E, Greifova H, Mackovich A, Hashim F and Lukac N (2018). Curcumin offers antioxidant protection to cryopreserved bovine semen. Czech Journal of Animal Science 63(7):247-255.

Tvrda, E, Tusimova E, Kovacik A, Paal D, Greifova H, Abdramanov A and Lukac N (2016). Curcumin has protective and antioxidant properties on bull spermatozoa subjected to induced oxidative stress. Animal Reproduction Science 172:10-20.

Yadava, Chhote Lal, Saxena Atul, Yadav Brijesh, Singh Vipin, Reddy A Vidhya Sagar, Patel Akhil, Kumar Anuj and Yadav Sarvajeet (2018). Effect of sericin supplementation on the semen quality of cryopreserved hariana bull semen. Ruminant 7(1):93-96.

قدرة الكركومين على حفظ خصائص الحيوانات المنويه ونشاط مضادات الأكسدة للبلازما المنويه للسائل المنوى المجمد للماعز والمجمع فى الصيف والشتاء

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الملخص

أثناء حفظ السائل المنوي بالتجميد يؤدي الإجهاد التأكسدي (OS) إلى انخفاض ملحوظ في بنية ووظيفة خلايا الحيوانات المنوية. ويعمل الكركمين (CM) كمضاد للأكسدة لمسح الجذور الحرة الناجمة عن نظام التشغيل أثناء الحفظ بالتجميد. ويهدف البحث إلى تقييم تأثير CM على متغيرات الحيوانات المنوية لسائل منوى الماعز المحفوظة بالتجميد والتي تم جمعها في موسمى الصيف والشتاء في مصر. تم تجميع السائل المنوي من خمسة تيوس دمشقي، وتخفيفه (1:20) وموازنته (5 درجات مئوية لمدة ساعتين) وتقسيمه إلى ثلاث اقسام مكملة بـ CM عند 0- 10-20 ميكروغرام/مل في مخفف التريس يمثل E0- E10 - E20 على التوالي. تم تحضين السائل المنوي بعد إسالته وتقييمه عند 0- 1- 2- 5- 7 ساعات بعد التحضين على 37 درجة مئوية. أظهرت النتائج أن جميع مقاييس السائل المنوى كانت أفضل معنوياً في الشتاء عنها في الصيف. أدت مكملات (20 CM ميكروغرام / مل) إلى تأثيرات جيدة فى الحفاظ على الحركة التقدمية للحيوانات المنوية والحيوية وسلامة الاكروسوم للسائل المنوي بعد اسالة السائل المنوى للتيوس فى موسمى الصيف والشتاء المحتضن لمدة 7 ساعات. أظهر كلا مستويي CM تأثيرًا إيجابيًا على زيادة إجمالي قدرة مضادات الأكسدة وتقليل مستوى المالونديالدهيد في وسط الحيوانات المنوية في فصل الصيف والشتاء بعد الاسالة. ولوحظ أن عمل CM على تحسين متغيرات الحيوانات المنوية واكسدة الدهون وحالة مضادات الأكسدة يكون أكثر وضوحًا في الصيف منه في السائل المنوي في الشتاء.وتوصى الدراسى بإضافة الكركمين إلى السائل المنوي للتيوس حيث يمكن أن يسهل ويعزز جودة السائل المنوي وبروتوكولات التخزين لتحسين خصوبة الحيوانات المنوية المحفوظة بالتجميد في السائل المنوي للتيوس الذي تم جمعه في فصلى الصيف أو الشتاء.