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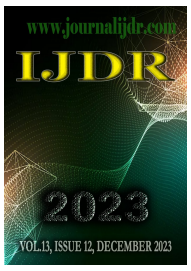

RESEARCH ARTICLE OPEN ACCESS

EFFECTS OF ADDING CURCUMIN ON QUALITY OF POST-THAWED ARABIAN STALLION SEMEN

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ABSTRACT

The objective of this study was to investigate the impact of including curcumin antioxidant on the viability of sperm after the processes of cooling and freezing. curcumin antioxidant was used in HF-20 extender at concentrations of 0.32, 0.60, 1.30, and 2.71 µm/L. HF-20 extender was a basic extender and used for control group. The post-thawed semen exhibited significantly higher total motility in the 0.32 curcumin treatment groups as compared to others. The extender containing 0.32 µm/L curcumin showed a significantly higher in total motility compared to others. Progressive motility values were similar among extenders. Speed motion of stallion sperm in extender containing curcumin showed significantly higher on VCL, VSL, VAP, LIN and STR compared to control. The findings of the plasma membrane integrity (HOST obtained with curcumin extenders at 0.32, 0.60, 1.30 µm/L were significantly high compared to control extender. Normal morphology and vital test sperm were significantly higher in control compared to curcumin extenders. Acrosome integrity was not significant difference among group. However, significantly higher DNA fragmentation was showed in curcumin 0.32 µm/L and control extenders. In conclusion, curcumin antioxidant has shown the capacity to enhance the quality of frozen-thawed sperm. On other hand, negatively influence on the viability and integrity of DNA inside the frozen-thawed spermatozoa. Therefore, more research to find the optimal level of curcumin andadditional research should be conducted to assess the fertility potential of cryopreserved stallion semen.

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INTRODUCTION

The use of artificial insemination (AI) technique in horses poses challenges compared with other animal species, such as cattle, as stallion semen subjected to this process became vulnerable to environmental and cellular factors, which ultimately becoming less fertile (Blottner *et al*., 2001). Further, many studies have indicated / suggested that one of the underlying reasons might be is that such practices in selecting stallion semen are based on morphological characteristics, e.g. fitness, rather than reproductive/ biological characteristics (Clulow *et al*., 2007). Despite improvements in preserving stallion semen through many available semen extenders, there are still challenges in preserving high quality stallion semen, i.e. retaining a high fertilizing characteristics/capacity; thus, it's a matter of finding suitable extender. Therefore, studies/researchers are on race for finding a suitable one.

Cryopreservation technique exposes collected semen to various environmental and cellular conditions rendering the fertility characteristics of preserved semen. This method triggers cellular oxidative stress through the removal of plasma from the sperm cells as well as the exposure of semen to the oxygen generating free radicals causing cellular injuries affecting collected semen quality (Paoli *et al*., 2017). Therefore, the use of antioxidants is warranted and many previous research provided evidence that might be beneficial on maintaining preserved stallion semen in enhanced quality through the measurements of various cellular as well as microscopic endpoints (Alamaary *et al*., 2021). However, previous work has mainly used synthetic antioxidants, which might be expensive and not widely covered as an antioxidant that alleviates the potential harm of cellular free radicals. Thus, the search for and use of natural antioxidants is worth pursuing given the reported potential positive findings. Curcumin/ turmeric plant is widely used as a food additive as well as in traditional medicine belonging to the plant

rhizomes of turmeric (*Curcuma longa* L.) from the ginger family plants (Kocaadam, and Şanlier, 2017). Curcumin is the main compound in the turmeric plant consisting of more than 70% of the curcuminoids in the plant powder. From its chemical structure, curcumin is a compound containing many hydroxy and methoxy groups belonging to the polyphenol's compounds and showing lipophilic characteristics (Menon and Sudheer, 2007). As a polyphenol's compound, many reports have indicated curcumin anti-inflammatory, antioxidants, as well as other features (Kocaadam, and Şanlier, 2017; Alizadeh, *et al*., 2018). There is a bulk of evidence indicating the ability of curcumin to alleviate the effect oxidative stress and the ability to neutralize many reactive oxygen species as well as interacting with many molecular targets (Jakubczyk, 2020). Further, the food and drug administration have categorized curcumin as a safe compound. Therefore, we propose here the adding of natural antioxidants to the egg yolk extender to provide a more economic as well as effective and efficient way of preserving stallion semen using the cryopreservation method. We propose the use curcumin as antioxidants to be added to the cryopreservation medium and studying the vitality characteristics of stallion semen compared to a control group.

MATERIALS AND METHODS

Animals: Four healthy stallions aged 4 to 10 years were selected for this study after a breeding soundness examination. All of the animals were housed individually and offered pellets, alfalfa hay, clean water, and integrated mineral licks. A rhythm semen collection was performed using estrus-mare twice a week per stallion.

Semen collection and processing: Estrus-mare was used for semen collection in this trial. After semen collection, the gel was removed immediately from the semen. The sample was collected using sterile gauze and transferred to a water bath at 37°C. The semen volume measured in a graduated cylinder. The ejaculate was then evaluated for general progressive motility and sperm concentration. Sperm concentration and motility were determined using the CASA system (ISAS program, Prosser R+D, Paterna, Valencia, Spain). Samples with a minimum concentration of 200 × 10^6 sperm/ml and motility > 60% used for this study. The filtered semen of each ejaculate was diluted (1:1) with a centrifuged medium and then divided into seven aliquots. The aliquots were centrifuged at 900 g for 10 minutes, the seminal plasma was removed, and each sample was re-suspended with FH-20 (0) without any supplementation as a control. HF-20 extender was supplemented with curcumin at concentration 0.32, 0.60, 1.30 and2.71 µm/L. The final semen concentration after dilution was 200 × 10^6 sperm/ml. All of the tubes were cooled to 4°C for 90 minutes before being assessed for motility, morphology, and sperm membrane integrity. Cooled semen was filled into 0.5 ml straws. The straws were frozen by putting straws horizontally on the liquid nitrogen surface (9 cm) for 9 minutes and then immersing immediately in liquid nitrogen. All straws were collected in goblets and kept in liquid nitrogen tank.

Extenders: The centrifuged medium was a mixture of 6.0 g glucose, 0.37 g ethylene-diamine-tetra-acetic acid (EDTA), 0.37 g sodium citrate, 0.18 g sodium bicarbonate, 100,000 IU penicillin, and 0.08 g streptomycin in 100 ml of distilled water. HF-20 as freezing extender will be used. Experiment I, freezing extender with egg yolk will be prepared by dissolving 5.0 g glucose, 0.3 g lactose, 0.3 g raffinose, 0.15 g sodium citrate, 0.05 g sodium phosphate, 0.05 g sodium potassium tartrate, and 3 mL glycerol in 50 mL of distilled water and EY at 10 mL will be added as a basic buffer, and then distilled water will be added to a total volume of 100 mL (control extender). Curcumin at different concentrations will be dissolved in the basic buffer. The pH of the extender was adjusted using sodium bicarbonate buffer. Measurements of pH was adjusted using a pH meter (Hanna, model HI-2212, Woonsocket, RI, USA).

Frozen semen evaluation: The frozen straws were thawed in a water bath at 38ºC for 60 seconds, and the contents were expelled into a small warm tube. Total and progressive motility were then evaluated using the ISAS program and assessed for plasma membrane integrity, morphology defects, acrosome integrity, and viability. Frozen semen evaluation was conducted at the AI Center for Animal Production and Breeding, Qassim University.

Assessment of sperm motility: The ISAS program (CASA system) was used to assess the motility patterns immediately after the dilution of the semen or the post-thawed semen. A sample (2.7 µL) from each tube was placed onto a slide and semen motility was assessed based on five digital images from different fields via a ×10 negative-phase contrast objective and warm stage at 38ºC. Motility patterns were measured according to the total motile sperm (TMS %), rapid progressively motile (PM %), curvilinear speed (VCL µm/s), rectilinear speed (VSL µm/s), average value (VAP µm/s), linearity index (LIN %), and straightness index (STR %). At least 300 sperm were analyzed from each sample, and the images were read within one second.

Plasma membrane integrity: A hypo-osmotic swelling test (HOST) was used to assess the plasma membrane integrity of the spermatozoa. A minimum of 100 sperm cells were analyzed for the coiled tail using phase contrast microscopy (×400). A mixture of a glucose-based solution at 100 mOsmol and 20 µL of semen was incubated at 37°C for 50 minutes in a water bath (Neild *et al*. 1999).

Viability test: Sperm viability was evaluated using an acridine orange (AO) and propidium iodide (PI) kit (Halotech DNA S.L., Madrid, Spain). First, the semen was diluted to 10-15 × 10^6 sperm/ml. Then 10 µL of diluted semen was placed on a slide. Then 1.0 µL of AO and PI was mixed with the diluted semen. Finally, the mixture was covered and then evaluated using a fluorescence microscope. Living sperm retained the AO, producing green fluorescence, while PI penetrated the damaged sperm, causing red fluorescence. A total of 300 sperm was assessed per sample.

DNA fragmentation test: The Halomax kit, which was created by Halotech SL in Spain, was used for the assessment of DNA fragmentation in equines. The technology used in the Sperm-Halomax® methodology is based on the sperm chromatin dispersion test (SCDt) as outlined in the study conducted by Cortés-Gutiérrez *et al*. in 2008. The initial volume of sperm was diluted in agarose to get a final concentration of 1 × 10^6 sperm/ml. The diluted sample was next moved to racks, where it underwent lysis. Following this, the sample was stained with propidium iodide (Halotech DNA S.L., Madrid, Spain) in order to produce slides for examination. The investigation of DNA fragmentation was conducted using a fluorescence microscope (Optika S.r.I, Italy) at a magnification of 1000X. In order to determine the sperm DNA fragmentation index, a count of 300 sperm was conducted on each slide. The distinguishing feature of this index is the occurrence of a halo around the head of the sperm, resulting from the dispersion of chromatin inside agarose. The presence of a halo signifies the presence of DNA damage, which may manifest as either single-strand or double-strand breaks. The dispersion of DNA fragments from the sperm head is seen, with larger chromatin fragments displaying relatively less displacement than smaller pieces, leading to the creation of a distinct "halo" around the sperm head. The phenomenon of sperm fragmentation is considered to occur when the diameter of the halo exceeds twice the radius of the sperm head.

Morphology shape of sperm: Sperm morphology will be examined using the Hancock's solution. A 15 µL drop of semen was mixed with 15 µL of in a clean and warm tube. The mixture was dropped and covered, and evaluated under oil immersion at 1000 × magnification. The spermatozoa's morphological defects were classified sperm abnormalities according to their effect on fertility: major defects include most abnormalities of the head and midpiece, proximal cytoplasmic droplets and single abnormalities present in a high percentage, whereas minor defects include looped tails, detached sperm heads, and distal cytoplasmic droplets (Alamaary *et al*., 2021).

Acrosome integrity: A drop of semen sample was mixed with a drop of isotonic Hancock's solution with 6% of formaldehyde on a clean and warmed microscope slide. The mixture was then covered and assessed in a light microscope at x1000 at least 200 sperm for acrosome defects.

Statistical analysis: Descriptive analyses (data were expressed as the means and SEM) were determined for the evaluated variables: total motility (TM), progressive motility (PM), path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), straightness (STR), linearity (LIN), wobble (WOB) acrosome integrity, vitality, HOST, morphological defect and DNA fragmentation. Statistical comparisons between groups were performed with one-way analysis of variance (ANOVA) ($P<0.05$). The data were considered statistically different if $P<0.05$ (SPSS, version 21).

RESULTS

Effect of adding curcumin on viability of sperm after cooling: Motility and speed parameters of stallion sperm after cooling are showed in Table 1. The extender with 0.32µm/L curcumin showed significant in total motility and progressive motile. However, the speed motion showed higher in extender with curcumin compared to control extender. The minor abnormalities were significantly higher in extender with curcumin. Whereas, there was not significant that showed in major abnormalities.The normal morphology of sperm was significantly higher in controlcompared to curcumin groups HOST. did not show a significant among groups. The percentage of acrosome integrity was not different among groups.

Effect of adding curcumin on viability of sperm after freezing: Motility and speed parameters of stallion sperm after deep-freezing in liquid nitrogen are showed in Table 2. The extender containing 0.32 µm/Lcurcuminshowed a significantly higherin total motility compared to others. Progressive motility values were similar among extenders. Speed parameters of stallion sperm diluted in extender containing curcumin showed significantly high on VCL, VSL, VAP, LIN and STR compared to control. Except WOB did not showed significant among groups. The significant higher plasma membrane integrity (HOST) obtained with curcuminextendersat 0.32, 0.60, 1.30 µm/L compared to control extender. Normal morphology sperm were significantly higher in control compared to curcumin extenders. Acrosome integrity was not significant difference among group. However, the percentage of minor and major abnormalities of sperm were similar among groups. The percentage of vitality was significantlyhigh in control group compared to curcumin groups. However, significatly higher DNA fragmentation was showed in curcumin 0.32 µm/L and control extenders.

DISCUSSION

Curcumin, derived from the turmeric plant (Curcuma longa), is a natural phytochemical molecule known for its antioxidant qualities. Curcumin may enhance sperm motility by augmenting antioxidant processes and regulating lipid peroxidation and nitric oxide generation. There is a lack of information about the use of curcumin in freeing stallion semen. The objective of this research was to examine the impact of curcumin on the quality of sperm in stallions.

Table 1. effect of adding curcumin at different concentrations on sperm kinetics and defect of morphology in cooled stallion semen.

Parameters	Curcumin (µm/L)				Control
	0.32	0.60	1.30	2.71	
Total motility (TM) (%)	94.12±1.15^{a}	86.38±1.16^{b}	85.37±2.45^{b}	84.50±3.20^{b}	86.25±1.25^{b}
Progressive motility (PM) (%)	33.15±1.57^{a}	21.22±5.30^{b}	27.17±4.52^{b}	23.40±5.81^{b}	27.35±7.67^{b}
VCL (µm/s)	94.12±2.72	93.35±3.32	86.27±5.59	92.51±3.29	91.27±1.73
VSL (µm/s)	27.30±1.13^{a}	27.18±1.07^{a}	24.67±2.34^{ab}	26.48±0.66^{ab}	23.22±0.55^{b}
VAP (µm/s)	46.95±1.76	46.58±2.15	42.50±2.58	45.08±1.89	46.24±0.86
LIN (%)	29.27±1.66^{ab}	29.38±1.49^{ab}	34.91±5.92^{a}	29.28±1.59^{ab}	26.37±0.28^{b}
STR (%)	58.22±3.75^{ab}	58.32±3.50^{ab}	57.65±2.98^{ab}	59.21±3.93^{a}	51.93±0.46^{b}
WOB (%)	50.42±1.23	50.46±0.79	50.02±0.54	49.51±1.17	52.04±0.30
HOST (%)	75.54±5.13	81.50±2.96	81.71±3.58	81.38±2.07	77.38±2.75
Acrosome integrity (%)	82.30±1.98	83.97±3.36	81.55±4.31	84.21±3.53	88.32±1.24
Normal morphology (%)	68.42±1.46^{b}	68.36±3.13^{b}	69.13±1.08^{b}	71.66±5.00^{b}	81.13±1.58^{a}
Major abnormal (%)	13.39±0.93	13.55±3.38	8.78±1.84	14.52±4.52	14.43±1.76
Minor abnormal (%)	16.52±0.21^{a}	17.37±2.10^{a}	22.07±2.93^{a}	13.76±0.43^{a}	6.19±2.29^{b}

^{a,b,ab} Values with different superscripts in the same row and experiment differ significantly at $P<0.05$, ANOVA.

Table 2. The effect of adding curcumin at different concentrations on sperm kinetics parameters and sperm morphology in post thawed-frozen stallion semen

Parameters	Curcumin (µm/L)				Control
	0.32	0.60	1.30	2.71	
Total motility (TM) (%)	57.50±2.5^{a}	46.00±2.4^{b}	33.33±4.9^{b}	32.50±1.70^{b}	37.85±1.01^{b}
Progressive motility (PM) (%)	26.13±8.00	22.43±6.52	23.40±9.03	24.13±9.16	20.55±1.89
VCL (µm/s)	90.18±1.28^{a}	88.98±1.71^{ab}	87.71±5.15^{ab}	90.96±1.82^{a}	73.70±8.43^{b}
VSL (µm/s)	41.38±10.13^{a}	34.25±2.72^{a}	29.90±4.43^{ab}	33.16±3.41^{a}	20.39±1.43^{b}
VAP (µm/s)	47.85±1.37^{a}	48.41±2.07^{a}	46.50±2.77^{ab}	48.21±3.77^{a}	39.80±3.34^{b}
LIN (%)	35.03±1.16^{ab}	39.10±2.16^{a}	32.75±4.29^{ab}	36.36±2.63^{ab}	29.58±1.80^{b}
STR (%)	65.13±3.44^{a}	70.70±4.21^{a}	63.26±5.47^{a}	67.86±1.51^{a}	52.18±0.97^{b}
WOB (%)	54.03±2.15	55.36±1.57	51.50±2.16	53.46±2.87	56.15±2.35
HOST (%)	57.81±2.17^{a}	59.07±2.36^{a}	59.43±2.43^{a}	52.98±1.78^{ab}	47.70±0.87^{b}
Vitality test (%)	25.16±2.9^{b}	17.00±2.07^{b}	19.85±2.9^{b}	13.33±2.6^{b}	40.26±5.25^{a}
Acrosome integrity (%)	84.33±5.2	82.33±6.3	81.00±6.0	81.00±2.6	90.75±1.6
Normal morphology (%)	68.35±2.67^{b}	68.55±2.85^{b}	70.72±6.81^{b}	74.52±1.14^{ab}	81.36±0.89^{a}
Major abnormal (%)	13.09±4.83	13.65±6.20	16.28±4.20	11.80±0.10	6.78±1.06
Minor abnormal (%)	18.54±4.38	17.77±3.47	12.98±3.04	13.44±0.83	11.95±1.46
DNA fragmentation (%)	35.46±5.22^{a}	24.07±1.34^{ab}	19.91±1.80^{b}	19.29±3.25^{b}	49.35±9.88^{a}

^{a,b,ab} Values with different superscripts in the same row and experiment differ significantly at $P<0.05$, ANOVA.

The beneficial impact of curcumin on the quality of thawed sperm (specifically, motility and morphology) in goat, ram, and bulls has been documented by Bucak *et al*. in 2009, 2010, and 2011, respectively. This has led to the utilisation of curcumin as an antioxidant additive and its demonstrated ability to protect sperm during the freezing process when included in the freezing extender. However, a research conducted by Rossi *et al*. (2018) shown that curcumin had a harmful impact on stallion sperm at a lower dose (10 µm/L) and for a shorter duration compared to other species. The observed phenomenon may be attributed to variations in the composition of the sperm plasma membrane and the role of protein kinase C (PKC) in regulating sperm motility, which is distinct to each species. Thus, they concluded that the addition of curcumin did not provide any advantages for horse sperm in this particular experimental context. Nevertheless, our present investigation aims to examine the impact of including curcumin at reduced dosages, in comparison to a prior research, on the quality and viability of frozen stallion semen. The ability of sperm to move is essential for them to pass through the cervix and uterotubal junction. Furthermore, it is particularly critical for the sperm to be able to penetrate the cumulus cells and zona pellucida of the ovum (Fujihara *et al*., 2018). During the current study, the addition of the antioxidant curcumin at a concentration of 0.32 µm/L resulted in notable variations in sperm motility and motion characteristics, with the exception of progressive motility and LIN. The findings regarding sperm motility and acrosome abnormality do not align with the findings reported by Bucak *et al*. (2010) for ram spermatozoa and Salman *et al*. (2021) for bulls. These studies did not observe any difference in the supplementation of curcumin for the cryopreservation of ram spermatozoa.

The HOST test evaluates the sperm plasma membrane's capacity to withstand damage caused by increased permeability resulting from swelling caused by hypo-osmotic therapy. This method offers a means of conducting a stress test on a membrane, specifically designed for evaluating the stabilising effect of antioxidants (Bucak *et al*., 2011). The research found that the best HOST rates were achieved when sperm samples were cryopreserved using curcumin at concentrations of 0.32, 0.60, and 1.30 µm/L. This suggests that the utilisation of curcumin preserves the structural integrity of the sperm plasma membrane while undergoing the process of freezing. This conclusion is consistent with the findings reported by Bucak *et al*. (2010) about the freezing of ram spermatozoa. The disparity between the production of reactive oxygen species (ROS) and the protective mechanisms of antioxidants leads to an excessive accumulation of ROS. This accumulation contributes to the fragmentation of sperm DNA, malfunction of mitochondria, and peroxidation of membrane lipids, all of which are linked to infertility. Mammalian spermatozoa are very susceptible to lipid peroxidation (LPO), which is the process of membrane lipids being oxidised by partly reduced oxygen molecules, such as superoxide, hydrogen peroxide, and hydroxyl radicals.

Administration of antioxidants may inhibit this process (Su *et al*., 2019). According to some authors (Aitken *et al*., 1993; Su *et al*., 2019), the membranes of mammalian spermatozoa undergo spontaneous lipid peroxidation (LPO), which leads to the destruction of the lipid matrix structure. This occurs due to the invasion of reactive oxygen species (ROS). These assaults eventually caused damage to the activities of sperm, such as their ability to move, maintain a healthy membrane, and reproduce. This damage was a result of oxidative stress and the generation of harmful aldehydes. In this research, there was a significant difference in sperm motility and functional membrane integrity between the low dosages of curcumin and the control group. Verify that the occurrence of ROS may be averted by cryopreserving the samples using curcumin. The present findings are consistent with previous studies conducted on ram and goat (Bucak *et al*., 2008, 2010), which shown that the antioxidant curcumin may help preserve the integrity of the sperm membrane after thawing. This stability was assessed using the HOST method and was found to be effective against oxidative stress. The presence of tGSH, GSH-Px, and SOD in semen serves as a defence mechanism against lipid peroxidation (LPO) and plays a crucial role in preserving sperm functions (O *et al*., 2006). However, the antioxidant capability in the sperm cell may not be enough to avoid lipid peroxidation (LPO) during the freeze/thawing process (Storey, 1997). Glutathione is a tripeptide thiol molecule that plays a crucial role in cellular physiology. It serves to protect cells from oxidative stress, aids in the production of proteins and DNA, and facilitates gamete fertilisation (Irvine, 1996). This finding aligns with previous studies conducted on ram and goat spermatozoa, which observed an elevation in tGSH levels when exposed to GSH, curcumin, and dithioerythritol during freezing or storage (Bucak *et al*., 2008, 2010; Coyan *et al*., 2010; Atessahin *et al*., 2008; Bucak *et al*., 2007). Nevertheless, the current research found that the addition of curcumin did not preserve the vitality and integrity of DNA during the thawing process, in contrast to the control group. The study observed that the curcumin content led to an increase in viability reduction and DNA fragmentation in post-thawed horse semen. The seminal plasma consists of several components such as proteins, fatty acids, and minerals. These substances act as a medium to safeguard and provide nourishment to sperm until fertilisation occurs (Mehrotra *et al*., 2013). Additionally, seminal plasma is a multifaceted fluid containing many antioxidant mechanisms that safeguard sperm against reactive oxygen species (ROS) and DNA damage caused by cryopreservation or internal or external stressors (Eini *et al*. 2021).

In addition, seminal plasma fluid has an enzymatic defence mechanism consisting of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase. It also contains nonenzymatic antioxidants such glutathione, ascorbic acid, vitamin E, albumin, and taurine (Calamera *et al*., 2003). Additionally, polyunsaturated fatty acids have a vital impact on the capacity of sperm to survive and move (Martínez-Soto *et al*., 2013). Additionally, there exists a correlation between the content of fatty acids and the antioxidant capacity of seminal plasma. Equine spermatozoa are typically separated from seminal plasma before being subjected to cryopreservation. Consequently, sperm experience a reduction in the advantages provided by seminal plasma. In a research conducted by Moore *et al*. in 2005, it was shown that samples containing 5% seminal plasma exhibited a significant difference in the total and progressively motile spermatozoa compared to those exposed to 20% seminal plasma. Hence, more investigation is required to elucidate the impact of incorporating curcumin in the presence of seminal plasma on the viability and integrity of DNA in post-thawed stallion semen.

CONCLUSIONS

Curcumin at 0.32 µm/L resulted in higher motility, motion speed HOST values whilelevel at 0.32, 0.60, and 1.30µm/L provided lesscryoprotective effect on minor and major sperm abnormality. Supplementation with curcumin at all level showed significantly low in vitality and DNA fragmentation in comparison with the control groups. Except level 0.32 µm/L showed significantly affect in DNA fragmentation compared to other level. Supplementation with curcumin prior to the cryopreservation process may be recommended to facilitate the enhancement of sperm cryopreservation techniques. Furthermore, future research should focus on concentrations lower than those found in this study and on the molecular mechanisms of the antioxidative effects of the antioxidants curcumin during cryopreservation.

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