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Effects of Vitamin C and Melatonin on the Viability and Mitochondrial Distribution of In vitro Matured Dromedary Camel Oocytes

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Abstract

The purpose of this work was to look into the impact of antioxidants on the viability and mitochondrial distribution of dromedary camel oocytes, through 1) Study the effect of vitamin C (ascorbic acid) and melatonin on the maturation rate and fertilization rate of dromedary camel oocytes, 2) Study the impact of vitamin C and melatonin on the viability, mitochondrial distribution, and intensity of in-vitro matured dromedary camel oocytes. Camel oocytes were aspirated from ovaries. Oocytes of excellent and good quality were in-vitro matured using in vitro maturation media (TCM-199 + 10 ug/ml FSH + 10%fetal calf serum + 100 IU/ml Pregnant mare serum + 50 mg/ml gentamycin) without (control group) or with antioxidant groups (50 µg/ml vitamin C or 100 µM/ml melatonin) and incubated in CO2 incubator (38.5 ̊C, 5% CO2, 20% O2 and 95% humidity) for 40 hours. In vitro matured oocytes with 1st polar body were either in vitro fertilized using epididymal spermatozoa for detection the fertilization rate or stained with Hoechst 33342 (bis-Benzamide H33258) dye and Mito Tracker red dye for detection the viability and mitochondrial distribution and intensity using confocal microscope. Results showed that, the nuclear maturation rate of dromedary camel oocytes in vitro matured with antioxidants were significantly ($P<0.001$) higher than the control group. The fertilization rate of in vitro matured oocytes in the control group (56.26 ± 0.63) was lower than vitamin C and melatonin group (70.88 ± 0.87 and 76.63 ± 0.82, respectively). The mitochondrial intensity was significantly increased in the vitamin C (118.3 ± 1.13) and melatonin (231.1 ± 4.06) groups when compared with control group (102.5 ± 2.16). The melatonin group was significantly higher in peripheral mitochondrial distribution when compared with control and vitamin C groups, while the diffused distribution of mitochondria was markedly greater in the vitamin C and control group when compared with melatonin group. In-conclusion, Antioxidant's supplementation on in vitro maturation media were significantly improved the maturation rate and fertilization rate through enhancing the mitochondrial intensity and distribution in Dromedary camel oocytes.

Keywords: Dromedary camel oocytes; Vitamin C; Melatonin; Mitochondria; oocyte viability.

1. Introduction

Dromedary camels, sometimes known as Arabian camels, account for 90% of the camelus genus (1). Due to its ability to supply milk, meat, and transportation in tough, arid climate, the camel has been regarded as a valuable animal in desert regions for centuries. Camels' reproductive efficiency is widely thought to be low in natural situations. This could be attributed to a short breeding season, a long prepubertal stage, a 13-month gestation period, and a protracted period of lactation-related anestrus (8–10 months) resulting to a long inter-calving gap (2). In vitro embryo production (IVEP) is an essential biotechnology in livestock breeding. The in vitro fertilization (IVF) and the development of in vitro embryo generation have recently been regarded as another alternative for camel genetic enhancement. The use of these methods, however, is contingent on the accessibility of developed and completely competent oocytes. As a result, oocyte maturation in vitro is utilized to produce a large number of mature oocytes capable of developing into embryos and leading to live births following the transfer to recipient animals. The maturity of the oocyte is a requirement for a successful in vitro fertilization procedure (3). In in vitro fertilization technique, oxidative stress is a significant component that

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influences the overall yield of viable embryos. Antioxidant defense mechanisms exist in both male and female reproductive systems, which quench reactive oxygen species (ROS) and maintain a healthy balance of pro- and antioxidants. This protects the gonadal cells and gametes from oxidative damage, which is necessary for optimal reproductive activity (4). Oocytes and embryos are damaged by oxidative stress caused by events such as light exposure, manipulation of gametes and embryos, high oxygen concentrations, and uncommon metabolites and substrates concentrations during assisted reproductive technologies procedures (5). Owing to the shortage of natural defensive mechanisms within the body, the lack of natural antioxidant defenses, and the availability of multiple possible ROS-inducing sources, the deleterious impact of in vitro generated oxidative stress may be magnified (6). In order to acquire the in vitro fertilizing ability, the balance maintaining between ROS and scavenging is critical. Mammalian oocytes mature through a series of discrete and complex nuclear and cytoplasmic maturation mechanisms (7). Cytoplasmic maturation entails a series of events such as reorganization of organelles and storing proteins, mRNAs, and factors of transcription, all of which are crucial in a serial of processes initiated with process of maturation and fertilization, and early embryogenesis, whereas nuclear maturation is primarily concerned with chromosomal segregation (8). During in vitro embryo production, supplementing in vitro maturation media with substances having antioxidant capabilities may avoid an excessive increase in ROS, enhance the quality of developed oocytes, and hence boost the efficiency of in vitro embryo creation (9). Vitamin C (ascorbic acid) is a powerful reducing agent and free radical scavenger in the biological system, limiting damage from these dangerous chemicals. Ascorbic acid serves two biological functions: antioxidant and enzymatic cofactor (10). It is a water-soluble antioxidant present in the ovaries (11). Female infertility is affected by the availability of ascorbic acid in vivo as its deprivation is associated with abnormal follicular growth and failure of meiosis resumption (12). In vitro, appropriate cytoplasmic maturation (13) and embryo developmental competence would require a threshold intracellular concentration of ascorbic acid in the oocyte (14). The pineal gland synthesizes melatonin (5-methoxy-N-acetyltryptamine) from tryptophan, an important amino acid. Melatonin, in addition to its role as a biological clock synchronizer and a regulator of seasonal reproduction, has a variety of other functions (15). It affects the reproductive system by lowering oxidative stress, changing the shape of follicles and ovarian steroidogenesis capacity of follicle and corpus luteum (16). Because of its high lipophilicity and hydrophilicity, it can transfer into other organs and fluids through cell membranes (17). Melatonin and its metabolites are effective free radicals' scavengers (18) as well as indirect antioxidants (17) due to their stimulatory action on the antioxidant system. Melatonin can boost the effectiveness of the mitochondrial electron transport chain by working in tandem with other antioxidants (19). Melatonin also acts as a chelator, which may help to reduce metal-induced toxicity (20). In vitro fertilization of matured dromedary camel oocytes necessitates proper sperm and oocyte preparation in order to get high-quality results. In camels, sperm preparation is regarded as one of the most necessary aspects of in vitro embryo formation. Different animals, such as the llama (21), camel (22,23), and cattle (24), have employed epididymal spermatozoa with high fertilization rates. Oocyte maturation, fertilization, and development are all dependent on mitochondrial maturation and reconfiguration (25). Mitochondria are reallocated to a variety of locations throughout oocyte maturation and early embryo development, which is important for oocyte quality since oocytes with low developmental potential have an irregular mitochondrial distribution and lower ATP levels (26). The effectiveness of an in vitro fertilization treatment depends heavily on proper nuclear and cytoplasmic maturation. The goal of this work was to study the impact of supplementation of in vitro maturation media with antioxidants (vitamin C/Melatonin) on fertilization rate, mitochondrial distribution, and intensity of in vitro matured dromedary camel oocytes.

2. Materials and methods:

The present experiments were carried out at Embryo and Genetic Resources Conservation Bank, National Research Centre, Giza, Egypt. All chemicals and reagents were bought from Sigma-chemical Company, unless stated mentioned. Data expressed for 5 replicates in each group

Collection of dromedary camel oocytes:

We obtained our samples, Dromedary camel ovaries, from camels which slaughtered in Giza's El-Warraq. The ovaries were kept at 37°C in sterile normal saline (0.9% sodium chloride, Nacl) supplemented with 100,000 IU/l penicillin and 1g/l streptomycin in a thermos container. Within 2-3 hours of the animals' slaughter, the ovaries were delivered to the laboratory.

A 21-gauge needle linked to a 10 ml syringe was a device that used to aspirate oocytes from follicles (3-8 mm in diameter). The aspiration medium was modified phosphate buffer saline (m-PBS) with Bovine serum albumin (4 mg/ml) added. Under stereomicroscope, recovered oocytes were divided into four groups according to Kandil et al. (27):- excellent: oocytes with homogeneous granulated cytoplasm and more than three layers of dense cumulus cells, good: 3 layers of tight cumulus cells surround oocytes with equally granulated homogeneous cytoplasm. fair oocytes that are partially surrounded with cumulus cells and partial granulation in cytoplasm and finally denuded oocytes without cumulus cells and granulation, only Excellent and Good quality oocytes were used for further experiments.

In vitro maturation of Dromedary camel oocytes:

Selected excellent and good quality oocytes were washed twice in m-PBS and once in maturation medium TCM-199 supplemented with 10% fetal calf serum (FCS), 50 mg/ml gentamycin, 10 µg/ml FSH (Foltrobin, Agtech, USA) and 100 IU/ml Pregnant mare serum (PMSG, Intervit, Holland). Maturation media, without any additive, acts as control group. The other groups in which antioxidants were added as following; 50 µg/ml Ascorbic acid (vitamin C) CH-9471 Buchs and 100 µM/ml Melatonin, M063178 (28).

Oocytes were cultured in 500 µL of in vitro maturation medium in Nunc™ 4-well dishes for 40 hr at 38.5 °C in 5% CO2 incubator with 95% humidity air. In vitro matured decumulated dromedary camel oocytes showed cumulus cells expansion and extrusion of the 1st polar body (3) Cytoplasmic maturation was judged through cumulus expansion according to Kandil et al. (27) into 4 grades (G0, GI, GII, GIII), Grade 0: no expansion of cumulus cells, Grade I: slight expansion, Grade II: moderate expansion, Grade III: full expansion.

Nuclear maturation rate **% =** $\frac{No.of\ Oocytes\ with\ 1st\ pb}{Total\ no\ of\ oocytes}$ **x 100.**

In vitro fertilization of in vitro matured Dromedary camel oocytes:

Testicles from mature dromedary camels were collected post-mortem at a local abattoir (El-Warraq) in Giza. They are transported to the laboratory in an ice box. Testes and epididymides were removed from the scrotal sac. Collection of epididymal sperm by using the retrograde flushing technique as performed by Turri et al. (29) using insulin syringe loaded with 1 ml of warmed (37°C) Sperm TALP (30). Sperm quality was analysed immediately after flushing. Individual motility ranged from 60-70 %. The obtained sperm suspension was centrifuged for 10 min at 1800 rpm with SP-TALP then the supernatant was thrown away, and the pellet was rinsed with F-TALP and centrifugated at 1800 rpm for 5 min. The in vitro matured oocytes were washed in the F-TALP then transferred 4-well dishes. The motile spermatozoa 1x 106 / ml were added to oocytes. Addition of PHE (penicillamine 20 µm/ml, hypotaurine 10 µm/ml and epinephrine 1µm/ml) in 4 well and incubated for 20 hr at 38.5 °C in 5% CO2. Detection of fertilized oocytes by extrusion of the 2nd polar body.

Fertilization rate% = $\frac{\text{No.of fertilized oocytes (2nd polar body)}}{\text{No. of matured oocytes}}$ x 100

Evaluation of mitochondrial intensity and distribution of in vitro matured dromedary camel oocytes

In vitro matured camel oocytes were washed in PBS+ 0.1% PVP, stained in Hoechst 33342 (bisbenzimide H33258) in concentration 1 µg/ml for staining DNA of matured oocytes, incubated for 15 minutes in CO2 incubator at 37°C, then washed using PBS 2 times and stained in Mito Tracker red FM (Invitrogen) in concentration 100 nM, incubated for 30 minutes/ 37°C, then oocytes were washed 2 times in PBS and examined for viability, mitochondrial intensity and distribution of in vitro matured Dromedary camel oocytes under Confocal microscope (Zeiss,710). The Hoechst Excitation is 346 nm and Emission is 460 nm while Mito Tracker Excitation is 581 nm and Emission is 644 nm**.**

Statistical analysis:

Data were fed to the computer and analysed using IBM SPSS software package version 20.0

Qualitative data were described using number and percent while quantitative data was described as mean± standard error (SE). The significance of differences was tested by paired t-test and analysis of variance (ANOVA) followed by post hoc test. Usage of Chi-square test for categorical variables.

Results:

Experiment 1: Effect of antioxidants on maturation rate of Dromedary camel oocytes

The mean percent (%) of GIII cumulus expansion of camel oocytes matured in maturation media supplemented with melatonin (66.10±0.37) was significantly higher than control (60.82±0.66) and vitamin C (61.98±0.75), cumulus expansion GII in vit C (22.27±0.44) and melatonin (23.85±0.47) were significantly higher than control (19.54±0.85), the

cumulus expansion in oocytes of control group (11.81±0.64) with GI was significantly higher than vit c (8.79±0.82) and melatonin (5.89±0.57) . The G0 cumulus expansion was significantly higher in control (7.83±0.84) and vit c (6.95±0.30) when compared with melatonin (4.16±0.44) (Table 1, Fig.1). The first polar body extrusion was studied as a nuclear maturation indication (Table 2, Fig. 2,3). The matured camel oocytes with polar body were significantly higher (P<0.001) in oocytes in vitro matured with vitamin C and melatonin (80.99 ± 0.97 and 85.67 ± 0.89 respectively) when compared to the control group 75.28 ± 0.71.

Table 1: **Effect of antioxidants on cytoplasmic maturation rate of Dromedary camel oocytes**

Cytoplasmic Maturation Rate (mean±SE) %				
Groups	**GIII**	**GII**	**GI**	**G0**
Control	60.82±0.66[b]	19.54±0.85[b]	11.81±0.64[a]	7.83±0.84[a]
Vitamin C	61.98±0.75[b]	22.27±0.44[a]	8.79±0.82[b]	6.95±0.30[a]
Melatonin	66.10±0.37[a]	23.85±0.47[a]	5.89±0.57[c]	4.16±0.44[b]
F(P)	**20.276 (<0.001)**	**12.569 (0.001)**	**18.769 (<0.001)**	**11.231 (0.002)**

a-b-c superscribe the significant difference in the same column.

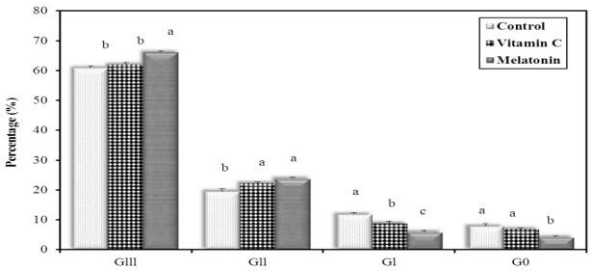

Figure (1): Effect of antioxidants on cytoplasmic maturation rate of dromedary camel oocytes

Table2: Effect of antioxidants on nuclear maturation rate of Dromedary Camel oocytes

	Nuclear maturation rate (mean ± SE) %		
Groups	**No of oocytes**	**Metaphase II (oocytes with 1st polar body)**	
		No	**(mean±SE) %**
Control	**311**	**234**	**75.28 ± 0.71[c]**
Vitamin C	**318**	**258**	**80.99 ± 0.97[b]**
Melatonin	**322**	**275**	**85.67 ± 0.89[a]**
F(P)		**35.783 (<0.001)**	

a-b-c superscribe the significant difference in the same column

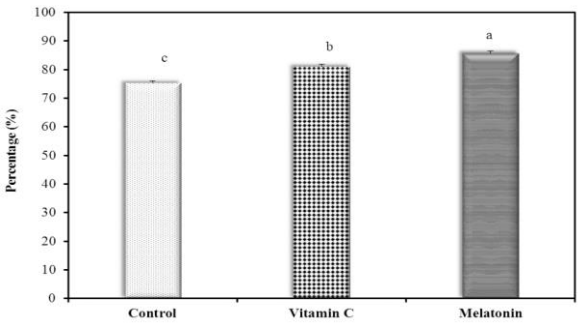

Figure (2): Effect of antioxidants on nuclear maturation rate of dromedary camel oocytes

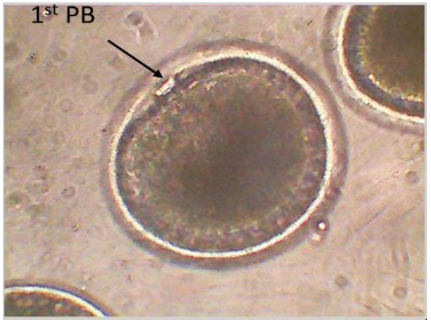

Fig 3, Nuclear maturation of dromedary camel oocytes (1st polar body)

Experiment 2: Effect of antioxidants on fertilization rate of in vitro matured Dromedary camel oocytes

We investigated the number of in vitro fertilized oocytes by extrusion of the 2nd polar body as shown in Table 3, Fig. 4,5. There was a considerable difference in the mean % of fertilized camel oocytes in melatonin group (76.63 ± 0.82) and vitamin C group (70.88 ± 0.87) when compared with control group (56.26 ± 0.63).

Table 3: Effect of antioxidants on fertilization rate of in vitro matured dromedary camel oocytes

	Fertilization Rate (mean ± SE) %		
Group	**No of matured oocytes**	**No of fertilized oocytes (2nd polar body)**	
		No	**(mean ± SE) %**
Control	**234**	**131**	**56.26 ± 0.63c**
Vitamin C	**258**	**183**	**70.88 ± 0.87b**
Melatonin	**275**	**211**	**76.63 ± 0.82a**
F (P)			**181.03 (<0.001)**

a-b-c superscribe the significant difference in the same column

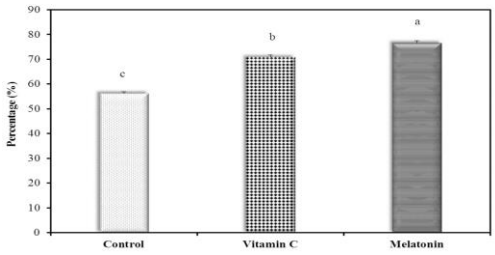

Figure (4): Effect of antioxidants on the fertilization rate of in vitro matured Dromedary camel oocytes

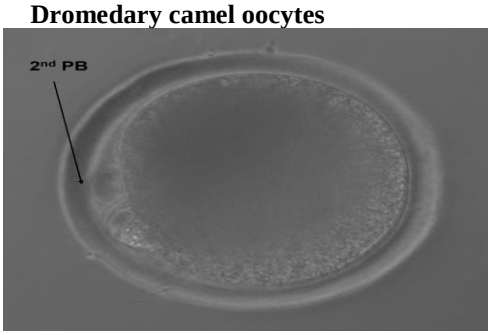

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Fig 5, Fertilized dromedary camel oocyte (2nd polar body)

Experiment 3: Effect of antioxidants on mitochondrial intensity and mitochondrial distribution of in vitro matured Dromedary camel oocytes

The effects of vitamin C and melatonin supplementation to IVM medium on the mitochondrial intensity and distribution were investigated. There was a significant difference between the three groups in Mito Tracker Red FM uptake among the oocytes as measured by the fluorescence intensity using confocal microscope (Table 4, Fig. 6). The mitochondrial intensity was significantly higher in melatonin group (231.1 ± 4.06) and vit c group (118.3 ± 1.13) when compared with control group (102.5 ± 2.16). At the same time there was significant difference in the mitochondrial intensity in melatonin group when compared with vit c group. Additionally, there was also a significance between them in the distribution of active mitochondria in the in vitro matured oocyte cytoplasm among oocytes groups (Table 5, Fig. 7). Significantly higher proportions of oocytes with peripheral mitochondria (fig, 8) were observed in oocytes in vitro matured with melatonin (57.1%) when compared with vitamin C (27.3%) and the control (6.3%) while the oocytes with diffused mitochondria was significantly higher in oocytes in vitro matured with vitamin C (63.6%) and control group (56.3%) when compared with melatonin group (4.8%). Melatonin and control groups were significantly higher in semi-peripheral distribution of active mitochondria 38.1% and 37.5%, respectively when compared with vit c group (9.1%).

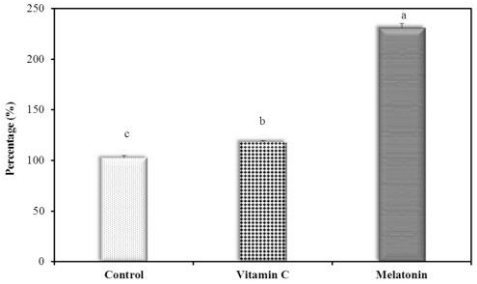

Figure (6): Effect of antioxidants on the mitochondrial intensity of in vitro matured Dromedary camel oocytes

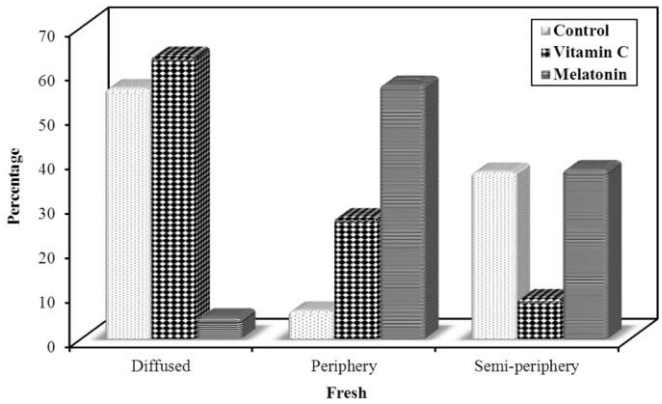

Figure (7): Effect of antioxidants on the mitochondrial distribution of in–vitro matured Dromedary camel oocytes

Table 4: Effect of antioxidants on mitochondrial intensity of in vitro matured Dromedary camel oocytes

	control	**Vitamin C**	**Melatonin**
No of in vitro matured oocytes	**32**	**33**	**42**
Mitochondrial intensity (mean±SE)	**102.5 ± 2.16^{c}**	**118.3 ± 1.13^{b}**	**231.1 ± 4.06^{a}**
F (P)	**657.584 (<0.001)**		

a-b-c superscribe the significant difference in the same column

Table 5: Effect of antioxidants on mitochondrial distribution of in vitro matured Dromedary camel oocytes

	Control (n = 32)		**Vitamin C (n = 33)**		**Melatonin (n = 42)**		**p**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Diffused	18	56.3^{a}	21	63.6^{a}	2	4.8^{c}	
Periphery	2	6.3^{c}	9	27.3^{b}	24	57.1^{a}	<0.001*
Semi-periphery	12	37.5^{b}	3	9.1^{c}	16	38.1^{b}	

a, b, c, p value for Chi square test for comparing between the different columns

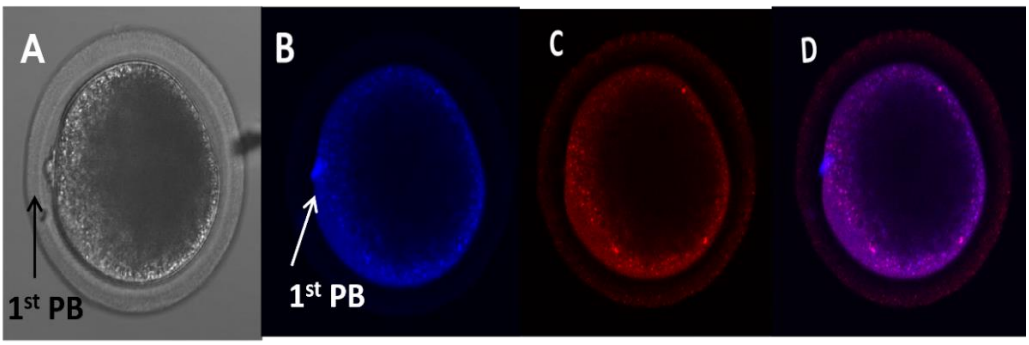

Fig 8: Viability and mitochondrial distribution on in-vitro matured dromedary camel oocytes (A) matured oocyte without staining, (B) matured oocyte stained with Hoechst dye showed 1st polar body (C) matured oocyte stained with Mito Tracker red FM showed peripheral distribution (D) matured oocytes stained with Hoechst dye and Mito Tracker red FM.

Discussion:

Reactive oxygen species are produced during normal aerobic metabolism in all cells, such as during intermediate steps of oxygen reduction in the mitochondrial electron transport chain and multiple enzymatic processes (31). ROS operate as signaling molecules in a variety of physiological mechanisms when they are in their balanced state (32). ROS, on the other hand, in large concentrations have harmful effects on cells, resulting in cell death and damage. (33). Antioxidants were introduced to boost the efficiency of embryo generation by efficiently controlling reactive oxygen species and the formation of oxidative stress (9). When comparing the maturation rate and fertilization rate of dromedary camel oocytes in vitro matured in maturation medium containing 50 µg vitamin C to the control group, the current study found a significant difference. Our results reflect the positive impacts of antioxidants addition on the maturation rate and fertilization rate of in vitro matured dromedary camel oocytes. The present results agree with previous reports such as Miclea et al. (37) and Huang et al. (38) discovered that using 50 µg/ml vitamin C increased conception rates and improved the growth of embryos derived from somatic cell with nuclear transfer. Pernes et al. (34) found that adding ascorbic acid to an in vitro maturation system of canine oocytes at a specific concentration (250 M) improves resumption of meiosis and maturation rates. According to Mallol et al. (35) and Eppig et al. (36) there was an improvement in the quality of mice oocytes as well as an increase in embryo production. Supplementation the in vitro maturation medium with 50 µg/ml vitamin C improves the maturation rate and blastocyst development in bovine (39). Also, Khattab et al. (40) found that vitamin C can improve oocytes maturation and embryo development in camel. Ascorbic acid showed a very positive impact on improving developmental competence of caprine oocytes and increased maturation rates during heat stress (41). These results are returned to the positive role of vitamin C on cells. It protects lipid membranes and proteins from oxidative damage, making it the initial line of antioxidant defense. It can work not only inside the cells, but also outside them, as it is water soluble molecule, neutralizing free radicals and preventing the damages caused by these harmful molecules (42). Ascorbates are relatively stable in their oxidized state and do not cause cell damage (43). In contrast to our results, according to Nohalez et al. (45), adding 50 µg/ml vitamin C to maturation media had no effect on the maturation rate of swine oocytes, fertilization rates, or embryo growth following IVF., but it is reported that its addition on vitrification-warming media increased the embryo survival. Previous bovine research has yielded similar findings (46). According to Cordova et al. (47), in-vitro maturation of prepubertal bovine oocytes with L-ascorbic acid had no influence on maturation rates but dramatically lowered the embryos proportion that progressed to the blastocyst stage. The difference in results may be due to species difference, the dose of vitamin C and the season. According to several studies, supplementing in vitro maturation media with a high concentration of vitamin C has some negative side effects, such as slowing down the maturation process and that may be due to the two different action of vitamin C: lower amounts have an antioxidant effect, while greater quantities have a pro-oxidant effect (48). Melatonin is a multifunctional and a universal natural potent antioxidant and free radicals' scavenger (18). we found in our study that supplementation of the in vitro maturation media with 100 µM/ml melatonin improves maturation rate and fertilization rate of Dromedary Camel oocytes. Our findings corroborate with those of Fathi et al. (49) who found that 25 M melatonin increased the in vitro developing capability of dromedary camel oocytes. Numerous investigations have revealed that melatonin supplementation has a positive impact on IVM of oocytes and enhances their development in different species such as in bovine (51). Also, Lin et al. (52) discovered that melatonin addition in combination with prolonged IVM can boost the quality and growth of porcine oocytes with low quality. These findings are comparable to those of Park et al. (53) and Yang et al. (54) who found that melatonin promotes porcine oocyte meiotic maturation and cumulus cell growth. Additionally, its beneficial effects on maturation rate of ovine oocytes promoting their meiotic competence (55). The addition of 1.0M melatonin to in vitro maturation conditions promotes nuclear maturation of GV mouse oocytes while lowering cumulus cell death. (56). Takada et al. (57) found that adding 10-9 M to in vitro maturation media protects bovine cumulus cells from DNA induced damage while having no effect on in vitro embryo development. Melatonin at 10-6 M supports conception and early embryo development in mice following in vitro fertilization, according to Ishizuka et al. (58). Recently, An et al. (59) discovered that 10-9 M melatonin boosted bovine oocyte maturation and greatly improved the subsequent development. In contrast to our results, Farahavar and Shahne (60) found that melatonin failed to promote the maturation of bovine oocytes. Antioxidant supplementation could enhance oocyte quality and embryo development by improving

mitochondrial activity. Our results found that vitamin C improve the developmental competence of dromedary camel oocytes and increase the mitochondrial intensity to become 118.3 ± 1.13 compared to control 102.5 ± 2.16. Besides, 63.6% of oocytes in vit C group with diffused mitochondrial distribution manner in the cytoplasm. Vitamin C is a potent antioxidant that scavenges free radicals (9). By modulating some key genes involved in mitochondrial redox state, its addition to culture media increases the quality and survival rates of pig cryopreserved embryos (10). KC et al. (44) found that the dehydroascorbic acid (DHA), oxidized vitamin C form, enters mitochondria by facilitative glucose transporter 1 (Glut1) and accumulates in mitochondria as ascorbic acid protecting the mitochondrial genome and membrane from oxidative damages. The function of vitamin c in mitochondrial activities in matured oocytes need further studies. The cytoplasmic and nuclear maturation of oocytes are influenced by mitochondrial distribution and metabolic activity (mitochondrial intensity) (61). Melatonin is an antioxidant that targets mitochondria (62). It accumulates at a higher concentration in mitochondria than in other organelles or subcellular sites, despite a concentration gradient. This could be accomplished by the active transport of melatonin by the mitochondrial melatonin transporter (s). Melatonin safeguards mitochondria by scavenging ROS and blocking the permeability transition pore in mitochondria (MPTP). Melatonin preserves mitochondrial functioning by maintaining the optimum mitochondrial membrane potential (63). Thus, in our research, we discovered that labelling mitochondria with Mito Tracker Red revealed a significant difference between oocytes in vitro matured in melatonin-supplemented maturation conditions and the control group. The mitochondrial intensity was significantly higher in melatonin-treated oocytes (231.1 ± 4.06) when compared with the control group (102.5 ± 2.16). Also, the distribution of oocytes in melatonin-treated group are changed to be most of oocytes became with periphery mitochondria. These results agree with An et al. (59) and Zhoa et al. (51) who found that melatonin raised the normal mitochondrial distribution in bovine. Using confocal analysis, Matured camel oocytes displayed a highly polarized mitochondrial distribution pattern in the subcortical area, according to Abdoon et al. (7). Kafi et al. (64) also found that the majority of mitochondria were found in the matured camel oocyte's periphery.

In conclusion:

Vitamin C and melatonin significantly increase the maturation rate and fertilization rate through enhancing the mitochondrial intensity and distribution in Dromedary camel oocytes.

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Conflicts of interest

There is no conflicts of interest in this work.

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