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# Effect of L-carnitine on the in vitro maturation and fertilization of bovine oocytes

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**ABSTRACT**

The present study pointed out to clarify the effects of different L-Carnitine concentrations (0, 0.3, 0.6, and 1 mg/ml) on the *in-vitro* maturation, fertilization and blastocyst formation rates of bovine oocytes, in addition to assessing the lipid peroxidation rate and total antioxidant enzymes' capacity. Bovine ovaries were collected from the El-Mounib abattoir in Giza Governorate, Egypt. The oocytes were collected by using the aspiration technique. The oocytes with (4-8mm) diameters were selected, washed, and matured in TCM-199 supplemented with different L-Carnitine concentrations. Afterward the period of maturation, the oocytes were evaluated for nuclear and cytoplasmic maturation; the oocytes were analyzed to determine the levels of Malondialdehyde (MDA), Catalase (CAT), Glutathione (GSH), and Superoxide dismutase (SOD) activities. The *in-vitro* matured oocytes were fertilized and assessed for the fertilization rate and the embryo developmental potentials. The results revealed that L-Carnitine at a concentration of 0.6 mg/ml significantly ($P \leq 0.05$) improved the oocyte maturation (M-II) and fertilization rates besides the different stages of embryo development. In addition, L-Carnitine noticeably ($P \leq 0.05$) diminished the lipid peroxidation (MDA) and increased the antioxidant enzymatic activity including: Catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) mainly at a concentration of 0.6 mg/ml. In conclusion, 0.6 mg/ml of L-Carnitine has potentially improving impacts on *in-vitro* matured and developmental competence of bovine oocytes

## 1. INTRODUCTION

Cattle production systems can improve genetic gain and fertility by using *in-vitro* embryo production (IVEP) (Hansen and Block, 2004). IVEP is a useful research tool for understanding embryo development (Hansen et al., 2014). IVEP system is crucial for the application of other technologies such as somatic cell nuclear transfer and the production of transgenic cows' production (Maksimenko, et al., 2013). Despite these advantages, several problems are associated with IVEP that limit its practical application in cattle production systems, including sub-optimal embryonic and fetal survival (Hasler, 2000).

The oocyte maturation and culture conditions are key parameters for successful IVEP in mammals (Zhao et al., 2024). Energy metabolism is essential for oocyte maturation and embryonic development as these processes require a high level of energy from various substances, such as carbohydrates, amino acids, and lipids (Downs, 2015). Because fatty acids have higher energy levels than carbohydrates, they are an essential energy source for oocyte maturation and development (Dunning and Robker, 2012). They are carried to the mitochondria, where β-oxidation breaks them down to generate ATP (Aitken, 2020), oxidative phosphorylation produces reactive oxygen species (ROS) that lead to lipid peroxidation as well as apoptosis, and mitochondrial dysfunction (Yin et al., 2016).

For maintaining the equilibrium between production and removal of ROS, the oocyte possesses an antioxidant mechanism "either enzymatic or non-enzymatic" (Silva and Silva, 2023). Enzymatic systems include superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) (Von-Mengden et al., 2020). The non-enzymatic compounds are substances from exogenous sources which prevent the effect of oxidative stress such as vitamin E, cysteamine, vitamin C, vitamin A, pyruvate, taurine, and hypotaurine antioxidants (Khazaei and Aghaz, 2017; Uddin et al., 2020). One of the worst effects of free radicals' attack is the oxidation of unsaturated fatty acids (lipid peroxidation) that results in the production of Malondialdehyde (MDA) which is a byproduct of lipid peroxidation and has the stability to be employed as an accumulative marker of lipid peroxidation (Rakha et al., 2022).

L-Carnitine (LC) is an organic vitamin-like, water-soluble quaternary ammonium molecule, that contributes to β-oxidation and potentially enhances mitochondrial function (Zolini et al., 2019; Carrillo-Gonz´alez and Maldonado-Estrada, 2020). To facilitate the β-oxidation process, it transports long-chain fatty acids from the cytosol to the mitochondria (Chankitisakul et al., 2013). Additionally, it behaves like an antioxidant for preventing embryo apoptosis and preserves the oocyte from DNA damage (Mansour et al., 2009). Moreover, L-Carnitine stimulates lipid metabolism and ATP synthesis (Zare et al., 2017; Zolini et al., 2019).

Numerous researches were conducted on the beneficial effects of L-Carnitine supplementation on mammalian oocytes. L-Carnitine was reported to increase the mitochondrial activity in the *in-vitro* matured oocytes in mammals (Placidi et al., 2022), buffalos (Liang et al., 2020) and sheep (Mishra et al., 2016), lowering lipid droplet and

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ROS levels in pigs (Somfai et al., 2011; Xu et al., 2020). L-Carnitine supplementation during *in-vitro* maturation procedure (IVM) improved the nuclear maturation and subsequently embryo developmental potentials of oocytes in bovine (Phongnimitr et al., 2013; Liang et al., 2020) and mouse (Moawad et al., 2014).

The goal of the current study was to ascertain how L-Carnitine incorporation in the *in-vitro* maturation media of bovine oocytes affects their cytoplasmic and nuclear maturation, lipid peroxidation and oxidative stress, as well as the embryos' developmental potentials.

## 2. MATERIAL AND METHODS

This study was conducted in the IVF unit of the Animal Reproduction Research Institute, El Haram, Giza Governorate from November 2023 to March 2024. All experiments and samples` manipulations were authorized by the Ethics Committee of the Faculty of Veterinary Medicine, Benha University (BUFVTM01-05-29).

### *2.1 Chemicals and media*

The Media was prepared using standard protocol following aseptic measures. Only cell culture tested chemicals were used to formulate the media. All the media were filtered using a 0.22 µm pore size filter (Durapure® membrane filter, Ireland) and equilibrated at 38.5°C with 5% $CO_2$ in a humidified air for at least 2 hours before use for media PH calibration.

### *2.2. Samples*

A total number of 90 cattle ovaries (336 oocytes) were collected in the period from November 2023 to March 2024. The ovaries were harvested from the El-Mounib abattoir in Giza Governorate, Egypt, and transferred to the lab within 2–3 h after slaughtering. In a thermal flask with pre-warmed normal saline solution (0.9% NaCl) supplemented with 10 mg/ml streptomycin, and 10,000 IU/ ml penicillin (sigma-Aldrich, A5955, USA) (Pang, 2019). In the lab, the ovaries were trimmed with sterile scissors, washed 3–5 times with sterile warm (37°C) 0.9% saline (Zhao et al., 2024), and maintained at this temperature till aspiration.

### *2.3. Oocyte recovery and selection*

Using a needle "18-gauge" attached to a sterile 10-milliliter syringe filled with 0.5 ml of the aspiration media, Modified Dulbecco's phosphate-buffered saline (M-DPBS) supplemented with bovine serum albumin (BSA) 6 mg/ml (Biowest, France, P6154) and gentamicin (Gentacure-10, Pharma Swede, Egypt, 221207) 50 mg/ml, Oocytes were aspirated from the follicles with 4–8 mm diameter on the surface of the ovaries (Yousaf and Chohan, 2003). The aspirated follicular fluid was left for 5 minutes in a 37°C water bath to settle down (Rahman et al., 2020). The Follicular fluid content settling down took place by placing the collecting tube in a warmer block at 37°C for 10-15 minutes, the sediments was transferred by a plastic Pasteur into a clear falcon© petri dish containing the aspiration media and examined using a stereo microscope (Nikon, Tokyo, Japan) for Cumulus-oocyte complexes (COCs) selection (Elmetwally et al., 2022; Zhao et al., 2024). COCs with compact, granular, homogenous, and several layers (at least 4 layers) of cumulus cells and uniform cytoplasm were picked up, and washed three times in each the aspiration medium, then the maturation media (Elmetwally et al., 2022; Zhao et al., 2024).

### *2.4. In vitro maturation*

TCM-199 (199E, VACSERA, Egypt,1) with phenol red enriched with 10% inactivated fetal calf serum (FCS) (Sigma-Aldrich, USA, 0001659514), 10µg/ml LH, 5µg/ml FSH, 1µg/ml Estradiol-17β (E2) (Sigma-Aldrich, USA, 77H0666), 2.2mg/ml sodium pyruvate (oxford lab chem, India, 113-24-6),10 mg/ml streptomycin, and 10,000 IU/ ml penicillin (sigma-Aldrich, A5955, USA) were used to perform the *in-vitro* maturation of the selected oocytes (Bahgat et al., 2023). L-Carnitine (Sigma-Aldrich, USA, C0158-5G) was prepared with the IVM media in different dilutions according to the experimental design (control (0.0), 0.3, 0.6 and 1 mg/ml) (Liang et al., 2020; Elmetwally et al., 2022). A disposable NUNC™ culture dish was filled with 100µL droplets of the maturation medium (IVM), overlaid with sterile mineral oil (Biomedia, LVO-720), and incubated for two hours prior to use at humidified atmosphere with 38.5°C, 5% $CO_2$ then the oocytes were cultured at a rate of 15-20 oocytes/drop in the dish and incubated for 20 hrs (Bahgat et al., 2023; Zhao et al., 2024).

#### *2.4.1. Assessment of the cytoplasmic and nuclear maturation*

The oocyte cytoplasmic maturation rates were evaluated under a stereomicroscope to assess the cumulus cells expansion (Abeydeera, 2002; Alvarez et al., 2009). According to Mostagir (2019), the oocytes with a complete or moderate cumulus expansion and a first polar body extruded into the perivitelline space, were considered mature. Only healthy oocytes were used in the experiment and the degenerated oocytes were discarded.

##### *2.4.1.1. Oocyte staining by Aceto-Orcein stain for nuclear maturation assessment*

The final matured COCs were pipetted for complete cumulus cell removal after being gently denuded by using low-speed Vortex for two to three minutes (Pang, 2019; Zolini, 2019). The 1%Aceto-Orcein stain (Nice, 306029, India) applied to a subset of oocytes according to Moawad et al., 2021; Masoud (2023). The staining was done in 3 steps, fixation step: through transferring the COCs to a solution of sodium citrate "1% hypotonic" for about three minutes then 5–10 oocytes were placed on a sterile glass slide and covered with a glass coverslip that was fastened to the slide's four corners with four spots of Vaseline for at least 24 hours, and stored with a fixative solution [Ethanol: Acetic acid (3:1)]. The staining step: was applied in a jar containing 1% aceto-orcein stain (wt/vol) in 45% acetic acid (v:v) for 30 min. Finally, the washing step: was done by running a washing solution was applied (20% glycerol [v:v] and 20% acetic acid [v:v] in distilled water), between the slide and coverslip then assessed under a phase contrast microscope to evaluate the status of nuclear maturation based on nuclear chromatin configuration (Fig. 1) (Masoud et al., 2023).

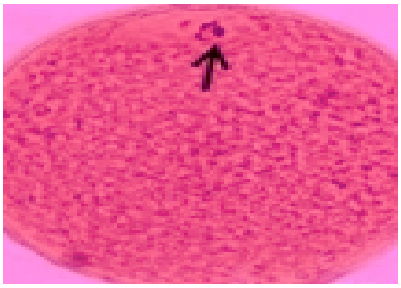

Fig. (1). Mature oocyte after staining with Aceto-Orcein stain. The arrow indicates the second metaphase spindle as a sign of oocyte nuclear maturation.

*2.4.2. Assessment of the effect of L-Carnitine impact on anti-oxidant enzymatic activities and Lipid peroxidation*

The effect of LC on ROS and lipid peroxidation levels was detected by the determination of the Malondialdehyde (MDA), Catalase (CAT), Glutathione (GSH), and Superoxide dismutase (SOD) levels after oocyte maturation. The tested samples from about 45 oocytes were prepared according to the kits' instructions, homogenized with buffer, then centrifuged for 15 min at 4000 rpm at 4 ◦C. At the end of the test, the level of each enzyme's activity was measured according to the kit's instructions at a specific wavelength.

*2.4.2.1. Determination of Malondialdehyde (MDA) level*

It was estimated by the MDA-Colorimetric method (Biodiagnostic, MD2529, Egypt). The final precipitate of tested sample was stored on ice in accordance with the kit's instructions, the principle of the test was a reaction between Thiobarbituric Acid (TBA) and Malondialdehyde (MDA) in the sample, resulted in a pink colored end product of Thiobarbituric Acid-Reactive Substances (TBARS). The absorbance of TBARS can be estimated at 535 nm (Satoh K., 1978; Olszewska-Słonina et al., 2011).

*2.4.2.2. Determination of Catalase (CAT) activity*

Catalase assay is an antioxidant biomarker which provides another useful tool for oxidative stress investigations. CAT was detected according to Aebi (1984) and Banerjee et al. (2010), using a catalase-Colorimetric method (Biodiagnostic, CA2517, Egypt). The principle depends on the decomposition action of CAT on $H_2O_2$. Since, each unit of catalase decomposes 1 µM of $H_2O_2$ per minute at 25°C and pH 7.0. This reaction is stopped after exactly one minute with catalase inhibitor then the remaining $H_2O_2$ form a chromophore with color intensity inversely proportional to the amount of catalase in the original sample. The optical density was measured at 510 nm.

*2.4.2.3. Determination of reduced glutathione level*

GSH concentration was measured by Glutathione Reduced-Colorimetric method (Biodiagnostic, GR2511, Egypt). The idea depends on the reduction of Dithionitrobenzoic acid (DTNB) with glutathione (thiol group) to produce a chromogenic yellow compound that directly proportional to GSH concentration. Glutathione concentration measured according to Beutler et al. (1963) and Kozer et al. (2003) at a wavelength of 405 nm.

*2.4.4.4. Determination of superoxide dismutase activity*

In accordance with Nishikimi et al. (1972) and Hamed (2016) the SOD concentration was estimated by Superoxide Dismutase -Colorimetric method (Biodiagnostic, SD2521, Egypt) . It relies on the ability of SOD enzyme to inhibit the reduction of nitroblur tetrazolium dye by phenazine methosulphate-mediated. At the end of the procedures, the level was detected at 560 nm wavelength.

*2.5. In vitro fertilization:*

*2.5.1. Preparation of IVF media*

Fertilization media used consists of fertilization-Tyrode's albumin lactate pyruvate medium (IVF-TALP) supplemented with 10µg/ml heparin for sperm capacitation (Badr, 2009). Fertilization media droplets of 100µl were allocated in a sterile culture dish overlaid with mineral oil and kept in the incubator with 5% $CO_2$ and maximum humidity (85-95%) at 38.5 ◦C for about two hours before sperm-oocytes co-incubation (Rahman et al., 2020). Only the healthy and mature oocytes were cultured in the fertilization drops at a rate of 10 oocytes/drop after being washed three times in the TALP medium (Elmetwally et al., 2022).

*2.5.2. Sperm preparation*

The most motile sperm were separated for the *in vitro* fertilization process using the swim-up approach (Mostagir et al., 2019; Rahman et al., 2020; Elmetwally et al., 2022). Shortly, a frozen semen straw thawed in a water container set at 37°C for 30 seconds (Badr, 2009; Najafi et al., 2014; Bahgat et al., 2023). Then swim-up processed by using 1ml of sperm-TALP medium supplemented with 6mg/ml of BSA (Biowest, France, P6154), incubated for 30 minutes at 37°C and followed by 2 centrifugations for 5 min for purifying the motile sperms. After the last centrifugation, the supernatant was disposed of, and 1 milliliter of TALP medium with 10 mg/ml heparin was added for capacitation, mixed and the pellet incubated in $CO_2$ incubator for 10 minutes at 38.5°C, 5% $CO_2$ and maximum humidity. The sperm parameters were evaluated and the sperm diluted to a concentration of $2\times10^6$ sperm/ml with 60-80% motility. In the fertilization droplets, 20 µl of prepared semen sample co-incubated (Elmetwally et al., 2022; Bahgat et al., 2023). We examined after 6 hrs the penetration rate, then extended the co-incubation for 18 hours, and oocytes with polyspermywere discarded. After 18 hrs, successfully penetrated oocyte is indicated when the sperm tail is visible through the egg zona pellucida (fig. 2) and the fertilized oocytes were detected by the presence of 2 pronuclei (2PN) (Rahman et al., 2020; Elmetwally et al., 2022).

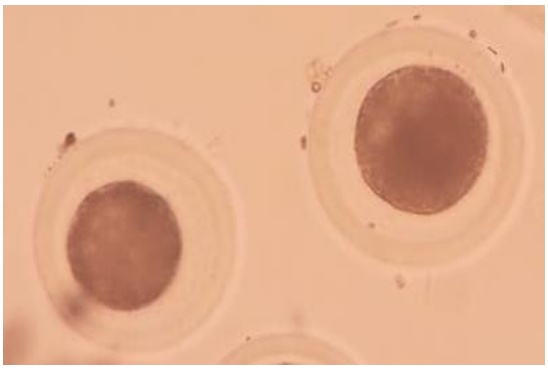

Fig. (2). Cattle oocytes with sperm Penetration

*2.5.3. In vitro culture (IVC) and embryo development*

50µl droplets of culture medium (synthetic Oviductal Fluid-SOF) that covered by mineral oil were prepared in a disposable sterile 60ml petri dish, which incubated in the incubator at 38.5◦C with 5% $CO_2$, 7% $O_2$ and 90-95% humidity (Räty etal., 2011; Elmetwally et al., 2022). The fertilized oocytes were gently denuded to remove any loosely linked spermatozoa and any attached cumulus cells, and then transferred to IVC media at a rate of 5 oocytes/ droplet. The culture medium was replaced with fresh medium every 48 hours until Day 7 post-incubation. 48 hours post-insemination, the cleavage percentage was recorded then at Day-5 and Day-7 post-insemination, the developed morula and blastocyst stages were recorded, respectively (Rahman et al., 2020; Elmetwally et al., 2022; Bahgat et al., 2023).

*2.6. Statistical analysis*

The data statistical analysis has been performed using SPSS ver. 27 (IBM Corp. Released 2013) according to Steel et al. (1997). One-Way ANOVA with Duncan's post hoc test was used to determine the significant difference in the different values of antioxidant enzymes.

Qui-square was used to determine the statistical difference in maturation and fertilization rates as well as the rate of embryo development. The statistical significance was set at $P \leq 0.05$

## 3. RESULTS

*3.1. The Effects of L-Carnitine supplementation to in vitro maturation media (IVM) on nuclear maturation rate of bovine oocytes*

As shown in Table 1, there was a significant difference at ($P < 0.05$) between the different cattle oocytes LC-treated groups. LC at a concentration of 0.6 mg/ml significantly ($P < 0.05$) elevated the percentage of oocytes with MII (48.27%) compared to the control and the other groups (0.3 and 1 mg/ml) that showed the lowest records. The 0.3 mg/ml LC concentration represented a moderate effect (39.29 %) while 0 and 1mg/ml concentrations showed the lowest effect on oocyte maturation improvement. On the other hand, the LC addition in concentrations of 0.3 and 0.6 mg/ml during *in vitro* maturation of cattle oocytes declined the degenerated oocytes percentage in contrast to the other groups.

*3.2. The effects of L-Carnitine supplementation to IVM of bovine oocytes on their fertilization rates*

The fertilization rate has been highly improved ($P < 0.05$) with LC at a concentration of 0.6 mg/ml with a percentage of 38.77% compared to the other experimental groups (Table 2). Lower enhancement of fertilization rate occurred at the concentration of 0.3 mg /ml, but no effect of 0 and 1.0 mg/ml concentration has been reported. No difference has been recorded regarding the penetration rate between the 4 different groups.

*3.3. The effects of L-Carnitine supplementation to IVM of bovine oocytes on their embryo development*

Table 3 demonstrated that the effect of LC on the different stages of embryo development. A highly significant improvement ($p < 0.05$) in the percentage of different stages of embryo development, cleavage stage, Morula stage to blastocyst formation stage, has been found with LC at a concentration of 0.6 mg/ml compared to the other experimental groups. On the other hand, the 0.3 concentration of LC weakly affects embryo developmental competence while 0 and 1.0 mg/ ml concentrations represented no effect on the embryo developmental stages.

*3.4. The effects of L-Carnitine supplementation to IVM of bovine oocyte on their lipid peroxidation & oxidative stress state*

As presented in Table 4, LC at concentrations of 0.3 and 0.6 mg/ml markedly ($P < 0.05$) decreased the level of MDA during the IVM of bovine oocytes compared to the other groups. In the meantime, LC at the concentration of 0.6 mg/ml significantly improved ($p < 0.05$) the levels of catalase, GSH, and SOD compared to the other groups.

Table 1 The Effects of L-Carnitine supplementation to *in vitro* maturation media (IVM) on nuclear maturation rate of bovine oocytes

| Experimental groups | No. oocytes | Nuclear status | | | Degenerated oocytes No. (%) |
|---|---|---|---|---|---|
| | | GV No. (%) | GVBD and MI No. (%) | MII No. (%) | |
| Control (0.0) | 52 | 2 (3.84) | 17 (32.69) | 14 (26.92) | 19 (36.53) |
| 0.3 mg/ml | 56 | 5 (8.93) | 23 (41.07) | 22 (39.29) | 6 (10.71) |
| 0.6 mg/ml | 58 | 3 (5.17) | 21 (29.31) | 28 (48.27) | 6 (10.34) |
| 1.0 mg/ml | 57 | 7 (12.28) | 19 (33.33) | 15 (26.32) | 16 (28.07) |
| Chi-square | - | 5.467 | 2.235 | 9.429 | 24.419 |
| P-value | - | 0.141 | 0.525 | 0.024 | 0.000 |

The experiment was replicated at least three times /group.

Table 2 The effects of L-Carnitine supplementation to IVM of bovine oocytes on their fertilization rates.

| Experimental groups | No. of mature oocytes | Penetration rate No. (%) | Fertilization rate No. (%) | Unfertilized oocytes No. (%) |
|---|---|---|---|---|
| Control (0.0) | 31 | 7 (22.58) | 5 (16.13) | 19 (61.29) |
| 0.3 mg/ml | 44 | 13 (29.54) | 10 (22.72) | 21 (47.72) |
| 0.6 mg/ml | 49 | 21 (42.86) | 19 (38.77) | 9 (18.36) |
| 1.0 mg/ml | 34 | 6 (17.64) | 4 (11.76) | 24 (70.58) |
| Chi-square | - | 12.386 | 18.889 | 32.101 |
| P-value | - | 0.006 | 0.000 | 0.000 |

The experiment was replicated at least three times /group.

Table 3 Effects of L-Carnitine supplementation to IVM on preimplantation embryo development of bovine oocytes

| Experimental groups | No. of fertilized oocytes | 2-8 cell stage No. (%) | Morula stage No. (%) | Blastocyst stage No. (%) | Uncleaved fertilized oocytes No. (%) |
|---|---|---|---|---|---|
| Control (0.0) | 29 | 3 (10.34) | 1 (3.45) | 0 (0.00) | 25 (86.21) |
| 0.3 mg/ml | 34 | 7 (20.59) | 3 (8.82) | 1 (2.94) | 23 (67.65) |
| 0.6 mg/ml | 26 | 8 (30.77) | 6 (23.07) | 3 (11.53) | 9 (34.62) |
| 1.0 mg/ml | 30 | 3 (9.68) | 1 (3.33) | 0 (0.00) | 27 (90.00) |
| Chi-square | - | 17.000 | 28.105 | 5.400 | 27.022 |
| P-value | - | 0.001 | 0.000 | 0.020 | 0.000 |

The experiment was replicated at least three times /group.

Table 4 Effect of L-Carnitine on lipid peroxidation & oxidative stress during in vitro maturation of bovine oocytes.

| Experimental groups | MDA nmol/ml | CAT U/L | GSH mM/L | SOD U/ml |
|---|---|---|---|---|
| Control (0.0) | $6.86 \pm 1.28^{ab}$ | $20.60 \pm 1.86^{b}$ | $2.95 \pm 0.71^{b}$ | $19.34 \pm 3.50^{b}$ |
| 0.3 | $3.52 \pm 0.36^{b}$ | $24.98 \pm 1.17^{b}$ | $3.78 \pm 0.83^{b}$ | $21.03 \pm 2.87^{b}$ |
| 0.6 | $2.63 \pm 0.66^{b}$ | $33.87 \pm 3.21^{a}$ | $8.22 \pm 0.96^{a}$ | $35.55 \pm 1.88^{a}$ |
| 1.0 | $5.66 \pm 1.19^{a}$ | $20.95 \pm 1.89^{b}$ | $2.65 \pm 0.42^{b}$ | $15.55 \pm 2.45^{b}$ |

The experiment was replicated at least three times /group. Data are presented as Mean±SEM. Values with different superscripts within the same column differed significantly at $P < 0.05$.

## 4. DISCUSSION

Oocyte maturation is an important step in assisted reproductive technology affected by various factors as the culture medium metabolites, oxygen concentration, and temperature (Nagai, 2001). It is a crucial stage that is affected by several variables, including temperature, oxygen content, and metabolites in the culture medium (Nagai, 2001). Lipid metabolism is an important energy source for the maturation of bovine oocytes and the development of embryos (Dalbies-Tran et al., 2020; Khan et al., 2021; Zhao et al., 2024). Cattle oocytes are distinguished by the presence of lipid contents in

the appearance of lipid droplets, which have a direct correlation with the oocytes' susceptibility to oxidative damage and may enhance oocyte maturation, fertilization, embryonic development, β-oxidation, and ATP generation (McKeegan and Sturmey, 2011; Liang et al., 2020; Zhao et al., 2024).

According to the current study results, the oocyte's nuclear maturation (M-II) fertilization rate, and embryo development were enhanced when LC (0.6 mg/mL) was supplemented to the bovine oocyte maturation medium. These results are coordinated with those reported by Phongnimitr (2013), Spricigo (2017), and Liang (2020). Furthermore, the supplementation of LC at a concentration of 0.5 mg/ml in the maturation media of porcine oocytes improved the rate of blastocyst formation with a marked decrease with the higher LC concentration (Wu et al., 2011). LC reduced DNA damage and improved oocyte chromosomal structure and blastocyst development (Abdelrazik et al., 2009; Knitlova et al., 2017). Elmetwally et al. (2022) reported that LC concentration of 0.3 mg/ml is the most effective concentration that improved recovery, survival rates of vitrified bovine oocytes and minimized abnormalities in zona pellucida in combination with Ethylene Glycol (EG) and Dimethylsulfoxide (DMSO) as cryoprotectants. On the other hand, Chankitisakul (2013), Xu et al. (2018), and Zhao (2024) reported a non-significant impact of LC on the bovine oocyte nuclear maturation due to differences in the maturation media components and in vitro maturation period.

It had been stated that LC concentrations of 0.6 and 1.2 mg/mL had the same blastocyst formation rate in cattle (Liang et al., 2020), however, it was suggested to use LC at a concentration of 0.6 mg/mL as higher concentrations may detrimentally deplete lipid density that adversely affect the embryo development (Sutton-McDowall et al., 2012). The transfer of free fatty acids from the cytosol into the mitochondrial β-oxidation cycle, which includes nuclear and cytoplasmic oocyte maturation, is stimulated by L-Carnitine (Paczkowski et al. 2013; del Collado et al., 2017). In the current study, LC at a concentration of 0.6 mg/ml reduced MDA levels and improved the activity of catalase, GSH, and SOD. It has been recorded that L-Carnitine decreased the intensities of lipid droplets and the ROS in the *in vitro* matured porcine (Wu et al., 2011; Somfai et al., 2011), cattle (Takahashi et al., 2013; Baldoceda et al., 2015), and sheep oocytes (Mishra et al., 2016).

L-Carnitine has been stated to improve the quantity of activated mitochondria in the oocytes that induce mitochondrial translocation which is related to a considerable reduction in intracellular lipid content (Yamada et al., 2006; Somfai et al., 2011). Furthermore, L-Carnitine acts as a potent antioxidant with a scavenging function of reactive oxygen species (ROS) (Somfai et al., 2011; Dunning and Robker, 2012). In addition, it pursuits antioxidant enzymes, such as SOD, catalase, and GPX, which comprise a natural defense system against redox imbalance (Rizzo et al., 2010; Mishra et al., 2016).

## 5. CONCLUSIONS

In summary, L-Carnitine (LC) incorporated into the bovine oocyte maturation medium at a concentration of 0.6 mg/ml. It significantly enhanced oocyte maturation and fertilization rates, as well as promoting embryonic development at various stages. Furthermore, LC was found to elevate antioxidant enzymatic activity, while concurrently reduced lipid peroxidation.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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