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L-carnitine Supplementation Enhances Nuclear and Cytoplasmic Maturation Rates of Sheep Oocytes *In Vitro*

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ABSTRACT

The aim of the present study was to determine the effectiveness of l-carnitine (LC) supplementation on nuclear and cytoplasmic maturation rates of sheep oocytes. In experiment 1, oocytes were maturated for 24 hours in tissue culture medium 199 supplemented with LC at doses of 0.3 mg/mL, 0.6 mg/mL, and 0.9 mg/mL. In experiment 2, oocytes were maturated and fertilized in a media supplemented with LC at a dose of 0.3 mg/mL and incubated with 5x10^6 sperm/mL for 12 hours. The treatment group consisted of LC supplementation only in maturation medium (P_1), only in fertilization medium (P_2), and in both maturation and fertilization media (P_3). In experiment 3, sperm motility patterns were assessed using CASA after being exposed to fertilization medium supplemented with LC at a dose of 0.3 mg/mL for 0 and 3 hours. Our results showed that supplementation of LC at a dose of 0.3 mg/mL significantly ($p<0.05$) increased the percentage of oocytes reaching metaphase II (86.7±4.1%) compared to those supplemented with LA at doses of 0, 0.6, and 0.9 mg/mL (73.6±1.2%, 81.4±1.3%, and 70.5±1.6%, respectively). The LC treatment in the fertilization medium only did not influence the number of two pronuclear formations (62.1±2.5%) compared to supplementation either in the maturation medium only (72.0±4.7%) or a combination of both in maturation and fertilization media (68.2±2.7%) ($p<0.05$). Further results after 3 hours of incubation compared to the control group showed the total motility (24.8±2.04% vs. 17.49±2.37%), progressive motility (14.17±2.03% vs. 6.49±1.64%), and curvilinear velocity (VCL) (119.70±3.73% vs. 71.15±10.59%) ($p<0.05$) were increased in the fertilization medium containing LC but it did not improve the fertilization rate. It is concluded that supplementation of LC at a dose of 0.3 mg/mL in the maturation medium only could better improve the nuclear and cytoplasmic maturation rates of sheep oocytes.

Keywords: fertilization; l-carnitine; maturation; oocyte; sheep

INTRODUCTION

The systems of *in vitro* embryo production (IVEP) have been widely used for livestock to improve quality production with the various benefits provided (Egashira *et al*., 2019). However, IVEP in sheep is quite low compared to the other livestock species such as cattle or pigs (Paramio & Izquierdo, 2014). In general, the low rate of IVEP in livestock is caused by the low rate of oocyte nuclear and cytoplasmic maturation (El-Raey & Nagai, 2014). The rate of nuclear maturation usually is assessed based on the number of oocytes reaching the stage of metaphase II. Meanwhile, one of the criteria that is generally used to assess cytoplasmic maturation is the number of oocytes forming pronuclear after fertilization (Curnow *et al*., 2010). Therefore, synchronization between nuclear and cytoplasmic maturation is required to support fertilization and subsequent development after fertilization (El-Raey & Nagai, 2014).

The incidences of low rates of nuclear maturation and fertilization occur due to the negative effects of reactive oxygen species (ROS) (Lopes *et al*., 2010), which is produced during *in vitro* culture (Troung & Gardner, 2017). It is reported that increased levels of ROS during the process of *in vitro* maturation (IVM) can trigger oxidative stress in the oocytes (Lobo *et al*., 2010). Oxidative stress can cause damages to carbohydrates, lipids, proteins (Wang *et al*., 2017), and DNA in the oocytes (Lobo *et al*., 2010) that eventually decrease the oocyte competence. In addition, the increased levels of ROS during the process of *in vitro* fertilization (IVF) can result in the failure of fertilization (Opuwari & Henkel, 2016). These conditions can be minimized by the supplementation of an antioxidant in the media (Shafiei *et al*., 2020). One of the potential antioxidants, which has an important role either as scavenging of ROS or as an agent to support nuclear maturation and fertilization, is l-carnitine (Phongmitr *et al*., 2013).

L-carnitine is a water-soluble, small, and highly polar molecule that is crucial for lipid metabolism (Jiang *et al*., 2020). Furthermore, many researchers (Phongmitr *et al*., 2013; Fathi & El-Shahat, 2017) have been associated

the useful effects of LC with its roles as an antioxidant (ROS scavenger), an enhancer of lipid metabolism via β-oxidation, and in increasing glutathione (GSH) levels. As an antioxidant, LC has an activity to protect the damages of mitochondrial membrane and DNA induced by ROS and inhibited cell apoptosis (Wu *et al*., 2011). L-carnitine has a higher antioxidant activity than the other antioxidants such as tocopherol and Trolox (Kelek *et al*., 2019). Furthermore, LC plays a crucial role in the metabolism of lipid by transporting long-chain fatty acids from the cytosol into the mitochondria for further used in the β-oxidation reactions to produce the adenosine triphosphate (ATP), which is required for the metabolism of an oocyte (Phongmitr *et al*., 2013; Agarwal *et al*., 2018). The strong source of ATP in the cell is the metabolism of fatty acids via β-oxidation that improve the processes of oocytes maturation and development after fertilization (Dunning & Robker, 2017).

In addition, the benefit of LC could increase the activity and levels of glutathione (GSH) (Sovergino *et al.,* 2017). GSH improves the development competence of mammalian oocytes at the cytoplasmic level (Curnow *et al.,* 2010). Cytoplasmic maturation is required during early fertilization and embryogenesis after fertilization (Reader *et al*., 2017). Dokmeci (2005) showed the beneficial effect of LC on spermatozoa in IVEP of mice by improving motility and quality of chromatin. However, information concerning the effects of LC during the maturation and fertilization of sheep oocytes *in vitro* is still limited. Therefore, this study was conducted to examine the effectiveness of LC supplementation on nuclear and cytoplasmic maturation of sheep oocytes.

MATERIALS AND METHODS

Assessment of Functional Activity of L-carnitine on Nuclear Maturation

Oocytes collection and maturation *in vitro*. The maturation process was carried out according to Riyuska *et al.* (2019) with minor modifications. Sheep ovaries were collected from a local slaughterhouse and then carried to the laboratory in 0.9% NaCl with 0.1 mg/mL of streptomycin (MEIJI, Indonesia) and 100 IU/mL of penicillin (MEIJI, Indonesia). The oocytes were collected by repeatedly slicing the ovaries with a scalpel blade (slicing technique) in a sterile petri dish containing phosphate-buffered saline (PBS), supplemented with 0.1 mg/mL of streptomycin (Sigma–Aldrich. Inc, S9137. USA), 100 IU/mL of penicillin (Sigma–Aldrich. Inc, P-4687. USA), and 0.3% bovine serum albumin (BSA) (Sigma-Aldrich. Inc, A7030. USA). Only oocytes surrounded by more than three layers of cumulus cells and with homogeneous cytoplasm were used in this experiment.

Selected Cumulus oocyte complex (COCs) were then washed three times in the maturation medium. The oocytes were matured in 100 μL drop of maturation medium for 10-15 oocytes covered by mineral oil. The composition of the maturation medium is tissue culture medium 199 (TCM-199) (Sigma-Aldrich, St. Loius, M4530, USA) supplemented with BSA at a dose of 0.3%, human chorionic gonadotrophin (hCG) (Intervet Boxmeer-Holland) at a dose of 10 IU/mL, gentamycin (Sigma-Aldrich, G1264, USA) at a dose of 50 μg/mL, and follicle stimulating hormone (FSH) (Vetoquinol N,-A Inc, Canada) at a dose of 10 IU/mL. The oocytes were maturated in 4 different concentrations of LC (C0158, Sigma-Aldrich, St. Louis, USA) treatments, namely 0 (control), 0.3, 0.6, and 0.9 mg/mL, respectively. The oocytes were maturated under 5% CO_2 at 38.5°C in humidified air for 24 hours.

Assessment of nuclear maturation. The oocytes were separated from their cumulus cells using the 0.25% hyaluronidase enzyme (Sigma-Aldrich, St. Louis, H3506, USA) in a sterile petri dish and repeated pipetting with a fit pipette adjusting to the measure of the oocytes. The oocytes were completely denuded, then washed within PBS + 0.3% BSA, and placed on a glass slide under coverslips. The slide was placed in a fixative solution mixture of acetic acid (K45626263 420, Merck, Germany) and absolute ethanol (K44151883 303, Merck, Germany) in the ratio of 1:3 for 48-72 hours. Then, the slide was stained using 2% aceto-orcein (Sigma-Aldirch, St. Louis, O7380, USA) and examined under a phase-contrast microscope (Olympus IX 70, Japan). The oocytes were classified based on the changes of chromosome configuration and nuclear membrane, namely germinal vesicle (GV), germinal vesicle break down (GVBD), metaphase I (MI), anaphase and telophase I (A/TI), and metaphase II (MII) (Figure 1). The germinal vesicle was characterized by condensed or slightly diffused chromatin, whereas GVBD stage was characterized by diffused chromatin in which the nuclear membrane was fragmented before rapidly disappearing to leave only small sacs with double walls. Metaphase I (MI) stage was characterized by strongly condensed chromatin that formed an irregular network of individual bivalents (prometaphase) or a metaphase plate without the polar body. Oocytes classified as Metaphase II (MII) stage was characterized by the presence of either a polar body or two chromatin masses (Shirazi & Sadeghi 2007).

Assessment of Functional Activity of L-carnitine on Cytoplasmic Maturation

***In vitro* fertilization.** L-carnitine was supplemented into the medium either in IVM or IVF and a combination using the best concentration obtained from the results of previous experiments (0.3 mg/mL). The LC treatments were divided into four groups, namely without LC supplementation either in IVM or IVF medium (P0) as controls, LC supplementation only in IVM medium (P1), or in IVF medium (P2), and supplementation both in the IVM and IVF media (P3). Frozen semen of sheep obtained from an artificial insemination center, Lembang, Bandung, West Java, Indonesia, was used for *in vitro* fertilization. The fertilization process was done according to Riyuska *et al.* (2019) with minor modifications. The frozen semen was thawed in a water bath at 37°C for 30 seconds and then diluted with IVF medium (Suzuki *et al*., 2000) of 0.5 mM $MgSO_4$ containing 12 mK KCl, 0.5 mM NaH_2PO_4, 90 mM NaCl, 25 mM $NaHCO_3$, 10 mM sodium lactate, 5 mM caffeine, 3 mg/mL BSA, and 50 μg/mL gentamicin. The diluted semen was then centrifuged at 500 x g for 5 minutes. After centrifuga-

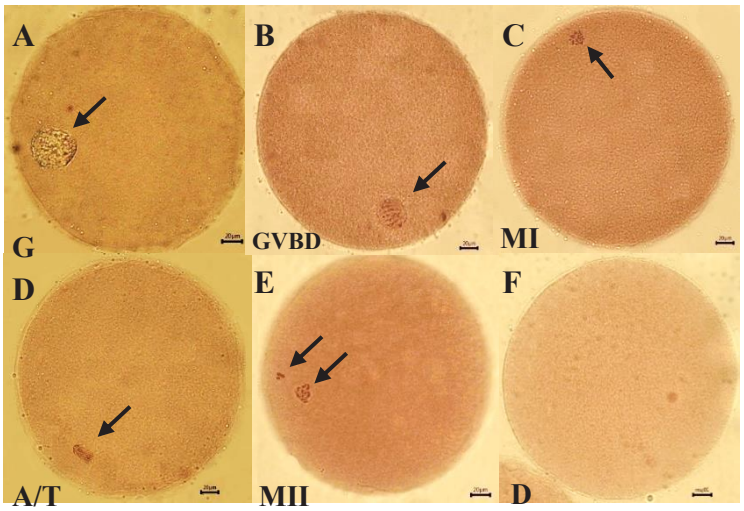

Figure 1. Nuclear maturation stage of oocytes in various l-carnitine concentrations in the maturation medium. A= germinal vesicle; B= germinal vesicle breakdown; C= metaphase I; D= anaphase/telophase, E= metaphase II; F= degeneration; GV= germinal vesicle; GVBD= germinal vesicle break down; MI= metaphase I; A/TI= anaphase and telophase I; MII= metaphase II; D= degeneration.

tion, the supernatant was discarded, then the pellet was diluted with the IVF medium to a final concentration of $5x10^6$ sperm/mL and put in a droplet (100 µL). After the maturation process, the oocytes were washed 3 times using the fertilization medium without sperm, then incubated into the droplet of sperm (100 µL) in 5% CO_2, at 38.5°C in humidified air for 12 hours.

Assessment of cytoplasmic maturation. The assessment of the cytoplasmic maturation was determined by the formation of pronuclear (El-Raey & Nagai, 2014). The normal fertilization was classified by the formation of two pronuclear, while the oocyte having more than two pronuclear was classified as polyspermy. The cytoplasmic maturation rate was evaluated with 2% aceto-orcein (Sigma-Aldirch, St. Louis, O7380, USA) after 12 hours of fertilization process and examined under a phase-contrast microscope (Olympus IX 70, Japan).

Assessment of sperm motility. In this experiment, LC was supplemented into the fertilization medium with the best concentration in the previous experiment (0.3 mg/mL). Samples of each treatment were assessed for the pattern of sperm motility using the CASA system (Sperm Vision™ 3.7 Minitube, Germany). Parameters measured were percentages of total motility, progressive motility, the amplitude of lateral head displacement (ALH), linearity (LIN), and curvilinear velocity (VCL). Sperm sample for analysis was obtained from sperm sample derived from IVF process according to the treatment. Analysis of the pattern of sperm motility was conducted 0 and 3 hours after the incubation period to record the pattern of motility as an indicator of the capacitation process to improve fertilization rate.

Statistical Analysis

Nuclear maturation and fertilization rates were analyzed statistically with One-Way Analysis of Variance (ANOVA). The patterns of sperm motility were assessed using Two-Way ANOVA. Differences at a probability value of $p<0.05$ were considered to be statistically significant.

RESULTS

Nuclear Maturation Rate

The percentages of oocytes in MII stage in the maturation medium supplemented with various concentrations of LC (0, 0.3 mg/mL, 0.6 mg/mL, and 0.9 mg/mL) were 73.6±1.2%, 86.7±4.1%, 81.4±1.3%, and 70.5±1.6%, respectively (Table 1). The results of our study revealed that supplementation of LC at a dose of 0.3 mg/mL was the best result in improving the nuclear maturation compared to the other treatments and control ($p>0.05$).

Cytoplasmic Maturation and Pattern of Sperm Motility Rate

The success of cytoplasmic maturation *in vitro* could be assessed based on the formation of pronuclear (El-Raey & Nagai, 2014). The formation of two pronuclear was categorized as normal fertilization. Meanwhile, if the oocyte had more than two pronuclear was categorized as polyspermy (Figure 2). The result of the experiment revealed that the formation of two pronuclear was higher with the supplementations of LC in the maturation medium (P1) (72.0±4.7%) and in the combination of maturation and fertilization media (P3) (68.2±2.7%) compared to the supplementation of LC only in the fertilization medium (P2) (60.5±5.1%) and control without LC supplementation (62.1±2.5%) ($p<0.05$). Supplementation of LC only in the fertilization medium did not improve the normal fertilization. In addition, the results of the experiment showed the increasing number of polyspermy (14.8±2.3%) ($p<0.05$) in the LC supplementation in the fertilization medium (P2)

Table 1. Nuclear maturation rate of sheep oocytes in different concentrations of L-carnitine in the maturation medium

Concentration of LC (mg/mL)	No of oocytes	Nuclear maturation rate (%, mean ± SD)					
		GV	GVBD	MI	A/T	MII	D
0	110	1.8±0.5	7.3±4.0	12.7±4.7	0.0±0.0[a]	73.6±1.2[a]	4.5±1.5[a]
0.3	113	0.9±0.0	3.5±2.6	8.0±3.3	0.9±0.0[a]	86.7±4.1[b]	0.0±0.0[b]
0.6	102	2.9±5.9	2.9±3.7	12.7±5.5	0.0±0.0[a]	81.4±1.3[c]	0.0±0.0[b]
0.9	105	2.9±0.6	5.7±5.4	13.3±3.4	7.6±6.3[b]	70.5±1.6[d]	0.0±0.0[b]

Note: LC= L-carnitine; GV= germinal vesicle; GVBD= germinal vesicle break down; MI= metaphase I; A/T= anaphase and telophase; MII= metaphase II; D= degeneration. Means in the same columns with different superscripts differ significantly (p<0.05).

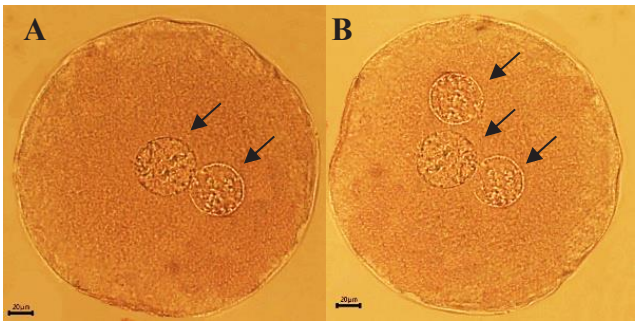

Figure 2. Pronuclear development of sheep oocyte after fertilization in different l-carnitine supplementations. A= 2 pronuclear; B= >2 pronuclear.

only compared to the control and the other treatments (Table 2).

The patterns of sperm motility (total motility, progressive motility, and curvilinear velocity (VCL)) in the medium supplemented with LC at a dose of 0.3 mg/mL revealed the significantly higher at 0 hour of incubation (55.12±4.13%, 40.66±7.92%, and 129.53±11.50%, respectively) and after 3 hours of incubation (24.8±2.04%, 14.17±2.03%, and 119.70±3.73%, respectively) compared to the controls at 0 hour of incubation (42.05±1.79%, 25.21±6.74%, and 1.73±1.56%, respectively) and after 3 hours of incubation (17.49±2.37%, 6.49±1.64%, and 71.15±10.59%, respectively) (p<0.05) (Figure 3). However, the parameters of linearity (LIN) and amplitude of lateral head displacement (ALH) of the sperms were not significantly different in the medium supplemented with 0.3 mg/mL LC either at 0 hour (0.54±0.04% and 4.80±0.62%) and after 3 hours of incubation (0.61±0.15% and 3.80±0.95%) compared with control without LC treatment at 0 hour of incubation (0.68±0.23% and 3.93±1.70%) and after 3 hours of incubation (0.69±0.16% and 3.20±0.95%) (p>0.05).

DISCUSSION

Rate of Nuclear Maturation

Nuclear maturation is an essential prerequisite for the success of fertilization (Holubcová *et al.*, 2019) and early embryonic development (Varghese *et al.*, 2011). The results of our study revealed that nuclear maturation was improved by supplementation of the LC at a dose of 0.3 mg/mL in the maturation medium. These results are in line with the study in the oocytes of swamp buffalo (Phongmitr *et al.*, 2013), mouse (Zare *et al.*, 2015), and camel (Fathi & El-Shahat, 2017) reporting that supplementation of LC in the medium of maturation could improve the nuclear maturation (MII stage) rate. The improvement of nuclear maturation may be related to the ability of LC to generate ATP (Agarwal *et al.*, 2018), act as a ROS scavenger, and increase GSH levels, which are necessary for nuclear maturation (Phongmitr *et al.*, 2013; Fathi & El-Shahat. 2017).

The energy in the form of ATP is a critical point in the oocyte development for meiosis resumption (Leoni *et al.*, 2015). Many researchers (Phongmitr *et al.*, 2013; Zare *et al.*, 2015; Fathi & El-Shahat, 2017; Agarwal *et al.*, 2018) state that LC plays an important role in lipid metabolism through its function as a cofactor for transporting fatty acids into the mitochondria through ß-oxidative processes to generate ATP production. Additionally, it has been shown that in the maturation process, the lipid content in the porcine oocytes has decreased, indicating that the utilization of lipid was the key energy source during oocyte maturation (Somfai *et al.*, 2011; Phongmitr *et al.*, 2013).

Table 2. Fertilization rate of sheep oocytes after treatment of L-carnitine in the different media

Group	LC treatment		No of oocytes	Total fertilization rate (%, mean ± SD)	Pronuclear formation rate (%, mean ± SD)	
	IVM	IVF			2PN	>2PN
P0	-	-	103	70.9±2.2[a]	62.1±2.5[a]	8.8±3.9[a]
P1	+	-	101	80.2±2.8[c]	72.0±4.7[b]	8.1±3.7[a]
P2	-	+	101	75.2±3.5[b]	60.5±5.1[a]	14.8±2.3[b]
P3	+	+	98	80.4±3.4[c]	68.2±2.7[b]	12.2±2.0[ab]

Note: IVM= *in vitro* maturation; IVF= *in vitro* fertilization; 2PN= 2 pronuclei; >2PN= polyspermy (more than two pronuclei); LC= L-carnitine (0.3 mg/mL); P0= without LC supplementation in IVM and IVF medium as a control; P1= LC supplementation only in IVM medium; P2= LC supplementation only in IVF medium; P3= combination of LC supplementation in IVM and IVF media; Means in the same columns with different superscripts differ significantly (p<0.05).

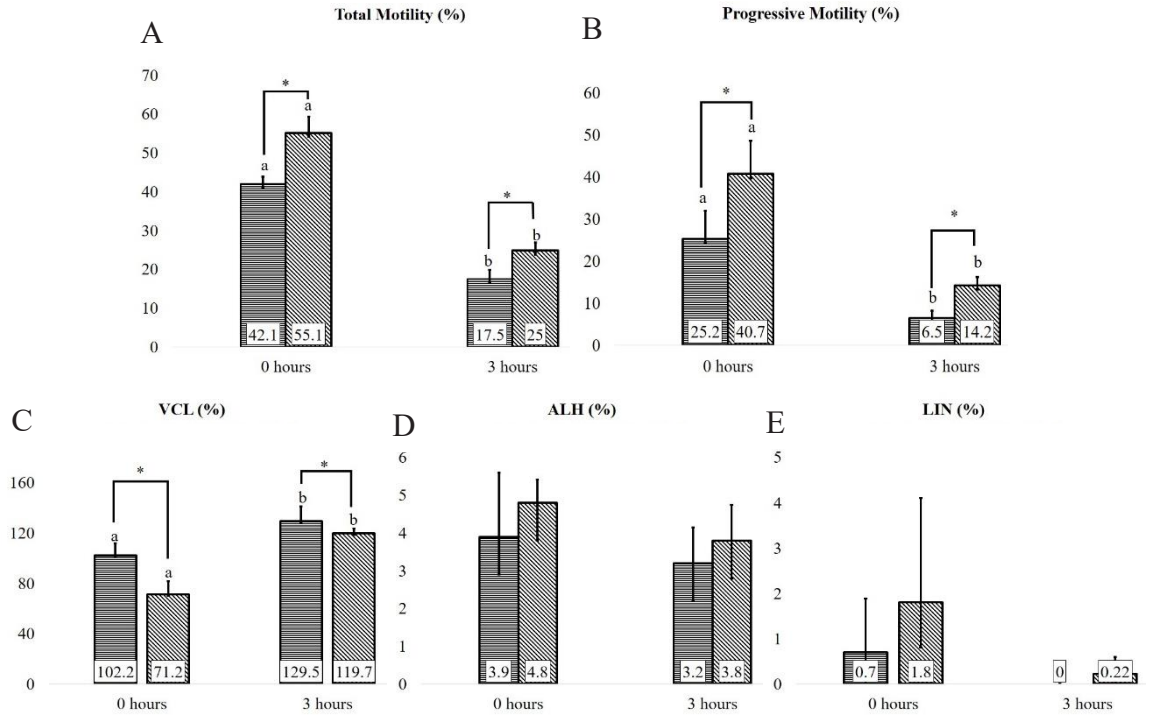

Figure 3. Sperm motility and movement pattern of sperm in the fertilization medium. A= Total Motility; B= Progressive Motility; C= Curvilinear velocity (VCL); D= Amplitude of lateral head displacement (ALH); E= Linearity (LIN). *= [a,b] Superscript shows means different significantly ($p<0.05$). ▤= Control; ▨= LC 0.3 mg/mL.

The ATP generating process was initiated by LC transfer into the tissue through the electrogenic force of the voltage-gated Na^+-channels. The process utilizes organic cation transporter-2 (OCT2) to transport LC into the oocyte (Agarwal *et al.*, 2018). In the oocytes, LC is converted into Acetyl-L-carnitine (ALC) by carnitine palmitoyl transferase-I (CPT-I) outside the membrane of mitochondria (Dunning & Robker, 2017), and CPT-II promote the regeneration of carnitine from acyl-carnitine after translocation of long-chain fatty acids into the mitochondrial matrix. After conversion, LC will maintain the balance ratio of acetyl CoA/CoA for maintaining glucose metabolism via the tricarboxylic acid cycle to produce higher energy (Agarwal *et al.*, 2018).

On the other side, LC also has an antioxidant activity required to reduce ROS level in the oocytes during the in vitro maturation process (Wu *et al*., 2011; Fathi & El-Shahat, 2017). Kelek *et al*. (2019) state that LC has a high antioxidant activity compared to the other antioxidants such as tocopherol and trolox. Furthermore, Zare *et al.* (2015) state that the antioxidant activity mechanism of LC may be done via a scavenging effect on radical anion, superoxide hydrogen peroxide, and 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH). On the other side, it was reported that the level of apoptosis could be decreased after supplementation of LC in the maturation medium through its antioxidant action (Mansour *et al*., 2019).

Many researchers (Wu *et al.*, 2011; Zare *et al*., 2015; Sovergino *et al*., 2017) state that LC can increase the level of GSH. In the oocyte nucleus, the accumulation of GSH has the potential to regulate the structure of chromatin and the dynamics of chromatin condensation (Vivancos *et al*., 2010). Those mechanisms are believed that supplementation of LC in the maturation medium improves the nuclear status of the oocyte by GSH generation.

Results of this study showed that supplementation of LC at a dose of 0.3 mg/mL had a higher nuclear maturation rate (86.7%) compared to the LC supplementation at doses of 0.6 and 0.9 mg/mL (81.4% and 70.4%). It seems that a higher concentration of LC reduces the nuclear maturation rate. This result is similar to the result reported by Wu *et al.* (2011) that the higher concentration of LC causes a lower rate of maturation in the pig oocytes. Terada *et al.* (2002) reported that supplementation of antioxidants with improper concentrations would have toxic effects on the oocytes. Furthermore, Spinaci *et al*. (2019) reported that excessive supplementation of antioxidants, a dangerous condition called the antioxidant paradox, leading to oxidative stress that eventually reduced the percentage of nuclear maturation rate. In addition, Fathi & El-Shahat (2017) reported that excessive LC concentrations would decrease lipid density that may not be beneficial for the further development of oocytes after fertilization.

Cytoplasmic Maturation Rate and Pattern of Sperm Motility

The success of *in vitro* fertilization would be optimal not only depend on the nuclear maturation but also on the cytoplasmic maturation (El-Raey & Nagai, 2014; Riyuska *et al*., 2019). The cytoplasmic maturation of oocytes is required during fertilization and can be assessed based on the success of pronuclear formation (Kim *et al*., 2011; El-Raey & Nagai, 2014). In the present study,

it was revealed that supplementation LC at a dose of 0.3 mg/mL only in the maturation medium (P1) and in the combination of the maturation and fertilization media (P3) showed that the formation of two pronuclear was higher than the other treatment and control. However, the levels of two pronuclear formations between those treatments were not significant. Therefore, it seems that the role of LC is greater in supporting pronuclear formation during the maturation process than fertilization. Therefore, supplementation of LC in the maturation medium is more effective to support pronuclear formation compared to the supplementation in the fertilization medium. The results found in our study are similar to the results of the study in camel (Fathi & El-Shahat, 2017), reporting that the supplementation LC in the maturation medium can increase the in vitro fertilization rate.

The role of LC to support cytoplasmic maturation presumably through its effect on increasing the concentration of GSH after its supplementation in the maturation medium (Wu *et al*., 2011; Zare *et al.,* 2015; Sovergino *et al.,* 2017). The increasing concentration of GSH may be related to the role of LC as an antioxidant and thus can maintain GSH storages in the oocytes (Zare *et al*., 2015). On the other side, the increase in GSH concentration can be contributed by the role of ATP-dependent enzymes in the synthesis of GSH (Sovergino *et al*., 2017). In β-oxidation of long-chain fatty acids, l-carnitine plays a critical role in generating ATP by providing a system of transmission for free fatty acids into the mitochondria (Jeulin & Lewin. 1996). Therefore, the process of β-oxidation is required by the oocytes to support nuclear and cytoplasmic maturation (Dunning *et al*., 2010). Furthermore, our finding strongly suggested that the formation of two pronuclear in the treatment group of LC supplementation in the fertilization medium only (P2) was lower than that in the LC supplementation in the maturation medium only (P1). This result indicates that the role of LC is more beneficial when it is supplemented in the IVM medium compared to in the IVF medium to support the cytoplasmic maturation.

Our additional finding revealed that supplementation of LC in the fertilization medium only (P2) could increase polyspermy (14.8%) compared to the other treatments, which has an impact on the failure of early embryonic development. The incidence of polyspermy in IVEP systems is not yet clearly understood. However, it might be caused by various factors, either from the spermatozoa or oocytes (Goncalves *et al*., 2010). Furthermore, Itoi *et al*. (2018) suggest that a higher number of motile sperms results in more frequent contact with an ovum, thus increasing the likelihood of polyspermy. Therefore, an assessment pattern of sperm motility was carried out in this study to observe total motility, progressive motility, VCL, ALH, and LIN.

The results showed that the supplementation of LC in the fertilization medium significantly increased the total motility, progressive motility, and hyperactivity assessed by increasing the percentage of curvilinear velocity (VCL) compared to controls. Those results may be the factors that could be the cause of increasing the incidence of polyspermy in the treatment group of LC supplementation in the fertilization medium only (P2). Agarwal *et al*. (2018) suggested that the increased sperm motility and hyperactivity could be related to the role of LC in providing energy in the form of ATP used by the sperms.

CONCLUSION

L-carnitine supplementation at the concentration of 0.3 mg/mL in the maturation medium can improve nuclear maturation, therefore support a better cytoplasmic maturation, which is characterized by the high fertilization rate. The further finding indicates that LC supplementation in the maturation medium only can support a better fertilization rate, which is presumably related to GSH production during maturation as a prerequisite for pronuclear formation. Meanwhile, total motility, progressive motility, and curvilinear velocity (VCL) increase in the fertilization medium containing LC, but it does not significantly improve the fertilization rate.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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