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ORIGINAL ARTICLE

Oral administration of L-carnitine improves the clinical outcome of fertility in patients with IVF treatment

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ABSTRACT

Age-dependent decline of mitochondrial function has been proposed to be a main cause of decline of embryo quality. Then, L-carnitine plays important roles in reducing the membranous toxicity of free-fatty acids by forming acyl-carnitine and promoting β-oxidation, preventing cell damage. Recent research revealed that L-carnitine played important roles *in vitro* in oocyte growth, oocyte maturation and embryo development. However, such beneficial effects of L-carnitine *in vivo* have yet to be verified. The effect of oral L-carnitine supplementation on embryo quality and implantation potential was examined. A total of 214 patients were included in this study. They all previously received *in vitro* fertilization-embryo transfer (IVF-ET) and failed to conceive. Then they were administered L-carnitine for 82 days on average and underwent IVF-ET again. There were no significant differences in the total number of retrieved oocytes, and their maturation and fertilization rates between before and after L-carnitine administration. The quality of embryos on Days 3 and 5 after insemination was improved following L-carnitine administration ($p < .05$) in cycles after L-carnitine administration compared with previous cycles. Healthy neonates were born after IVF-ET following L-carnitine administration. Our data suggested that oral administration of L-carnitine to fertility patients improved the developmental competence of their oocytes after insemination.

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Introduction

Assisted reproductive technology has become an indispensable technology for having children because of current social trends of women's late childbearing years. Fertility decreases with age [1]. One of the main reasons for the poor competence of embryos obtained from older patients is an increased rate of chromosomal aberrations [2] due to premature bivalent separation into univalent during meiosis [3]. Additionally, an age-dependent decline in mitochondrial function has also been suggested for this poor competence [4–7].

Mitochondrial dysfunction is triggered by damage of genomic and/or mitochondrial DNA, cellular membrane damage due to oxidative stress, endometriosis [8], and hormone imbalance [9]. Cell damage and aging are induced by impaired energy metabolism [10]. Although various factors have been shown to decrease cell damage *in vitro* [4,11], their beneficial effects *in vivo* remain unknown. Fatty acids provide large amounts of ATP via β-oxidation compared with glucose and amino acids. However, the free form of fatty acids has detergent-like activity, which impairs cell membrane/lipid bilayers and induces mitochondrial dysfunction and cellular aging [12]. L-Carnitine plays important roles in reducing the membranous toxicity of free-fatty acids by forming acyl-carnitine and promoting β-oxidation [13,14], leading to alleviation of cell damage. Long-chain fatty acids, such as palmitate and stearate, induce *de novo* synthesis of ceramide and cause apoptosis by inhibiting carnitine palmitoyl transferase I. Carnitine palmitoyl transferase I localizes in mitochondrial outer membranes and facilitates the transport of long-chain fatty acids by carnitine palmitoyl transferase II into mitochondria [15].

L-carnitine localizes ubiquitously in mammalian plasma and various tissues, particularly in skeletal and cardiac muscles, and suppresses mitochondrial damage and mitochondria-triggered apoptosis [16]. Recent studies have shown that L-carnitine also plays important roles in *in vitro* oocyte growth [17,18], oocyte maturation [19], and embryonic development [19,20]. However, whether such beneficial effects of L-carnitine also occur *in vivo* remains unknown. The present study aimed to investigate the effect of L-carnitine administration on embryonic development in patients who failed to conceive in previous *in vitro* fertilization-embryo transfer (IVF-ET) cycles.

Material and methods

Ethical approval

This study was approved by the local ethics Institutional Review Board of IVF Namba Clinic (Registry No. 201610) and the Japan Society of Obstetrics and Gynecology.

Ovarian stimulation

A total of 214 patients who failed to conceive in previous IVF-ET cycles and whose AMH value was higher than 1 ng/ml were administered 1000 mg/day L-carnitine (L-CAR Basic Premium; AA Project Co. ltd., Ishikawa, Japan) and were included in the present analysis after informed consent. The dose of L-carnitine was determined according to the recommendation of the Food Safety Commission of Switzerland in 2002 (https://www.admin.

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ch/opc/de/official-compilation/2002/573.pdf) and the Japanese Ministry of Health, Labor and Welfare (http://www.ffcr.or.jp/Zaidan/mhwinfo.nsf/a2a20b26f788ad86492565a700188056/ed3a3b81d61da5d54925733d000c3a22?OpenDocument). Patients were subjected to controlled ovarian stimulation according to their medical history as previously described [21]. For gonadotropin-releasing hormone (GnRH) agonist long cycles, patients received oral contraceptive pills (1 mg of norethisterone and 0.05 mg of mestranol; Aska Pharmaceutical, Co., Ltd., Tokyo, Japan) on Day 14 of the previous cycle and these were continued for 10 days. The GnRH agonist (600 µg/day, Suprecur® nasal solution 0.15%; Mochida Pharmaceutical, Tokyo, Japan) was administered on Day 21 of the previous cycle until induction of ovulation. On Day 3 of the cycle, patients received doses of follicle-stimulating hormone, ranging from 150 to 300 IU for 4 days. This was followed by administration of urinary human menopausal gonadotropin at doses of 150–450 IU until induction of ovulation. For GnRH antagonist cycles, a GnRH antagonist (2.5 mg, Ganirelix Acetate; MSD K.K., Tokyo, Japan) was administered daily after the leading follicles reached 13–14 mm in diameter as diagnosed by ultrasound. Ovulation was induced by human chorionic gonadotropin (hCG) administration when at least one leading follicle reached 18 mm in diameter. Transvaginal follicle aspiration was carried out 36 h after hCG injection. Ovum pick-up was performed between 2013 January and 2015 December.

IVF procedure

All oocytes were inseminated in GM-HTF (Gynemed GmbH & Co. KG, Lensahn, Germany) or injected with a sperm by intracytoplasmic sperm injection (ICSI) 40 h after hCG administration. Fertilization was confirmed 14–16 h after insemination by the presence of two pronuclei and extrusion of the second polar body. Fertilized ova were cultured in commercially available culture medium, such as Continuous Single Culture medium (Irvine Scientific Sales Company Inc., Santa Ana, CA). These ova were cultured until Day 5 at 37 °C under 5% O_2, 5% CO_2, and 90% N_2 with high moisture. Cryopreservation was performed with an open vitrification device [22].

Embryo classification

A transferable embryo was defined as having at least five blastomeres on Day 3, and <20% of its volume was filled with fragments. A morphologically good-quality embryo was defined as having at least seven blastomeres on Day 3, and <20% of its volume was filled with fragments as previously described [21]. Morphologically good-quality blastocysts were defined as reaching full-blastocyst stage on Day 5 with sufficient cells of the inner cell mass and the trophectoderm. To evaluate the effect of L-carnitine, we compared outcomes between the previous cycles (control) and after treatment cycles of patients.

Patients' background

The mean [±SD (standard deviation)] age of the patients was 38.3 ± 3.8 years in a previous cycle (control) and 38.5 ± 3.8 years in a subsequent cycle (cycle after L-carnitine treatment). The average period of L-carnitine administration until subsequent cycles was 83 days (7–437 days).

Developmental competence after embryo transfer

Single embryo transfer was performed in 96 patients under fresh or vitrified-warmed hormone replacement cycles between January 2013 and December 2015. For fresh cycles, a single embryo was transferred 3 days after oocyte pick-up in patients with endometrial thickness >8 mm on the day of hCG injection. Daily doses of 6 mg of chlormadinone acetate (Lutoral®; Shionogi & Co., Ltd., Osaka, Japan) were maintained until a pregnancy test. Chlormadinone acetate was administered until 8 weeks of gestation after confirmation of pregnancy. Progesterone (Progeston depot® 125 mg; Fuji Pharma Co., Ltd., Toyama, Japan) was injected intramuscularly on the day of ET with two additional injections after confirming pregnancy.

For hormone replacement cycles, the endometrium was prepared as described previously [22] by incremental doses of oral estradiol valerate (Progynova®; Bayer Schering Pharma Co., Ltd., Zürich, Switzerland) from 1 to 4 mg for 2 weeks following administration of GnRH agonist (600 µg/day, Suprecur® nasal solution 0.15%; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) for 3 weeks. A total of 6 mg per day of chlormadinone acetate was administered after confirming that the endometrial thickness was >8 mm by ultrasonography. Daily doses of 3 mg of estradiol valerate and 6 mg of chlormadinone acetate were maintained until a pregnancy test. A single vitrified-warmed blastocyst was transferred on the fifth day of chlormadinone acetate administration.

Pregnancy was defined beyond 8–10 weeks of gestation with a fetal heart beat confirmed by ultrasonography. The cases of two embryo transfer were not included in the analysis.

Statistical analysis

Data were compared by the χ^2 test, except for the number of retrieved oocytes and semen parameters. The number of retrieved oocytes and semen parameters were compared by the *t*-test. A *p* value <.05 was considered statistically significant.

Results

To assess the effect of L-carnitine, we compared the outcomes obtained from the cycles after L-carnitine administration with those from previous cycles (control group). All of the patients included in this study failed to become pregnant at the previous cycles.

The insemination method was not changed in 170 patients (ICSI: 136; conventional IVF: 34). The insemination method was changed in 44 patients from conventional IVF to ICSI because no fertilization was obtained in the previous cycles. There were no significant differences in the number of retrieved oocytes, the maturation rate, and the fertilization rate in cycles after L-carnitine administration compared with previous cycles (Table 1). Original semen parameters of male partners were also similar (Table 1). However, administration of L-carnitine significantly improved the rates of transferable embryos (L-carnitine versus control: 66.9 versus 60%; $p = .018$, Table 2), morphologically good-quality embryos (53.7 versus 46.4%; $p = .017$), and morphologically good-quality blastocysts (21.2 versus 11.4%; $p = .0037$). To assess the effect of dosing period of L-carnitine, we divided the outcomes on Day 3 after insemination into three and on Day 5 into two groups according to dosing period. The rates of transferable and morphologically good embryo after L-carnitine administration were higher in short-term group (32.4 days, $p < .05$, Table 2).

Table 1. Clinical changes after L-carnitine administration.

	Previous cycles (average ± SD)	Cycles after L-carnitine administration (average ± SD)
Clinical outcomes		
No. of retrieved oocytes	10.4 ± 5.7	10.3 ± 6.2
Maturation rate	81.5% (1835/2249)	83.1% (1862/2239)
Fertilization rates		
ICSI (136 patients)	78.1% (888/1136)	80.6% (927/1150)
cIVF (34 patients)	74.2% (237/319)	76.1% (258/339)
Original semen parameters		
Embryo development on Day 3 after insemination ($n = 79$[a])		
Sperm concentration (10^6 sperm/mL)	53.1 ± 47.5	61.6 ± 58.5
Sperm motility (%)	51.5 ± 17.4	52.9 ± 17.3
Abnormality of sperm morphology (%)	35.3 ± 11.4	35.4 ± 11.7
Blastocyst development on Day 5 after insemination ($n = 44$)		
Sperm concentration (10^6 sperm/mL)	80.5 ± 59.7	87 ± 71
Sperm motility (%)	53.8 ± 14.3	54.5 ± 15
Abnormality of sperm morphology (%)	33.6 ± 12.1	35.1 ± 11.2

[a]Testicular sperm extraction was conducted to two male patients because sperm was not detected in their semen. Their data were not included in the analysis.

Table 2. Effect of L-carnitine administration on embryo morphology.

	Previous cycles	Cycles after L-carnitine administration
Transferable embryo rate on Day 3	60%* (268/446)	66.9%* (331/495)
Short term (32.4 days, 7–44 days)	55.1%* (86/156)	66.3%* (116/175)
Medium term (65 days, 45–90 days)	60.4% (81/134)	70.8% (97/137)
Long term (144 days, 90–275 days)	64.7% (101/156)	64.5% (118/183)
Morphologically good embryo rate on Day 3	46.4%* (207/446)	53.7%* (266/495)
Short term (32.4 days, 7–44 days)	44.2%* (69/156)	55.4%* (97/175)
Medium term (65 days, 45–86 days)	44.8%* (60/134)	59.1%* (81/137)
Long term (144 days, 90–275 days)	50% (78/156)	48% (88/183)
Blastocyst rate on Day 5	35.1% (86/245)	43.3% (100/231)
Short term (38.7 days, 16–54 days)	36.2% (42/116)	46.9% (53/113)
Long term (142.2 days, 56–275 days)	34.1% (44/129)	39.8% (47/118)
Morphologically good blastocyst rate on Day 5	11.4%* (28/245)	21.2%* (49/231)
Short term (38.7 days, 16–54 days)	13%* (15/116)	26.5%* (30/113)
Long term (142.2 days, 56–275 days)	10% (13/129)	16.1% (19/118)

*$p < .05$ by the χ^2 test.

Table 3. Neonatal outcomes after L-carnitine administration.

	Single transfer of fresh Day 3 embryo	Single transfer of vitrified–warmed blastocyst
No. of cycles	41	55
Dosing period of L-carnitine	102 days (10–219)	104 days (27–275)
Detection of fetal heart beat (%)	17% (7/41)	32.7% (18/55)
Live birth rate (%)	17% (7/41)	25.5% (14/55)
Mean gestational age (days)	274.7	273.2
Mean birth weight (g)	3112.7	3074.9
Mean Apgar score	9.5	9.4
Proportion of congenital anomalies	0	0

The rate of morphologically good blastcyst after L-carnitine administration was also higher in short-term group (38.7 days, $p < .05$).

Twenty-one neonates were born after single transfer of fresh Day 3 embryos and vitrified-warmed blastocysts (Table 3). The mean (±SD) gestational age, birth weight, and Apgar score were 273.7 ± 8 days, 3087.5 ± 334.5 g, and 9.4 ± 0.6, respectively. To assess the effect of dosing period of L-carnitine, we divided the outcomes on implantation after single embryo transfer into three groups according to dosing period. The implantation rate in short (45 days), medium (80 days) and long-dosing period groups (189 days) were 21.8% (7/32), 28.1% (9/32) and 28.1% (9/32), respectively.

Discussion

The present study showed that oral administration of L-carnitine improved embryo quality in patients who failed to become pregnant in previous cycles. Healthy neonates were born after L-carnitine administration. The gestational age, birth weight, and Apgar score values in the present study were similar to those obtained after single transfer of vitrified-warmed Day 5 blastocysts [23]. No congenital anomalies were observed. The proportion of male neonates was 61.9%. This is the first report to examine the favorable effect of L-carnitine administration on fertility *in vivo*.

Mitochondria produce ATP, which is necessary for cell activity via oxidative phosphorylation. The tricarboxylic acid cycle has a higher ATP production ability per mole of substrate compared with the anaerobic glycolytic pathway. Glucose, amino acids, and fatty acids are major substrates for energy production. A large amount of ATP is produced from fatty acids via β-oxidation in mitochondria. However, oxidative stress enhances lipid peroxidation and degradation of phospholipids, increasing free forms of fatty acids [24]. Free forms of long-chain fatty acids are

hydrophobic anions that exhibit properties similar to those of anionic detergents. Therefore, a rapid increase in their tissue levels disturbs the structure and function of mitochondrial membranes [25]. Destruction of mitochondrial membranes also leads to leakage of electrons from the mitochondrial electron transport chain [26,27]. L-carnitine plays important roles in reducing the membranous toxicity of free-fatty acids by forming acyl-carnitine and promoting β-oxidation [13,14], resulting in alleviation of cell damage.

The quality of mammalian oocytes declines with maternal age because of mitochondrial dysfunction [4–7], which increases oxidative stress that perturbs redox-dependent metabolism in and around cells [28]. Therefore, mitochondria have been suggested to affect the quality of oocytes because mitochondria play critical roles in their growth and maturation, and embryonic development [12]. The quality of oocytes is a major factor that determines their developmental capacity. Various nutrients with an antioxidant nature have been tested to maintain and improve mitochondrial function in oocytes. L-carnitine decreases oxidative injury of mitochondria and cells in mice *in vitro* and *in vivo* [28]. Additionally, administration of L-carnitine successfully inhibits oxidative stress in various cells and decreases tissue injury in rodents that receive anticancer agents [29]. L-Carnitine administration also suppresses aging of senescence-accelerated rodents with amyotrophic lateral sclerosis (ALS) [30]. These observations suggest that L-carnitine has beneficial effects in suppressing the oxidative injury of mitochondria, cells, and tissues *in vivo*. Therefore, we hypothesize that L-carnitine also suppresses pathological events in ovarian cells and tissues of fertility patients.

In fact, in *in vitro* studies, L-carnitine supplementation of culture media supports *in vitro* growth of growing oocytes [17,18], *in vitro* maturation of oocytes [19], and *in vitro* embryonic development [19,20]. Furthermore, supplementation of L-carnitine of culture medium reduces abnormal distribution of mitochondria, increases mitochondrial membrane potential, and normalizes the spindle structure after *in vitro* maturation following vitrification [31,32]. Oral administration of L-carnitine successfully suppresses the pathological events induced by repeated ovulation and by natural aging in mice, such as aggregation of mitochondria and a decrease in the developmental capacity of oocytes [12]. The similar effects of L-carnitine that protect mitochondrial function and improve the quality and developmental competence of oocytes could be exerted in humans. These observations suggest that the protective effect of L-carnitine is exerted preferentially in and around mitochondria. Therefore, the protective effects of L-carnitine might reflect its activity to decrease fatty acids in and around mitochondria and/or to improve energy metabolism required for cell survival under oxidative stress [16].

It has been shown that the serum L-carnitine levels in polycystic ovary syndrome (PCOS) patients were decreased compared with healthy women [33,34]. In the present study, five PCOS patients were included in the analysis. Two of these PCOS patients became pregnant after L-carnitine. Oral administration of L-carnitine might increase the serum L-carnitine levels in PCOS patients. Further studies should be required to reveal a favorable effect of L-carnitine in PCOS patients.

The dosing period of L-carnitine in this study varied widely because of patients' situation. Thus, we assessed the effect of dosing period of L-carnitine on the outcome. In short-dosing period group, the development on Day 3 and 5 was improved. Furthermore, there was no difference in the detection rate of fetal heart beat among three dosing periods. Thus, L-carnitine administration for about 30 days might be enough to improve the fertility competence. A large-scale prospective study should be required to map out correct dosing schedule.

In this study, oral administration of L-carnitine to fertility patients improved the developmental competence of their oocytes after insemination, especially the rates of morphologically good-quality embryos and blastocysts. Healthy neonates were born without any congenital anomalies. Further studies are required to collect neonatal data and growth of neonates.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

1. Huang L, Sauve R, Birkett N, et al. Maternal age and risk of stillbirth: a systematic review. CMAJ 2008;178:165–72.
2. Nagaoka SI, Hassold TJ, Hunt PA. Human aneuploidy: mechanisms and new insights into an age-old problem. Nat Rev Genet 2012;13:493–504.
3. Sakakibara Y, Hashimoto S, Nakaoka Y, et al. Bivalent separation into univalents precedes age-related meiosis I errors in oocytes. Nat Comms 2015;6:7550.
4. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci USA 1994;91:10771–8.
5. Bartmann AK, Romão GS, Ramos Eda S, Ferriani RA. Why do older women have poor implantation rates? A possible role of the mitochondria. J Assist Reprod Genet 2004;21:79–83.
6. May-Panloup P, Chrétien MF, Jacques C, et al. Low oocyte mitochondrial DNA content in ovarian insufficiency. Hum Reprod 2005;20:593–7.
7. Bentov Y, Yavorska T, Esfandiari N, et al. The contribution of mitochondrial function to reproductive aging. J Assist Reprod Genet 2011;28:773–83.
8. Xu B, Guo N, Zhang XM, et al. Oocyte quality is decreased in women with minimal or mild endometriosis. Sci Rep 2015;5:10779.
9. Zárate S, Astiz M, Magnani N, et al. Hormone deprivation alters mitochondrial function and lipid profile in the hippocampus. J Endocrinol 2017;233:1–14.
10. Gonzalez F, Rafael de C, Michel B, et al. Reconsidering the role of mitochondria in aging. Gerona 2015;70:1334–42.
11. Zhang X, Wu XQ, Lu S, et al. Deficit of mitochondria-derived ATP during oxidative stress impairs mouse MII oocyte spindles. Cell Res 2006;16:841–50.
12. Miyamoto K, Sato E, Kasahara E, et al. Effect of oxidative stress during repeated ovulation on the structure and functions of the ovary, oocytes, and their mitochondria. Free Radic Biol Med 2010;49:674–81.
13. Bremer J. Carnitine-metabolism and functions. Physiol Rev 1983;63:1420–80.
14. Vanella A, Russo A, Acquaviva R, et al. L-propionyl-carnitine as superoxide scavenger, antioxidant, and DNA cleavage protector. Cell Biol Toxicol 2000;16:99–104.
15. Paumen MB, Ishida Y, Muramatsu M, et al. Inhibition of carnitine palmitoyltransferase I augments sphingolipid synthesis and palmitate-induced apoptosis. J Biol Chem 1997;272:3324–9.
16. Chang B, Nishikawa M, Nishiguchi S, Inoue M. L-Carnitine inhibits hepatocarcinogenesis via protection of mitochondria. Int J Cancer 2005;113:719–29.
17. Hashimoto S. Application of *in vitro* maturation to assisted reproductive technology. J Reprod Dev 2009;55:1–10.
18. Dunning KR, Akison LK, Russell DL, et al. Increased beta-oxidation and improved oocyte developmental competence in response to l-

carnitine during ovarian in vitro follicle development in mice. Biol Reprod 2011;85:548–55.
19. Dunning KR, Cashman K, Russell DL, et al. Beta-oxidation is essential for mouse oocyte developmental competence and early embryo development. Biol Reprod 2010;83:909–18.
20. Abdelrazik H, Sharma R, Mahfouz R, Agarwal A. L-Carnitine decreases DNA damage and improves the *in vitro* blastocyst development rate in mouse embryos. Fertil Steril 2008;91:589–96.
21. Hashimoto S, Nakano T, Yamagata K, et al. Multinucleation per se is not always sufficient as a marker of abnormality to decide against transferring human embryos. Fertil Steril 2016;106:133–9.
22. Hashimoto S, Amo A, Hama S, et al. Growth retardation in human blastocysts increases the incidence of abnormal spindles and decreases implantation potential after vitrification. Hum Reprod 2013;28:1528–35.
23. Iwahata H, Hashimoto S, Inoue M, et al. Neonatal outcomes after the implantation of human embryos vitrified using a closed-system device. J Assist Reprod Genet 2015;32:521–6.
24. Pacifici EH, McLeod LL, Sevanian A. Lipid hydroperoxide-induced peroxidation and turnover of endothelial cell phospholipids. Free Radic Biol Med 1994;17:297–309.
25. Furuno T, Kanno T, Arita K, et al. Roles of long chain fatty acids and carnitine in mitochondrial membrane permeability transition. Biochem Pharmacol 2001;62:1037–46.
26. Moraes CT, Ricci E, Petruzzella V, et al. Molecular analysis of the muscle pathology associated with mitochondrial DNA deletions. Nat Genet 1992;1:359–67.
27. Esposito LA, Melov S, Panov A, et al. Mitochondrial disease in mouse results in increased oxidative stress. Proc Natl Acad Sci USA 1999;96:4820–5.
28. Inoue M, Sato E, Nishikawa M, et al. Free radical theory of apoptosis and metamorphosis. Redox Rep 2004;9:237–47.
29. Chang B, Nishikawa M, Sato E, et al. L-Carnitine inhibits cisplatin-induced injury of the kidney and small intestine. Arch Biochem Biophys 2002;405:55–64.
30. Kira Y, Nishikawa M, Ochi A, et al. L-carnitine suppresses the onset of neuromuscular degeneration and increases the life span of mice with familial amyotrophic lateral sclerosis. Brain Res 2006;1070:206–14.
31. Moawad AR, Tan SL, Xu B, et al. L-carnitine supplementation during vitrification of mouse oocytes at the germinal vesicle stage improves preimplantation development following maturation and fertilization *in vitro*. Biol Reprod 2013;88:1–8.
32. Moawad AR, Xu B, Tan SL, Taketo T. L-carnitine supplementation during vitrification of mouse germinal vesicle stage–oocytes and their subsequent *in vitro* maturation improves meiotic spindle configuration and mitochondrial distribution in metaphase II oocytes. Hum Reprod 2014;29:2256–68.
33. Fenkci SM, Fenkci V, Oztekin O, et al. Serum total L-carnitine levels in non-obese women with polycystic ovary syndrome. Hum Reprod 2008;23:1602–6.
34. Celik F, Kose M, Yilmazer M, et al. Plasma L-carnitine levels of obese and non-obese polycystic ovary syndrome patients. J Obstet Gynaecol 2017;37:476–9.