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ORIGINAL ARTICLE

Alpha Lipoic Acid Upregulates Mitochondrial DNA Copy Number of Mouse Ovarian Follicles during *In Vitro* Culture

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KEYWORDS

Alpha lipoic acid;
Ovarian follicle;
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ABSTRACT: The objective of this study was to investigate the influence of alpha lipoic acid (ALA) on the expression of the *Tfam* gene and the copy number of mtDNA in preantral follicles (PFs) of mice during in vitro culture. PFs were mechanically isolated from 14-day-old National Medical Research Institute (NMRI) mice and subjected to a 12-day culture in the presence of 100µm ALA, followed by hCG-induced ovulation. The developmental parameters of PFs and oocyte maturation were evaluated, and the expression of the Transcription Factor A, Mitochondrial (*Tfam*) gene, along with mtDNA copy number in granulosa cells and oocytes, were measured using real-time PCR. The results showed that ALA-treated groups exhibited significantly higher diameter of PFs and survival rate, antrum formation, and MII oocytes rate in comparison to the control groups ($P<0.05$). Furthermore, *Tfam* gene expression significantly increased in granulosa cells and oocytes of the ALA groups in comparison with the control group, and the mtDNA copy number in granulosa cells and oocytes of the ALA-treated group was significantly higher than that of the control groups ($P<0.05$). Supplementing the culture medium with ALA demonstrated an improvement in PFs' developmental competence, likely mediated by the upregulation of *Tfam* gene expression and an increase in mtDNA copy number in oocytes and granulosa cells.

INTRODUCTION

Infertility is typically characterized as the inability to achieve pregnancy following 12 months of consistent unprotected sexual intercourse. According to the World Health Organization, approximately 1.5-2% of couples worldwide suffer from infertility [1]. To address this issue, assisted reproduction technology (ART) has been proposed, with in vitro culture of ovarian follicles being one of the methods used to treat infertility [1]. Nonetheless, the in vitro culture setting can potentially result in oxidative stress (OS), which arises from an imbalance between the generation of reactive oxygen species (ROS) and the cell's antioxidant defense system [2]. OS can adversely affect ovarian function, leading to an increase in degenerated oocytes and abnormalities in embryo development [3].

Antioxidants are divided into two types: enzymatic and non-enzymatic [4]. Alpha Lipoic Acid (ALA) falls into the non-enzymatic antioxidant category and is a disulfide compound derived from octanoic acid, also known as thioctic acid. ALA possesses both fat and water solubility, facilitating easy cell membrane penetration and absorption. Moreover, it can convert to its reduced form, Dihydrolipoic acid (DHLA) [4]. ALA and DHLA have demonstrated various beneficial properties, such as anti-aging, anti-mutagenic, anti-carcinogenic, and anti-inflammatory effects. Additionally, ALA acts as a

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coactivator for other antioxidants like glutathione, vitamin E, and C, further enhancing its effectiveness [5]. ALA is known to scavenge ROS, neutralize radicals, chelate metals, and regulate NF-KB activation [5].

While a controlled amount of ROS plays a vital role in various signaling pathways, excessive ROS levels can trigger apoptotic pathways in mitochondria [3]. Mitochondria have a significant impact on oocyte and embryo development, with mitochondrial DNA (mtDNA) being particularly vulnerable to OS compared to nuclear DNA [3]. Studies have confirmed a link between mtDNA dysfunction and infertility. Oocytes with deficient mtDNA copy numbers and reduced ATP production capacity are unable to undergo ovulation, leading to reproductive issues. Low mtDNA copy numbers have also been observed in degenerated oocytes, which are less likely to successfully fertilize [6-8]. Additionally, mtDNA copy number in granulosa and cumulus cells can serve as a marker for predicting competent embryos [9]. Mitochondrial transcription factor A, known as TFAM, plays a crucial role in mtDNA transcription and replication, essential processes for mitochondrial biogenesis. TFAM also acts as a protector of mtDNA against oxidative stress [10].

Prior research has suggested that ALA can enhance follicle development during in vitro culture [11-15]. Building upon these insights, the current study seeks to examine the impact of ALA on both mtDNA copy number and Tfam gene expression in preantral follicles of mice during in vitro culture.

MATERIALS AND METHODS

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Chemicals: All the chemicals employed in this study were procured from Sigma-Aldrich (Germany), unless specified otherwise.

Animals: Female and male National Medical Research Institute (NMRI) mice were obtained from the Pasteur Institute of Iran. The animals were kept and used in accordance with the guidelines of the animal ethics committee. The mice were accommodated under controlled conditions, which included a 12-hour light/12-hour dark cycle, unrestricted access to water and standard chow, room temperature held within the range of 20 to 25°C, and humidity levels maintained between 0% and 50%. The current study adhered to the guidelines of the Ethical Committee at the School of Biology, Damghan University, Iran. We followed all relevant institutional protocols for the ethical treatment and use of animals, in accordance with the Helsinki Declaration as revised in Tokyo 2004.

Preantral follicle isolation

We began with neonatal female mice (14 days old, n=20), and their euthanasia was performed through cervical dislocation. Subsequently, their ovaries were excised and placed in small pools of α-minimal essential medium (α-MEM; Gibco, UK), which was further enriched with 0.22g L^{-1} sodium bicarbonate, 0.0036 g L^{-1} sodium pyruvate, 10% fetal bovine serum (FBS), 100 IU ml^{-1} penicillin, and 100 µg ml^{-1} streptomycin. To acclimatize to the in vitro environment, the ovaries were then incubated at 5% CO_2, 37°C, and 95% humidity for duration of 15 minutes. Preantral follicles (PFs) were mechanically isolated using a 29-gauge needle from an insulin syringe under a stereomicroscope at 10X magnification, following established procedures [13]. Isolated PFs were selected based on specific criteria: round-shaped follicles with diameters ranging from 140 to 160 µm, containing an intact oocyte with numerous layers of granulosa cells. The isolated medium was kept at 37°C throughout the process.

Study design

The gathered preantral follicles (PFs) were randomly separated into two groups: control and ALA-treated groups. Both groups of PFs were cultured *in vitro* for 12 days in droplets of α-MEM medium. The study consisted of two sections: the first section evaluated follicular development, including follicular survival, diameter, antrum formation, and ovulation. The second section investigated the copy number of mtDNA and *Tfam* gene expression in granulosa cells and oocytes.

In vitro culture of PFs

Isolated PFs were individually transferred using glass Pasteur pipettes to 25 µl droplets of α-MEM

supplemented with 1% ITS (Insulin Transferring Selenium; Gibco, UK), 20 ng EGF (Epidermal Growth Factor), 100 mIU rhFSH (Recombinant human Follicle-Stimulating Hormone; Cinnal-f, Iran), 5% v v^{-1} FBS, and 100 µm ALA under sterile mineral oil. The PFs were then incubated at 5% CO_2, 37°C, and 95% humidity conditions for 12 days. Every 48 hours, we replaced 10 µl of the culture medium in each droplet with fresh medium. Follicle growth and development were assessed by measuring the follicle diameter under an inverted microscope at 10X magnification on the initial day, second, and fourth day of culture, as described previously [13]. Granulosa cell proliferation, antrum cavity formation, and overall survival rate were monitored using a stereomicroscope over the 12-day culture period. On the 12th day, we induced ovulation by introducing 1.5 IU ml^{-1} of human chronic gonadotropin (hCG; IBSA, Switzerland) into the culture medium. Following a 24-hour period, the oocytes were classified into three groups: those with a germinal vesicle (GV), those undergoing germinal vesicle breakdown (GVBD) without a germinal vesicle, and those at the metaphase II (MII) stage, characterized by the extrusion of the first polar body.

Oocyte and granulosa cells separation

At the end of the 12-day culture period, oocytes and granulosa cells were separated through incubation in a culture medium supplemented with 0.02 g collagenase for 1 hour. Oocytes were picked up using a mouth pipette and transferred to separate 1.5 mL microtubes. To detach granulosa cells attached to the bottom of the culture dish, 1 mL trypsin was added, and the mixture was centrifuged at 3000 g for 3 minutes. After centrifugation, all samples were washed with α-MEM supplemented with 10% FBS and centrifuged again at 3000 g for 3 minutes.

Absolute quantification of mtDNA copy number

DNA samples were extracted from oocytes and granulosa cells as previously described [16]. Specific primers for the mouse mitochondrial DNA sequence were designed using Primer3 plus software, and mtDNA amplification was performed with mitochondrial forward and reverse primers (Table 1). The PCR product was extracted from an agarose gel and cloned into the vector pTZ57R/T (Thermo Scientific Bio). Five serial dilutions of plasmid were prepared for the quantification of mtDNA copy number by real-time PCR for each oocyte and granulosa cell derived from both groups.

Table 1. Mouse mitochondrial specific primer sequences.

Gene	Primer pair sequence(5´-3´)	Accession numbers	PCR product size (bp)
MTR	GCTAGTGTGAGTGATAGGGTAG	NM-013523.3	79
MTR	CCAATACGCCCTGTAACAAC		

Real-Time PCR: Real-time PCR was conducted to determine Tfam gene expression in oocytes and granulosa cells. RNA was extracted from oocytes and granulosa cells separately using the RiboEx® (PCRlab, Germany) protocol. The extracted RNA samples were quantified and qualified through spectrophotometry and electrophoresis gel analysis, respectively. cDNA was synthesized using the Takara cDNA synthesis kit (Takara Bio, Japan). To evaluate Tfam mRNA expression, Rotor-gene 6000 (Qiagen) and QuantiTect SYBR Green RT-PCR kit (Qiagen, Hilden, Germany) were employed. The Livak method and 2-ΔΔCT were used for Tfam expression analysis, with the elongation factor 1 (Ef1) serving as the housekeeping gene (Table 2).

Table 2. Designed primer sequences used for real-time polymerase chain reaction (PCR)

Gene	Primer pair sequence(5´-3´)	Accession number	PCR product size (bp)
Ef1	Forward: AGTCGCCTTGGACGTTCTT Reverse: CCGATTACGACGATGTTGATGTG	NM_010106	124
Tfam	Forward: AAGGGAATGGGAAAGGTAGA Reverse: AACAGGACATGGAAAGCAGAT	NM_010106	76

Statistical Analysis: We conducted the statistical analysis utilizing the Statistical Package for the Social Sciences software (SPSS, IBM SPSS Statistics 16, USA). To assess the normal distribution of data, we applied the Shapiro test, and the Mann-Whitney (t-test) was employed. The threshold for statistical significance was set at $p<0.05$.

RESULTS

During the culture period, the growth and developmental rate of preantral follicles (PFs) were monitored morphologically every other day using an inverted microscope. The diameters of isolated PFs in the control group and in the presence of 100 µm ALA were summarized in Table 3. The initial diameter of PFs was not significantly different between both groups ($p > 0.05$). However, on the 2nd and 4th days of culture, the diameter of PFs in the ALA-treated group was significantly higher compared to the control group ($p < 0.05$; Table 3).

Table 3. Diameter of PFs during culture period.

Groups	Number of follicles *(n)*	Follicle diameters mean (µm ± SD)		
		Initial time	2nd day	4th day
Control	180	146.69 ± 3.77	202.37 ± 4.48	299.27 ± 11.55
ALA	180	146.29 ± 3.48*	216.68 ± 1.93*	323.52 ± 6.93*

In all cases at least 3 experimental replicates were performed.

*: Indicate different levels of significant difference with control groups ($p<0.05$)

During the culture process, the preantral follicles (PFs) underwent multiple developmental stages. By the second day of culture, as a result of granulosa cell proliferation, the follicles adhered to the culture dish and formed compact, spherical structures. On the fourth day, there was a notable increase in the number of granulosa cells. They extended throughout the culture medium and completely enveloped the oocyte, resulting in a dispersed or spread-out appearance. On the 6th day and onwards, an antrum cavity was formed. Surviving follicles were characterized by round and cohesive oocytes with intact zona pellucida and a uniform perivitelline space. On the other hand, follicles that released their oocytes before granulosa cell proliferation or experienced delayed granulosa cell proliferation were considered degenerate. The survival rate of PFs in the presence of ALA was 92%, significantly higher than the control group's rate of 79% ($p < 0.05$; see Table 4). The rate of antrum formation in the ALA-treated group was 68%, while the control group had a rate of 58%, with the difference being statistically significant ($p < 0.05$; Table 4). Moreover, the rate of follicles that ovulated in the ALA-treated group (48%) was significantly higher compared to the control group (40%; $p < 0.05$; Table 4). The maturation rate of oocytes derived from cultured PFs is presented in Table 4. The proportion of metaphase I (MI) and metaphase II (MII) oocytes released from preantral follicles (PFs) in the ALA-treated group was significantly greater in comparison to the control group ($p < 0.05$; see Table 4).

Table 4. Maturation rates of cultured pre-antral follicles.

Groups	NO. of follicles	Survived *n* (% ± SD)	Antrum formation *n* (% ± SD)	Ovulated follicles *n* (% ± SD)	Stages of oocyte development	
					MI oocytes *n* (% ± SD)	MII oocytes *n* (% ± SD)
Control	180	143 (79.44 ± 4.19)	105 (58.33 ± 3.34)	73 (40.56 ± 4.19)	17 (23.44 ± 3.39)	56 (76.56 ± 3.39)
ALA	180	166* (92.22 ± 4.20)	124* (68.89 ± 5.36)	88* (48.89 ± 2.55)	12* (13.62 ± 3.32)	66* (86.38 ± 3.32)

In all cases at least 3 experimental replicates were performed. *: Denote varying levels of significant difference compared to the control groups ($p<0.05$).

MI: metaphase I oocyte; MII: metaphase II oocyte.

Subsequently, we evaluated the relative mRNA expression of the Tfam gene in oocytes and granulosa cells originating from cultured preantral follicles (PFs). The relative expression of the Tfam gene in oocytes exhibited a notable increase in the ALA-treated group in contrast to the control group ($p < 0.05$; see Table 5). Similarly, the relative mRNA expression of Tfam in granulosa cells derived from the ALA-treated group demonstrated a significant increase compared to the control group ($p < 0.05$; see Table 5).

Table 5. The fold change in mRNA expression of *Tfam* (Mean±SD).

Groups	Granulosa cells	Oocyte
Control	1.02 ± 0.07	1.18 ± 0.18
ALA	1.48 ± 0.04*	1.84 ± 0.04*

In all cases at least 3 experimental replicates were performed.
*: Denote varying levels of significant difference compared to the control groups ($p<0.05$).

Moreover, we quantified the mitochondrial DNA (mtDNA) copy numbers in both oocytes and granulosa cells from the ALA-treated and control groups. The results are summarized in Table 6. Notably, the mtDNA copy number in oocytes obtained from ALA-treated preantral follicles (PFs) was significantly greater (288,430) in comparison to the control group (171,887; $p < 0.05$; Table 6). Likewise, the mtDNA copy number in granulosa cells derived from ALA-treated PFs (523,451) exhibited a significant increase compared to the control group (314,786; $p < 0.05$; Table 6).

Table 6. The mtDNA copy number of granulosa cells and oocyte (Mean±SD).

Groups	Granulosa cells	Oocyte
Control	314786 ± 7207.96	171887.66 ± 25221.60
ALA	523451 ± 7627.56*	288430.33 ± 21831.25*

In all cases at least 3 experimental replicates were performed.

*: Denote varying levels of significant difference compared to the control groups ($p<0.05$).

DISCUSSION

Assisted reproductive technology (ART) often involves *in vitro* maturation (IVM) as a strategy to overcome infertility. However, a major obstacle in IVM is the formation of reactive oxygen species (ROS) and oxidative stress (OS) during the culture period. OS can lead to reduced mitochondrial DNA (mtDNA) copy number, but this can be counteracted by antioxidant supplementation in the culture media. In this study, the potent antioxidant alpha lipoic acid (ALA) was investigated for its effect on mtDNA copy number and Tfam gene expression in preantral follicles (PFs) during *in vitro* culture.

The results showed that ALA significantly improved follicle development and increased Tfam gene expression and mtDNA copy number in both oocytes and granulosa cells. These findings align with previous research indicating that PFs are susceptible to ROS during *in vitro* culture, and ALA's antioxidant properties reduce ROS production and increase total antioxidant capacity [13, 17, 18]. Additionally, ALA has been shown to enhance antrum cavity formation, increase MⅡ oocyte rates, and improve survival in cryopreserving ovarian follicles [13]. It achieves this by reviving other antioxidants, such as glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), and enhancing the overall antioxidant defense system [11, 12].

Mitochondria play a crucial role in oocyte maturation and embryo development, and deficiencies in mtDNA can significantly impact normal development [16, 17]. ALA has been found to up-regulate nuclear respiratory factor 1 (NRF1), which, in turn, up-regulates SIRT3 and SOD2, maintaining ROS hemostasis in mitochondria. SIRT3 expression in granulosa cells protects the preimplantation embryo from OS, while decreased expression of SIRT family members in aged oocytes leads to increased ROS and mitochondrial dysfunction [19]. ALA's up-regulation of NRF1 is essential for TFAM activity, and TFAM itself is critical in

determining mtDNA copy number through deacetylation of SIRT1 and activation of PGC-1α [20].

Furthermore, ALA's association with acetyl-L-carnitine promotes the expression of PGC-1α, NRF1, and NRF2, as well as TFAM and mtDNA copy numbers. ALA also inhibits the NF-Kβ gene, which increases in the presence of OS, thereby preventing destruction of the inhibitor of nuclear factor kappa α (IkBα) [21, 22]. Sufficient TFAM and mtDNA copy number are vital factors in successful folliculogenesis and fertility. Studies have shown that mtDNA copy number in cumulus cells (CCs) may serve as a marker for collecting good-quality embryos after *in vitro* fertilization (IVF). Additionally, good-quality embryos typically have higher mtDNA copy numbers compared to lower quality embryos [7, 16, 23].

The study's findings also highlight that mature oocytes require a substantial number of mtDNA copies, and ALA supplementation in the maturation medium improves follicle developmental competence by increasing Tfam gene expression and subsequently boosting mtDNA copy number. Nonetheless, further research is needed to fully understand the cellular mechanisms behind how ALA up-regulates Tfam gene expression and increases mtDNA copy number in PFs.

In conclusion, this study provides evidence that the addition of ALA to the culture medium of mouse preantral follicles enhances mtDNA copy number and Tfam gene expression in oocytes and granulosa cells, resulting in improved PFs developmental competence. These findings shed light on the potential of ALA as an effective antioxidant in improving ART outcomes and warrant further investigations in this area.

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CONFLICT OF INTERESTS

The authors report there are no competing interests to declare.

ETHICAL CONSIDERATION

The protocol of this study has been reviewed and approved by ethical Committee of the School of Biology, Damghan University, Damghan, Iran that fulfills and follows Declaration of Helsinki as revised in Tokyo 2004 (Ref No: IR.DU.REC.1399.1.3).

The experiments were approved by the ethics committee of Damghan University, Damghan, Iran (IR.DU.REC.1399.23).

Author's Contributions

S.S.: Performed experiments and collected data. S.Z.: Participate in develop the concept and study designed, analyzed data, and authored the paper. M.N.: Contributed extensively to design of the study and conduction of molecular experiments, and revision of the manuscript. All authors participated in the finalization of the manuscript and approved the final draft.

Data availability

The article's data will be shared upon reasonable request to the corresponding author.

REFERENCES

1. Herta A.C., Lolicato F., Smitz J.E.J., 2018. In vitro follicle culture in the context of IVF. Reproduction. 156(1), F59-f73.

2. Agarwal A., Maldonado Rosas I., Anagnostopoulou C., Cannarella R., Boitrelle F., Munoz L.V., Finelli R., Durairajanayagam D., Henkel R., Saleh R., 2022. Oxidative Stress and Assisted Reproduction: A Comprehensive Review of Its Pathophysiological Role and Strategies for Optimizing Embryo Culture Environment. Antioxidants. 11(3), 477.

3. Sasaki H., Hamatani T., Kamijo S., Iwai M., Kobanawa M., Ogawa S., Miyado K., Tanaka M., 2019. Impact of Oxidative Stress on Age-Associated Decline in Oocyte Developmental Competence. Front Endocrinol (Lausanne). 10, 811.

4. Valko M., Rhodes C.J., Moncol J., Izakovic M., Mazur M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 160(1), 1-40.

5. Petca A., Bot M., Maru N., Calo I.G., Borislavschi A., Dumitrascu M.C., Petca R.C., Sandru F., Zvanca M.E., 2021. Benefits of α‑lipoic acid in high‑risk pregnancies (Review). Exp Ther Med. 22(5), 1232.

6. Gabriel M.S., Chan S.W., Alhathal N., Chen J.Z., Zini A., 2012. Influence of microsurgical varicocelectomy on human sperm mitochondrial DNA copy number: a pilot study. J Assist Reprod Genet. 29(8), 759-764.

7. St John J., 2014. The control of mtDNA replication during differentiation and development. Biochim Biophys Acta. 1840(4), 1345-1354.

8. Benkhalifa M., Ferreira Y.J., Chahine H., Louanjli N., Miron P., Merviel P., Copin H., 2014. Mitochondria: participation to infertility as source of energy and cause of senescence. Int J Biochem Cell Biol. 55, 60-64.

9. Desquiret-Dumas V., Clément A., Seegers V., Boucret L., Ferré-L'Hotellier V., Bouet P. E., Descamps P., Procaccio V., Reynier P., May-Panloup P., 2017. The mitochondrial DNA content of cumulus granulosa cells is linked to embryo quality. Hum Reprod. 32(3), 607-614.

10. Bouda E., Stapon A., Garcia-Diaz M., 2019. Mechanisms of mammalian mitochondrial transcription. Protein Sci. 28(9), 1594-1605.

11. Hatami S., Zavareh S., Salehnia M., Lashkarbolouki T., Ghorbanian M.T., Karimi I., 2013. Total Oxidative status of Mouse Vitrified Pre-Antral Follicles with Pre-Treatment of Alpha Lipoic Acid. Iranian Biomedical Journal. 18(3), 181-183.

12. Hatami S., Zavareh S., Salehnia M., Lashkarbolouki T., Ghorbanian M.T., Karimi I., 2014. The impact of alpha lipoic acid on developmental competence of mouse vitrified pre-antral follicles in comparison to those isolated from vitrified ovaries. Iranian Journal of Reproductive Medicine. 12(1), 57.

13. Talebi A., Zavareh S., Kashani M.H., Lashgarbluki T., Karimi I., 2012. The effect of alpha lipoic acid on the developmental competence of mouse isolated preantral follicles. Journal of Assisted Reproduction and Genetics. 29(2), 175-183.

14. Zavareh S., Karimi I., Salehnia M., Rahnama A., 2016. Effect of in vitro maturation technique and alpha lipoic acid supplementation on oocyte maturation rate: focus on oxidative status of oocytes. International Journal of Fertility & Sterility. 9(4), 442.

15. Zavareh S., Talebi A., Hasanzadeh H., 2015. Amending in vitro culture condition to overcome oxidative stress in assisted reproduction techniques (ART). Archives of Advances in Biosciences. 6(2), 135-148.

16. Ghorbanmehr N., Salehnia M., Amooshahi M., 2018. The Effects of Sodium Selenite on Mitochondrial DNA Copy Number and Reactive Oxygen Species Levels of In Vitro Matured Mouse Oocytes. Cell J. 20(3), 396-402.

17. Kasapoğlu I., Seli E., 2020. Mitochondrial Dysfunction and Ovarian Aging. Endocrinology. 161(2), 10.1210/endocr/bqaa001

18. Zoheir K., Harisa G., Allam A., Liguo Y., Xiang L., Liang A., Abd-Rabou A., Harrath H., 2016. Effect of alpha lipoic acid on in vitro development of bovine secondary preantral follicles. Theriogenology. 88, 10.1016/j.theriogenology.2016.09.013.

19. Deng X., Lin N., Fu J., Xu L., Luo H., Jin Y., Liu Y., Sun L., Su J., 2020. The Nrf2/PGC1α Pathway Regulates Antioxidant and Proteasomal Activity to Alter Cisplatin Sensitivity in Ovarian Cancer. Oxid Med Cell Longev. 4830418.

20. Zhang H., Wu B., Liu H., Qiu M., Liu J., Zhang Y., Quan F., 2013. Improving development of cloned goat embryos by supplementing α-lipoic acid to oocyte in vitro maturation medium. Theriogenology. 80(3), 228-233.

21. Fernández-Galilea M., Pérez-Matute P., Prieto-Hontoria P.L., Houssier M., Burrell M.A., Langin D., Martínez J.A., Moreno-Aliaga M.J., 2015. α-Lipoic acid treatment increases mitochondrial biogenesis and promotes beige adipose features in subcutaneous adipocytes from overweight/obese subjects. Biochim Biophys Acta. 1851(3), 273-281.

22. Kim C., Moon J., Jeung Y., Kim S., Chae H., Kang B., 2017. Lipoic acid supplementation increases the expression of pgc-1alpha gene in granulosa cells and improves IVF results in aging women undergoing IVF. Fertility and Sterility. 108(3, Supplement), e228.

23. Bogenhagen D.F., 2012. Mitochondrial DNA nucleoid structure. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms. 1819(9), 914-920.