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L-Carnitine-Mediated Antioxidant Defence in Buffalo Oocytes: A Novel Approach for Improving In Vitro Maturation and Embryo Developmental Competence

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Abstract

THE developmental competence of buffalo oocytes often declines during in vitro conditions, primarily due to oxidative stress. The current study was designed to evaluate the effects of L-Carnitine supplementation during in vitro maturation (IVM) of buffalo oocytes. Oocytes collected from ovaries of slaughtered buffaloes were matured in IVM media supplemented with L-Carnitine at 0 (control), 0.3, 0.6, or 1 mg.mL^{-1}, nuclear maturation, penetration, fertilization (IVF), embryo production and development were assessed. Additionally, antioxidant parameters including glutathione (GSH) level, superoxide dismutase (SOD) activity, catalase (CAT) activity, and lipid peroxidation (MDA) level in oocytes after IVM were measured. The outcomes indicated that significant improvements in oocyte maturation to metaphase II were observed in the 0.3 and 0.6 mg.mL^{-1} L-carnitine groups. Fertilization rates were enhanced within 0.3 and 0.6 mg.mL^{-1} L-carnitine groups in contrast to the control. The 0.6 mg.mL^{-1} L-carnitine group demonstrated significant enhancements in cleavage (2-8 cells) and morula formation. Level of GSH exhibited an elevation, and MDA level declined in L-carnitine groups, although not significantly. SOD and CAT activities were significantly elevated in the 0.6 mg.mL^{-1} l-carnitine treatment against the control. In conclusion, L-carnitine incorporation within IVM improves oocyte nuclear maturation, fertilization, embryo growth, and antioxidant activity in Egyptian buffalo oocytes. 0.6 mg.mL^{-1} emerging as the optimal concentration for overall improvements in oocyte quality and in vitro embryonic developmental competence.

Keywords: Buffalo, In vitro maturation, L-Carnitine, Antioxidant, ROS.

Introduction

The global population of buffalo has experienced significant growth, with Egypt playing a crucial role in African buffalo husbandry. Approximately 3.7 million buffaloes are found within the Egyptian livestock population [1]. Egyptian buffaloes are considered a valuable genetic resource, contributing substantially to meat and milk production. Buffalo milk dominates the Egyptian dairy sector, accounting for about 45–50% of the country's total milk output [2]. This prevalence is due to consumer preference for buffalo milk's white color, high-fat content (about 7%), and distinctive flavor [3]

However, buffalo exhibit notable physiological adaptations: strong immunity, extended reproductive longevity, efficient low-grade forage utilization, and tolerance of suboptimal management [4], buffalo face limitations due to inherent reproductive characteristics and physiological challenges. To address these issues and maximize the potential of this important livestock resource, various assisted reproductive technologies (ART) are being

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implemented, including in vitro embryo production (IVEP) [5].

IVEP popularity continues to grow despite persistent challenges in efficiency and pregnancy outcomes [6]. The complex physiological environment of the female reproductive tract presents a significant challenge for in vitro systems to mimic effectively [7].

IVM is an essential phase of the IVP process, encompassing a series of complex changes within the oocyte that culminate in the achievement of metaphase II [8]. The efficacy of IVM has been affected by multiple parameters, encompassing the constituents of the oocyte maturation medium, incubation temperature, atmospheric gas composition, and duration of maturation [9]. Optimizing IVM is crucial for enhancing oocyte developmental competence and improving IVP overall efficiency [10].

Oxidative stress (OS) significantly impacts the efficiency of IVM [11]. OS arises when disparity between antioxidant defence mechanisms and reactive oxygen species (ROS) generation occurs [6]. Culturing oocytes under high atmospheric oxygen tension (20% O2) can increase ROS production [11], leading to altering DNA and mitochondria, chromosomal misalignments, aneuploidy, increase lipid membrane damage, and apoptosis [6,11]. Buffalo oocytes, with their high lipid content, are especially susceptible to oxidative damage [8,12].

To mitigate these issues, researchers have incorporated various antioxidants into culture media [11,13,14]. One such antioxidant is L-Carnitine (LC), an aqueous-soluble vitamin-like quaternary ammonium compound found in nature [15]. β-oxidation is significantly modulated by L-Carnitine through improving transportation of fatty acids for ATP generation during lipid metabolism in in vitro oocyte maturation [15,16]. Recent research has highlighted L-carnitine's antioxidant properties, including the neutralization of free radicals, enhancement of antioxidant enzyme activity, and protection of cells from oxidative damage-induced apoptosis [17- 21].

However, it remains Unclear if enrichment with L-carnitine provides the combined benefits of modulating lipid metabolism and enhancing antioxidant activity in buffalo oocytes. So, this research aims to study the impact of different L-carnitine treatments on IVM, IVF, and IVC of buffalo cumulus oocytes complexes and developmental competence of resulted embryos, as well as assess changes in antioxidant/oxidant status of matured oocytes.

Material and Methods

Oocyte recovery

A total of 498 Buffalo ovaries were collected from local abattoir in Qalyubia Governorate, Egypt, from adult buffaloes with apparently normal reproductive organs. The reproductive status and ages of the animals were unknown. Ovaries were transported to the Animal Reproduction Research Institute (ARRI), Al-Haram, Giza, in a well-sealed isothermal container with physiological saline (0.9% NaCl supplemented with 100 IU/mL penicillin and 100 µg/mL streptomycin sulfate) at 35-37°C within 2 hours. Upon arrival, ovarian tissues were dissected, and ovaries underwent multiple washes in warm physiological saline (37°C) before aspiration [22].

Cumulus-oocyte complexes (COCs) were recovered from follicles (2-8 mm) using an 18G needle fitted to a sterile syringe (10 mL) containing phosphate buffered saline (PBS) enriched with 6 mg/mL bovine serum albumin (BSA, Fraction V) and 50 µg/mL gentamicin. Follicular fluid was collected inside Falcon tubes and left for sedimentation for 15 minutes in a water bath at 37°C. then COCs were washed three times in an aspiration medium and selected under a stereomicroscope [23]. Only oocytes that have a uniform ooplasm and a multilayered compact cumulus cells (≥ 3 cumulus layers) were used in IVM, following the classification criteria outlined by Rosario et al. [24].

In Vitro Maturation (IVM)

Selected COCs were washed thrice in aspiration medium at 37°C, with a final wash in pre-equilibrated IVM medium before transfer to the IVM dish. groups of 10-15 oocytes were deposited in maturation medium droplets (100 µL). The maturation media was TCM-199 with Earl's salts (Vacsera, Egypt) enriched with fetal calf serum (FCS): 10% heat-inactivated, sodium bicarbonate: 25 mM (Oxford, UK), sodium pyruvate: 0.2 mM (Oxford, UK), LH:5 µg/mL, estradiol 17β: 1 µg/mL, FSH: 0.5 µg/mL, and gentamicin: 50 µg/mL [25].

The medium was further enriched with L-carnitine (Sigma, MKBQ6348V) at concentrations of 0 (control), 0.3, 0.6, and 1.0 $mg.mL^{-1}$, based on previous studies [26-28]. Media droplets were prepared under sterilized light mineral oil (Sigma, USA) in 60 mm culture plates (Falcon, USA) and cultured in a CO_2 incubator (38.5ºC, 5% CO_2, 95% humidity) for 22 hours.

Assessment of Nuclear Maturation

Following 22 hours of incubation, oocytes were mechanically decumulated by gentle pipetting, washed three times in PBS, and were assessed for nuclear maturation [15]. Briefly, five denuded oocytes per group were mounted on glass slides with Vaseline spots at the corners, covered by cover glass, and fixed in aceto-ethanol solution (1:3) for 48 hours. Slides were subjected to 1% aceto-orcein (Nice chemicals, India) staining for 5 minutes, destained

with rinsing solution (distilled water: acetic acid: glycerol, 3:1:1), and air-dried. An inverted microscope was used for chromosomal configuration assessment at various magnifications [29,30]. oocytes categorized as: Germinal-vesicle (GV), Germinal-vesicle-break-down (GVBD), Metaphase 1 (M-I), and Metaphase 2 (M-II).

Oocytes exhibiting cytoplasmic or nuclear abnormalities were classified as degenerated.

Assessment of Antioxidant/Oxidative Status

•Antioxidant enzyme: levels of GSH, activities of CAT and SOD.

•Oxidative damage: MDA level.

The tested samples from about 20 oocytes per replicate after 22 hours of IVM were prepared according to the kits' instructions, homogenized with buffer then centrifuged at 3000 rpm for fifteen minutes. Samples were stored at -20°C until evaluation.

Evaluation of Antioxidant Enzyme Activity:

GSH level

Intracellular level of GSH were determined using a commercial GSH kit (Biodiagnostic, Egypt) [31,32]. GSH level was expressed as mM GSH/L.

SOD Activity

Activity of SOD was quantified by utilizing a commercial SOD kit (Biodiagnostic, Egypt) [33]. the SOD activity was expressed as U/mL.

CAT Activity

CAT activity was evaluated by utilizing a commercial CAT kit (Biodiagnostic, Egypt) [34,35]. The activity of CAT was expressed as U/L.

MDA levels

MDA, a final metabolite of lipid peroxidation, was evaluated by utilizing a commercial MDA kit (Biodiagnostic, Egypt) [36]. MDA levels were expressed as nmol MDA/mL.

In Vitro Fertilization (IVF)

In vitro fertilization was applied using frozen-thawed semen from fertile buffalo bulls. Semen was prepared using a swim-up procedure [37]. Briefly, three frozen semen straws underwent thawing in a water bath for 30 seconds at 37°C. The semen was then layered under SP-TALP enriched with 6 mg/mL BSA (Fraction V) in 15 test tubes and incubated at 37°C with 5% CO_2 at a 45° angle for 60 min. The motile spermatozoa were collected, washed twice with SP-TALP through centrifugation (500 x g for 10 minutes), and resuspended in fertilization TALP (F-TALP) medium improved with heparin 10 µg/mL for sperm capacitation. After 10 minutes of incubation in a CO_2 incubator, sperm motility was assessed. The final sperm count was 2 x 10^6 sperm cells/mL.

Matured oocytes were partially denuded mechanically to facilitate sperm penetration, washed twice in warm IVM medium, and then washed in IVF medium (F-TALP). 10 oocytes were added to 50 microliter drops of F-TALP medium. each drop was enriched with 2 x 10^6 sperm cells/mL. Sperms and oocytes were maintained together for 18 hours at 38.5ºC under 5% CO_2 (IVF=day 0) [38]. Penetration was assessed 6 hours after IVF by examining oocytes for the appearance of decondensed sperm heads, sperm tails with male pronuclei, or both [26]. Fertilization was assessed 18 hours after IVF, with oocytes displaying both female and male pronuclei and 2nd polar body identified as fertilized [26,39]

In Vitro Culture (IVC)

Following 18 hours, suspected zygotes were rinsed three times to cumulus cells and residual sperm elimination. They were then cultured in pre-adjusted culture medium (SOF) droplets (50 µL) under light mineral oil at 38.5ºC under 5% CO_2 and 95% humidity. Embryo cleavage (2-8 cells), morula and blastocyst stages were estimated on different Days 2, 5, and 7 (IVF=day 0) [40]. A part of the SOF medium was renewed with a fresh, pre-equilibrated SOF medium at least 2 hours prior embryo transfer to minimize the effects of waste byproducts.

Statistical Analysis

Three replicates at least of each experiment were carried out. SPSS version 22 was employed for analysis. The overall significance was evaluated by using The Kruskal-Wallis test. post Hoc test was applied for pairwise comparisons. Significance was defined as $P < 0.05$.

Results

The highest proportion of oocytes attaining MII was obtained in 0.6 $mg.mL^{-1}$ LC group (44.64%), followed by the 0.3 $mg.mL^{-1}$ group (32.87%). The proportion of MII oocytes in each of 0.6 $mg.mL^{-1}$ and 0.3 $mg.mL^{-1}$ groups were elevated significantly compared to 1.0 $mg.mL^{-1}$ L-carnitine (23.52%) and control (19.60%) ($P < 0.05$) (Table 1).

Furthermore, 1.0 $mg.mL^{-1}$ group exhibited a significantly higher percentage of GV (17.64%) in comparison to the control (7.84%). GVBD & MI were significantly higher in 0.3 $mg.mL^{-1}$ group (34.42%) in comparison to the control (35.28%).

The greatest rate of fertilization was obtained in the 0.6 $mg.mL^{-1}$ group (33.33%), followed by the 0.3 $mg.mL^{-1}$ mg group (21.95%). Fertilization rates in both the 0.6 $mg.mL^{-1}$ and 0.3 $mg.mL^{-1}$ groups were significantly greater than those in 1.0 $mg.mL^{-1}$ LC and control groups (15.15% and 13.15%, respectively). 0.6 $mg.mL^{-1}$ LC group also exhibited

the highest penetration rate (41.66%) in contrast to the control (Table 2).

A significant higher proportion of cleavage (2-8 cells) and morula stages, but not the blastocyst stages were observed following LC supplementation. 0.6 mg.mL^{-1} group exhibited significant improvement in 2-8 cell (30.76%), and morula (19.23%) stages in comparison to the other groups ($P < 0.05$). The blastocyst yield did not significantly vary between all groups (Table 3).

Although not statistically significant, the highest GSH concentrations were observed in 0.6 mg.mL^{-1} group, followed by 0.3 mg.mL^{-1} group. SOD activity was improved within both the 0.6 mg.mL^{-1} and 0.3 mg.mL^{-1} groups compared to the control. Nevertheless, a significantly higher SOD level was only observed in the 0.6 mg.mL^{-1} group in comparison to the other treatments and the control ($P < 0.05$). Similarly, L-carnitine treatment improved CAT activity, with a significantly higher CAT activity observed in the 0.6 mg.mL^{-1} L-carnitine group in comparison to the other groups and the control ($P < 0.05$) (Table 4).

Enriching with LC during IVM influenced lipid peroxidation, as evaluated by the level of malondialdehyde (MDA), in buffalo oocytes. Although the decrease was not statistically significant, the lowest MDA level was measured in the 0.6 mg.mL^{-1} L-carnitine group, followed by 1 mg.mL^{-1} L-carnitine group, in relation to control (Table 5).

Discussion

The current study comprehensively investigated the outcomes of L-carnitine (LC) supplementation during buffalo oocyte IVM, assessing nuclear maturation, fertilization, embryo development, antioxidant enzyme activity, and lipid peroxidation. Key findings highlighted that 0.6 mg.mL^{-1} LC consistently enhances oocyte competence, likely by modulating energy metabolism and redox balance to optimize developmental outcomes. The optimal L-carnitine concentration appears to be 0.6 mg.mL^{-1}. The data reveal a clear dose-dependent relationship between LC supplementation and antioxidant enzyme activity.

LC supplementation at 0.6 mg.mL^{-1} significantly improved nuclear maturation, achieving higher MII oocyte rates (44.64%) followed by 0.3 mg.mL^{-1} (32.87%). This result is consistent with prior studies in buffalo [15,16] with various concentrations (0.5, 0.375 mg/mL, and 2 mM,) and bovine [25] in concentrations 2.5, and 5 mM. Therefore, it is clear that LC promotes the miotic potential of buffalo oocytes and the response of oocytes to LC is dose-dependent.

LC supplementation at 0.6 mg.mL^{-1} also enhanced fertilization competence, as demonstrated by increased penetration (41.66%) and fertilization rates (33.33%). This aligns with a recent study in buffalo [16], which observed the highest significant fertilization rate obtained in 2 mM, followed by 3 mM.

Moreover, LC at 0.6 mg.mL^{-1} significantly improved cleavage and morula formation, supporting early embryo development. However, the improvements in blastocyst formation exhibited only marginal significance.

Modak et al. [15], demonstrated that the rate of cleavage on day 2 and blastocyst on day 7 was significantly higher following LC treatment in 0.5 mg/mL and 0.375 mg/mL in good-quality oocytes. El-Sokary et al. [42] reveal that frequencies of cleavage and morula rates were notably higher in 0.5 mM LC group in comparison to other treatments. A higher blastocyst yield was observed in the 1 mM and 0.5 mM groups rather than other groups. However, Omara et al. [16] concluded that marked elevation in blastocyst development in all LC groups relative to the control, even though there is no considerable variation between the LC groups and the control in the cleavage and morula stages.

0.6 mg.mL^{-1} LC supplementation enhanced oocyte antioxidant capacity. Specifically, it significantly elevated SOD and CAT activities, suggesting reduced oxidative stress. Such significant elevation in SOD and CAT expression following LC supplementation was observed in diseased rat and human [43-46]. LC (10 mM) supplementation to pig oocytes during IVM yielded a significant lowering of ROS level and improvement in the expression of SOD1 gene [47].

Although not statistically significant, the highest glutathione (GSH) concentrations were observed in the 0.6 mg.mL^{-1} group, followed by 0.3 mg.mL^{-1} L-carnitine groups. Similar outcomes were observed in bovines, where 3.8 mM did not considerably increase ROS levels or GSH activity. [48], cat in which 0.5 mg.mL^{-1} LC supplementation did not show significant influence on GSH level however, it reduced intracellular ROS levels [49]. Contradictory results were obtained in bovine [18], porcine [50], and ovine [51]. This could be due to variations in experimental conditions or assay sensitivity. Further, the outcomes are still variable among different cells and animal species, due to several factors. Examples of these factors are species-specific metabolism, mitochondrial activity, and ROS production [48].

The protective role of LC is underpinned by key mechanisms. LC facilitates fatty acid transport into mitochondria, enhancing β-oxidation and ATP synthesis [15,41]. providing the energy needed for oocyte meiotic progression [52]. LC mitigates superoxide anion radical generation through enhanced mitochondrial electron transport chain efficiency and reduced electron leakage [53].

LC mitigates reactive oxygen species (ROS) via its antioxidant properties, supposedly preventing oxidative damage to oocyte organelles [49-51]. This supposes reduced lipid peroxidation and DNA damage, which are common causes of oocyte degeneration during IVM [6].

These mechanistic insights are consistent with the established role of LC in optimizing energy metabolism and redox balance during oocyte maturation, as evidenced by improved nuclear maturation and developmental competence in prior studies likely through metabolic and antioxidant mechanisms.

Despite these positive findings, limitations must be acknowledged. The study's lack of significant effects on MDA levels.

The observed dose-dependent effects and the species-specific responses also highlight the importance of carefully optimizing the dose to tailor the process for unique systems. The inhibitory effects at higher LC concentrations (1.0 $mg.mL^{-1}$) indicate a narrow therapeutic window. Buffalo oocytes, known for their high lipid content [8,12], may have unique metabolic requirements.

The future work should involve efforts to build upon the findings and address the studies limitations through: Cytoplasmic Maturation and Epigenetic Markers, Synergistic Effects and In Vivo Validation.

Conclusion

The current research gives strong proof that 0.6 $mg.mL^{-1}$ LC is an optimal supplement for enhancing buffalo oocyte quality during IVM. By improving the activities of antioxidant enzymes, supporting mitochondrial function, and alleviating oxidative stress, LC supplementation holds significant promise for improving ART outcomes in buffalo. These findings contribute to the establishment of more potent approaches for improving reproductive efficiency in buffalo breeding programs. By mitigating these limitations and building upon our results, future research can further refine and optimize LC supplementation protocols for buffalo IVM, with significant implications for the efficiency and success of assisted reproductive technologies in this species.

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Declaration of Conflict of Interest

The authors report no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Agriculture, Assiut University, Egypt. (0320250026)

TABLE 1. Influence of L-Carnitine supplementation during IVM on nuclear status of Buffalo Oocytes

Treatment	No. Oocytes	GV No. (%± SD)	GVBD & MI No. (% ± SD)	MII No. (%± SD)
Control (0)	51	4 (7.84 ± 0.507) ^{b}	18 (35.28 ± 0.477) ^{b}	10 (19.60 ± 0.377) ^{b}
0.3 $mg.mL^{-1}$ LC	61	5 (8.91 ± 0.517) ^{b}	21 (34.42 ± 0.537) ^{a}	20 (32.87 ± 0.517) ^{ab}
0.6 $mg.mL^{-1}$ LC	56	4 (7.14 ± 0.447) ^{b}	16 (28.57 ± 0.337) ^{b}	25 (44.64 ± 0.217) ^{a}
1.0 $mg.mL^{-1}$ LC	51	9 (17.64 ± 0.337)^{a}	16 (31.37 ± 0.417) ^{b}	12 (23.52 ± 0.473) ^{b}
P-Value		0.059	0.031	0.017

* The percentage ± standard deviation is used to indicate values. Within a single column, various superscript letters differ significantly from each other ($P < 0.05$).
*GV; germinal vesicles, GVBD & MI; germinal vesicle breakdown and metaphase I, MII; Metaphase II.

TABLE 2. Influence of L-Carnitine supplementation during IVM on Penetration and Fertilization Rates

Treatment	No. matured oocytes	Penetration Rate No. (% ± SD)	Fertilization Rate No. (% ± SD)
Control (0)	38	12 (31.57 ± 0.577) ^{b}	5 (13.15 ± 0.507) ^{c}
0.3 $mg.mL^{-1}$ LC	41	14 (34.14 ± 0.517) ^{b}	9 (21.95 ± 0.477) ^{b}
0.6 $mg.mL^{-1}$ LC	36	15 (41.66 ± 0.537) ^{a}	12 (33.33 ± 0.547) ^{a}
1.0 $mg.mL^{-1}$ LC	33	10 (30.30 ± 0.527) ^{b}	5 (15.15 ± 0.587) ^{c}
P-Value		0.026	0.024

*The percentage ± standard deviation is used to indicate values. Within a single column, various superscript letters indicate statistically significant difference ($P < 0.05$).

TABLE 3. Influence of L-Carnitine supplementation during IVM on Buffalo Embryonic Development

Treatment	No. fertilized oocytes	2-8 cell stage No. (% ± SD)	Morula stage No. (% ± SD)	Blastocyst stage No. (% ± SD)
Control (0)	29	3(10.34±0.577)[a]	1 (3.44± 0.577)[a]	0 (0 ± 0.333) [a]
0.3mg.mL^{-1} LC	23	5(21.73±0.577)[a]	2 (8.69± 0.577)[a]	1 (4.34± 0.577)[a]
0.6mg.mL^{-1} LC	26	8(30.76±0.577)[b]	5(19.23±0.577)[b]	2 (7.69± 0.577)[a]
1.0mg.mL^{-1} LC	27	4(14.81±0.577)[a]	1(3.70 ± 0.577)[a]	0 (0 ± 0.333) [a]
P-values		0.035	0.059	0.151

* The percentage ± standard deviation is used to indicate values. Within a single column, various superscript letters significantly different ($P < 0.05$).

TABLE4. Influence of L-Carnitine supplementation during IVM on Antioxidant Enzyme Activity in Buffalo Oocytes

Treatment	Glutathione (mM/l) (Mean ± SD)	Superoxide Dismutase (U/ml) (Mean ± SD)	Catalase (U/l) (Mean ± SD)
Control (0)	3.9800 ± 1.31913[a]	19.2233 ± 4.69779 [a]	18.0367 ± 3.99463 [a]
0.3 mg.mL^{-1} LC	6.7300 ± 2.23336[a]	21.2533 ± 6.42679 [a]	24.8267 ± 4.12534 [a]
0.6 mg.mL^{-1} LC	6.9433 ± 4.22824[a]	36.3633 ± 4.47395 [b]	33.8233 ± 7.19889 [b]
1 mg.mL^{-1} LC	3.6400 ± 0.649 [a]	17.4267 ± 4.0942 [a]	20.6967 ± 5.09192 [a]

* mean ± standard deviation is used to indicate values. Within a single column, various superscript letters show significant difference ($P < 0.05$).

TABLE 5. Influence of L-Carnitine supplementation during IVM on malondialdehyde (MDA) levels in Buffalo Oocytes

Treatment	Malondialdehyde (nmol/ml) (Mean ± SD)
Control (0)	4.7367 ± 1.81131 [a]
0.3 mg.mL^{-1} LC	4.3833 ± 1.22321 [a]
0.6 mg.mL^{-1} LC	2.4167 ± 0.5523 [a]
1 mg.mL^{-1} LC	3.4700 ± 1.1221 [a]

*****mean ± standard deviation is used to indicate values. Within a single column, various superscript letters show significant difference ($P < 0.05$).

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الدفاع المضاد للأكسدة بواسطة الكارنيتين في بويضات الجاموس: نهج جديد لتحسين النضج في المختبر وكفاءة تطور الأجنة.

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الملخص

هدفت هذه الدراسة إلى تقييم تأثير إضافة مادة إل-كارنيتين أثناء عملية النضوج المعملي لبويضات الجاموس. تم جمع البويضات من مبايض الجاموس المأخوذة من المجازر ونضجت في وسائط معملية مضاف إليها إل-كارنيتين بتركيزات 0 (مجموعة التحكم)، 0.3، 0.6، أو 1 ملغ/مل. تم تحليل معدلات نضوج البويضات ، الإخصاب، وتطور الأجنة. بالإضافة إلى ذلك، تم قياس المؤشرات المضادة و الدالة على الأكسدة بعد فترة النضوج المعملي ، مثل مستوى الجلوتاثيون، نشاط إنزيم سوبر أكسيد ديسميوتاز، نشاط إنزيم الكاتالاز، ومستوى بيروكسيد الدهون.

أظهرت النتائج تحسنًا ملحوظًا في نضوج البويضات إلى الطور الاستوائي الثاني في مجموعتي 0.3 و 0.6 ملغ/مل مقارنة بمجموعة التحكم. كما ارتفعت معدلات الإخصاب في نفس المجموعتين. أظهرت مجموعة إل-كارنيتين بتركيز 0.6 ملغ/مل تحسينات ملحوظة في التطور الجنيني المبكر. زادت مستويات الجلوتاثيون وانخفضت مستويات بيروكسيد الدهون في البويضات المعالجة بإل-كارنيتين، رغم أن هذه التغيرات لم تكن ذات دلالة إحصائية. بالإضافة إلى ذلك، وجد ارتفاع ملحوظ في نشاط إنزيمي سوبر أكسيد ديسميوتاز والكاتالاز في مجموعة 0.6 ملغ/مل مقارنة بمجموعة التحكم.

في الختام، أثبتت الدراسة أن إضافة إل-كارنيتين أثناء النضوج المعملي يعزز النضوج النووي للبويضات، الإخصاب، وتطور الأجنة، كما يحسن النشاط المضاد للأكسدة في بويضات الجاموس المصري. يعتبر تركيز 0.6 ملغ/مل الأفضل لتحسين جودة البويضات والكفاءة التطورية.

الكلمات الدالة: الجاموس، النضج النووي للبويضات، إل-كارنيتين، الأكسدة.