Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 53, Number 2, p:143-152 June 2025
DOI: 10.21608/zvjz.2025.338452.1271

**RESEARCH ARTICLE**

# Impact of Cumulus-Oocyte Complexes Morphological Grades on In Vitro Maturation and Biochemical Profiles in Dromedary Camels

Mohamed Eleam[1], Gamal Shawki[1], Nasser Ghanem[2], Omar A. Farid[3], Beshoy S.F. Khalil[2], Ayman Mesalam[1*]

[1]Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt.
[2]Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt.
[3]National Organization for Drug Control and Research, Giza, Egypt.

*Correspondence: Corresponding author: Ayman Mesalam, E-mail: aymanmesalam@gmail.com.

## ARTICLE INFO

**Article History:**
**Received: 5 December 2024**
**Accepted: 15 January 2025**
**Published online: June 2025**

**Key words:**
Dromedary camels, Oocyte quality, *In vitro* maturation, Mitochondrial intensity, Antioxidants



## ABSTRACT

The dromedary camel holds significant economic value, but its reproductive limitations pose challenges to fertility. Assisted reproductive technologies (ARTs), such as in vitro maturation (IVM) and embryo production, offer potential solutions to enhance camel fertility. This study aimed to assess the impact of morphological properties of cumulus-oocyte complexes (COCs) on maturation rates of dromedary camel oocytes and biochemical markers in the spent IVM medium. COCs were classified into good- and poor-quality based on morphological assessment followed by IVM for 30 hours then cumulus expansion, polar body extrusion, and mitochondrial activity were evaluated. Biochemical markers, including antioxidants (superoxide dismutase (SOD), catalyze (CAT), glutathione (GSH)), adenosine triphosphate (ATP), nitric oxide (NO), and malondialdehyde (MDA), were also measured the spent IVM medium. Results showed significant differences between good- and poor-quality COCs in cumulus expansion (94.8% vs. 52.0%, $P < 0.0001$), polar body release (47.0% vs. 22.5%, $P = 0.0003$), and mitochondrial intensity (2.45 vs. 1.51, $P < 0.0001$). Good-quality COCs had significantly higher SOD, CAT, GSH, and ATP levels, while poor-quality COCs exhibited increased NO and MDA levels in the spent IVM medium where morphologically different grades of COCs were cultured. These findings highlight the critical role of COCs quality in determining the maturation success in dromedary camels.

## Introduction

The dromedary camel is of great economic significance, utilized for a variety of purposes such as transportation, entertainment, beauty pageants, and racing competitions [1]. However, concerns have been raised regarding the reproductive limitations of female dromedary camels including induced ovulation, seasonal breeding patterns, prolonged calving intervals, and inadequate expression of estrus signs [2]. To address these challenges, advancements in assisted reproductive technologies (ARTs), such as cryopreservation, *in vitro* fertilization (IVF), and embryo transfer, have been employed to enhance fertility and improve the genetic performance of camels [3]. Compared to other species, research on the dromedary camel is still in infancy. This marginality can be traced back to the low camel stock compared to other livestock, as they are found in limited geographic areas [4].

Oocyte maturation is tightly regulated by hormonal factors such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH), as well as by interactions between oocytes and granulosa cells [5-7]. *In vitro* maturation (IVM) is a critical step in producing mature oocytes capable of supporting successful embryonic development [8]. The proper execution of IVM is essential for the successful production of embryos through ART. This process involves the resumption and completion of the first meiotic division, the cytoplasmic, and molecular maturation. All the previous events are required for fertilization and subsequent embryonic development [9]. Over recent decades, significant progress has been made in improving oocyte maturation rates and quality *in vitro* [10]. Advances include regulating gene expression related to maturation, and stimulating maturation through hormonal interventions [10]. Research on camel oocytes is still evolving, with various efforts made to enhance their *in vitro* maturation [11].

Several factors influence oocyte maturation and early embryonic development, with oocyte quality being a pivotal determinant of success [12]. The ability of an oocyte to complete the maturation process, to be fertilizable, and produce healthy offspring is known as its quality [13]. The quality of the oocytes and the maturation conditions impact their developmental potential for further uses such as *in vitro* fertilization, culture, and embryo production [14]. Oocyte quality is determined by multiple interacting factors that must be carefully managed to optimize *in vitro* embryo production. These factors include the stage of the estrous cycle, ovarian morphology, follicle diameter, biochemical properties of the follicular fluid, hormonal profiles, and the cumulus-oocyte complex [15]. The association of cumulus cells (CCs) with oocytes is crucial for oocyte survival [16] and hence for IVF and IVM to be successful [17]. As the cumulus cells are in charge of providing the oocytes with nourishment during the latter stage of oocyte maturation [18]. The competence of oocytes is severely impacted when CCs separate from them [19].

Mitochondria are crucial organelles in the oocyte cytoplasm due to their multifunctional roles [20]. They are the primary source of adenosine triphosphate (ATP), the energy currency necessary for fertilization and the development of preimplantation embryos [21]. This energy supports the high metabolic demands of oocyte maturation, early embryogenesis, and the resumption of meiosis [10]. As the oocyte matures, mitochondria exhibit noticeable shifts in their distribution pattern from homogenous to heterogeneous [22]. According to recent research, poor-quality

oocytes exhibit several age-related dysfunctions [23] such as shifting mitochondrial gene expression, chromosomal aneuploidies, increased mitochondrial DNA damage, decreased mitochondrial membrane potential, and apoptosis. Any of these dysfunctions could result in severe developmental retardation and preimplantation embryo arrest [22]. Since oocyte maturation *in vitro* is accompanied with changes in the distribution of active mitochondria in addition to unique cumulus morphological changes, it has been suggested that mitochondria distribution may be utilized to determine cytoplasmic maturation [21].

During the handling of gametes and embryos *in vitro*, oxidative stress can pose a significant challenge due to the production of reactive oxygen species (ROS) [24]. ROS possess the ability to interact and modify any molecule, resulting in both structural and functional changes [25]. Oocytes are protected from ROS by a class of intracellular antioxidants that work to neutralize these harmful species. These antioxidants include enzymatic and non-enzymatic factors, such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutases (SODs), which are abundant in the follicular fluid and play a key role in safeguarding oocytes from oxidative damage [26].

The aim of this study was to investigate the impact of morphological properties of COCs on maturation rates and mitochondrial activity in dromedary camel oocytes after 30 hours of *in vitro* maturation. Additionally, we measured the concentrations of antioxidants (superoxide dismutase (SOD), catalyze (CAT), glutathione (GSH)), adenosine triphosphate (ATP), nitric oxide (NO), and malondialdehyde (MDA) in the spent IVM medium where morphologically different grades of COCs were cultured.

## Materials and methods

***Chemicals, Media, and Ethics Statements***

Unless otherwise noted, all chemicals, reagents, and media used in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA). All the methods and experimental procedures were reviewed and approved by Zagazig University Research Ethics Committee (approval number; ZU-IACUC/2/F/255/2024).

***Ovaries Collection and Oocyte Recovery***

Dromedary camel ovaries (n=100) were collected during the breeding season from local abattoirs and transported to the laboratory within 2 hours post-slaughter in a thermos flask containing sterile normal saline supplemented with 100 IU penicillin and 100 µg streptomycin/ml. After removing excess tissues, the ovaries were washed three times with warm saline and placed in a water bath at 38°C. Cumulus-oocyte complexes (COCs) were harvested by slicing the ovarian surface, followed by rinsing with warm phosphate-buffered saline (PBS) supplemented with 50 µg/ml gentamicin. COCs were retrieved and examined using a stereomicroscope at 90x magnification (Leica, Germany).

***COCs Categorization***

The recovered COCs (n=500, ten biological replicate) were morphologically classified based on the number and compactness of cumulus cell layers and cytoplasmic homogeneity [27]. Good-quality COCs (n=200), included COCs that consist of oocytes surrounded with at least two layers of compact cumulus cells with homogenous cytoplasm. Poor-quality COCs (n=300), include COCs that consist of oocytes surrounded with one layer of cumulus cells and partially or completely denuded oocytes.

**In Vitro *Maturation (IVM) of Oocytes***

COCs were cultured in IVM medium consisted of TCM-199 supplemented with 10% inactivated fetal bovine serum (FBS), 5 µg/ml follicle-stimulating hormone (FSH), 1 µg/ml estradiol-17β

(E2), 0.15 mg/ml glutamine, 22 µg/ml sodium pyruvate, and 50 µg/ml gentamicin and incubated in a humidified atmosphere at 38.5°C with 5% $CO_2$ for 30 hours [28].

***Assessment of cumulus expansion and polar body extrusion***

At the end of the *in vitro* maturation period, cumulus expansion was morphologically assessed under a stereomicroscope, and the percentage of expanded COCs was calculated based on the criteria of Amer and Moosa [29]. Furthermore, cumulus cells (CCs) were removed from the oocytes using 0.25% hyaluronidase and repeated pipetting, and the presence of the first polar body in the perivitelline space were examined under a stereomicroscope.

***Mitochondrial Activity Measurement***

The mitochondrial activity of the oocytes after the *in vitro* maturation was assessed using MitoTracker Green FM (Invitrogen, Eugene, USA) according to the manufacturer's instructions, with some modifications based on Ghanem *et al.* [30]. Oocytes were washed with PBS-PVP buffer for 15 minutes and incubated with 15 µl of 200 nM MitoTracker Green dye for 30 minutes, followed by two washes with PBS-PVP buffer. Oocytes were mounted on a clean slide with 1-2 µl of glycerol, and a cover slip was placed without compressing the oocytes. The edges were sealed with a paraffin-vaseline mixture. Fluorescence of the MitoTracker Green dye was excited at 580-596 nm, and images were captured using an epifluorescence microscope. Image J software (National Institutes of Health; https://imagej.net) was used to analyze the gray value mean, representing fluorescence intensity.

***Biochemical Analysis of Spent IVM Medium***

Biochemical components (ATP, SOD, CAT, NO, and MDA) in the spent IVM medium were analyzed using high-performance liquid chromatography (HPLC). The HPLC system (Agilent, Santa Clara, USA) was equipped with a column oven, quaternary pump, rheodyne injector, and UV variable wavelength detector with a 20 µl loop. Chromatograms and reports were generated using the Chemstation software (Agilent). The analysis was conducted at 210 nm with a flow rate of 2 ml/min using a Synerji RP Max column. The mobile phase was isocratic, consisting of potassium phosphate buffer and acetonitrile at pH 2.7.

***Statistical analysis***

Data were analyzed using SAS software (SAS Institute, 2004) with the Student's *t*-test. Results are expressed as mean ± standard error of the mean (SEM). Differences were considered statistically significant at $P \leqslant 0.05$.

## Results

***Effect of Oocyte Quality on Maturation Rates***

Representative images of cumulus expansion before and after maturation are presented in Figure 1A. The proportion of COCs that showed cumulus expansion after IVM was significantly ($P < 0.0001$) higher in good-quality COCs (94.8%) compared to poor-quality COCs (52.0%) (Figure 1B). Similarly, polar body extrusion was observed in 47.0% of good-quality COCs, whereas only 22.5% of poor-quality COCs showed extrusion ($P = 0.0003$, Figure 1C).

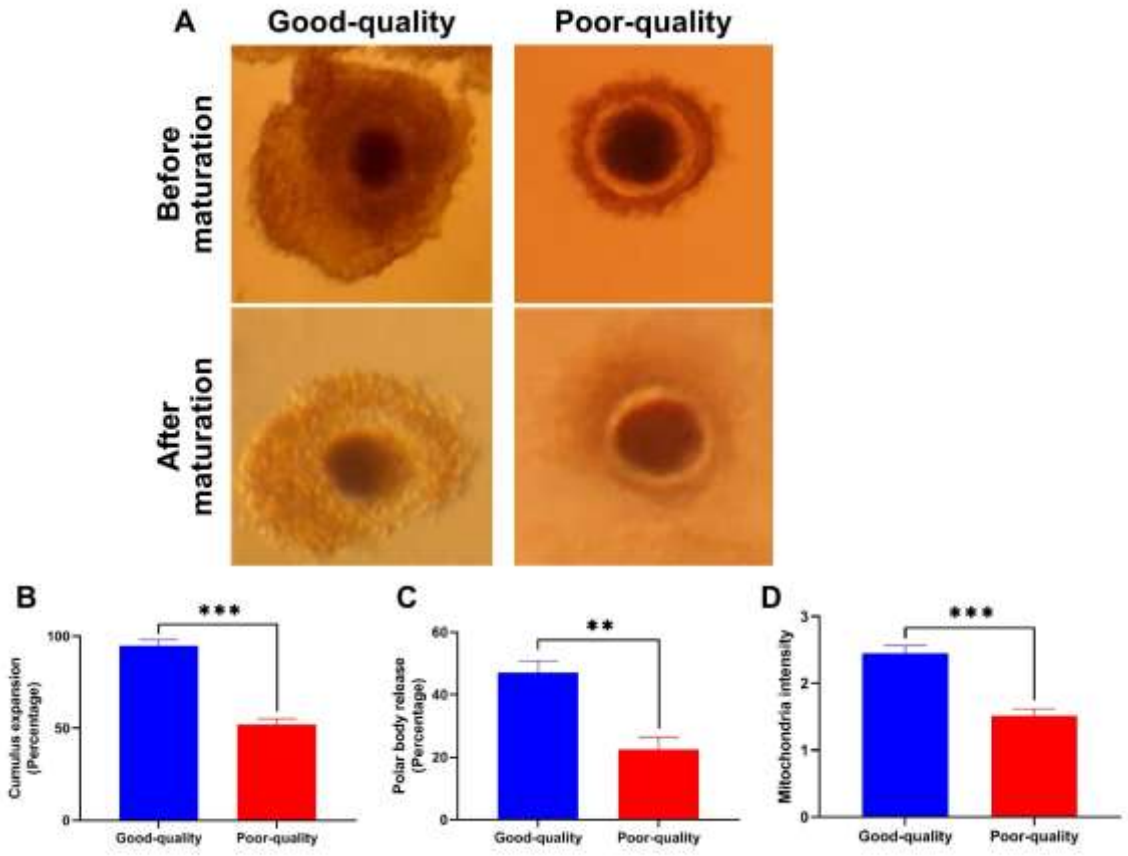


**Figure 1:** Comparison of key parameters between good-quality and poor-quality dromedary camel COCs. (A) Representative images showing cumulus expansion before and after maturation. (B) Percentage of cumulus expansion. (C) Percentage of polar body extrusion. (D) Integrated mitochondrial fluorescence intensity. Data are presented as mean ± SEM. ***, ** represent statistically significant differences between groups at $p \leq 0.001$ and $p \leq 0.01$, respectively.

***Effect of COCS Quality on Mitochondrial intensity***

As seen in Figure 1D, a significant ($P < 0.0001$) reduction in the integrated mitochondrial fluorescence intensity was observed in poor-quality COCS compared to good-quality COCs.

***Effect of COCs Quality on Antioxidants, ATP, Nitric Oxide, and Malondialdehyde Concentration in Spent* In Vitro *Maturation Medium***

Biochemical analysis revealed that good-quality COCs had significantly higher concentrations of SOD (1.78±0.07 U/ml vs. 1.34±0.02 U/ml, $P = 0.0009$), CAT (12.37±0.33 U/ml vs. 10.73±0.30 U/ml, $P = 0.0102$), GSH (3.16±0.09 µmol/ml vs. 2.59±0.02 µmol/ml, $P = 0.0010$), and ATP (18.20±0.66 µg/ml vs. 15.05±0.12 µg/ml, $P = 0.0033$) in the spent IVM medium where they were cultured (Table 1). In contrast, poor-quality COCs showed higher concentrations of NO (0.26±0.01 µmol/ml vs. 0.33±0.01 µmol/ml, $P = 0.0011$) and MDA (16.53±0.67 nmol/ml vs. 19.03±0.31 nmol/ml, $P = 0.0147$) in the spent IVM medium where they were cultured (Table 1).

**Table 1:** Antioxidants, ATP, Nitric Oxide, and Malondialdehyde concentrations in the spent IVM medium where good- and poor-quality COCs were cultured *in vitro*.

| Item | Good quality | Poor quality | P value |
|---|---|---|---|
| **SOD U/ml** | 1.78±0.07 | 1.34±0.02 | 0.0009 |
| **CAT U/ml** | 12.37±0.33 | 10.73±0.30 | 0.0102 |
| **GSH µmol/ml** | 3.16±0.09 | 2.59±0.02 | 0.0010 |
| **ATP µg/ml** | 18.20±0.66 | 15.05±0.12 | 0.0033 |
| **NO µmol/ml** | 0.26±0.01 | 0.33±0.01 | 0.0011 |
| **MDA nmol/ml** | 16.53±0.67 | 19.03±0.31 | 0.0147 |

SOD: superoxide dismutase, CAT: catalyze, GSH: glutathione, ATP: adenosine triphosphate, NO: nitric oxide, MDA: malondialdehyde.

## Discussion

Assisted reproductive technologies (ARTs), particularly *in vitro* embryo production, are increasingly significant in the reproductive management of camels. The success of *in vitro* maturation of camel oocytes is essential for improving embryo production outcomes in this species [31, 32]. Oocyte quality is heavily influenced by the morphological and functional characteristics of cumulus cells [21]. Cumulus cells communicate with the oocyte, supplying essential nutrients and supporting developmental competence [33, 34]. This interdependence suggests that oocytes surrounded by a higher number of cumulus cell layers will benefit from enhanced cellular interactions that promote nuclear maturation and developmental potential [18]. This study aimed to investigate the influence of the morphological properties of COCs after recovery on maturation rates and mitochondria intensity inside the dromedary camels' oocyte after *in vitro* maturation. Additionally, we evaluated the concentrations of antioxidants and other biochemical profiles in the spent maturation medium where morphologically different grades COCs were cultured.

Our results showed that good-quality dromedary camel COCS exhibited significantly higher rates of cumulus expansion and polar body extrusion compared to poor-quality COCs, indicating superior nuclear maturation. These findings align with previous study reported that cumulus expansion levels were significantly higher in good-quality oocyte compared to poor-quality group [28]. Similarly, study in buffalo showed that maturation rate and cumulus expansion increased with the proportion of good-quality oocytes [35].

Cumulus expansion plays a critical role in facilitating oocyte maturation by enhancing the communication between cumulus cells and the oocyte. This interaction ensures the transfer of essential metabolites, signaling molecules, and nutrients, which are vital for nuclear and cytoplasmic maturation [33, 34, 36, 37]. In our study, good-quality COCs exhibited significantly higher levels of cumulus expansion, which likely contributed to their superior mitochondrial activity and nuclear maturation. Cumulus expansion supports mitochondrial function by promoting ATP production and regulating oxidative phosphorylation, processes crucial for

meiotic progression and energy-dependent events during maturation [38-41]. Additionally, the enhanced cumulus-oocyte communication in good-quality COCs may have contributed to synchronized nuclear and cytoplasmic maturation, a key factor in achieving developmental competence [18, 20, 42]. These findings underscore the interdependence between cumulus expansion, mitochondrial activity, and nuclear maturation in determining oocyte quality and subsequent developmental potential.

Oocyte maturation *in vitro* involves changes in mitochondrial activity, suggesting that mitochondria may serve as markers for cytoplasmic maturation [43]. In our study, mitochondrial activity was significantly higher in good-quality COCs. This aligns with findings that mitochondrial function supports energy production crucial for meiotic progression and embryo viability [38]. In contrast, poor-quality COCs exhibited reduced mitochondrial intensity, likely linked to impaired ATP synthesis. Poor-quality oocytes are associated with various dysfunctions, including mitochondrial DNA damage, decreased mitochondrial membrane potential, and altered gene expression [22]. Oocyte quality has been shown to correlate with mitochondrial DNA and ATP levels [39], and elevated ATP levels in oocytes are associated with improved embryo development and implantation success [44]. Emerging research underscores the role of mitochondria as biomarkers of oocyte quality, influencing energy-dependent processes essential for meiotic completion and fertilization [20].

It has been established that follicular fluid contains free radical scavengers that protect oocytes and embryos by maintaining an optimal balance of reactive oxygen species (ROS) [45]. In our study, good-quality COCs maintained higher levels of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) in the spent maturation medium where they were cultured. This highlights the importance of an effective antioxidant system within the follicular fluid, protecting oocytes from oxidative damage caused by ROS generated during *in vitro* maturation (IVM) [46]. The observed higher antioxidant levels in the spent medium where good-quality COCs where cultured suggest a stronger oxidative defense, enhancing their maturation potential and development competence.

Conversely, medium where poor-quality COCs were cultured displayed elevated nitric oxide (NO) concentration, a marker of oxidative stress, which can compromise cellular integrity and maturation potential. Exposure to heat stress has been shown to increase ROS, lipid peroxides, and NO, correlating with poor oocyte quality [47-49]. Additionally, elevated NO concentration in the spent medium where poor-quality COCs were cultured may have adverse effects, as high NO concentrations are associated with reduced fertilization rates and increased embryo fragmentation [50]. Notably, malondialdehyde (MDA), a lipid peroxidation product [51], was significantly elevated in the spent medium where poor-quality COCs were cultured, indicating increased oxidative damage. High MDA levels are linked to reduced embryo quality [52], supporting our observation of lower developmental competence in poor-quality oocytes due to oxidative stress.

In conclusion, this study highlights the significant influence of dromedary camel COCs quality on *in vitro* maturation outcomes and biochemical profiles in the spent IVM medium. Good-quality COCs demonstrated superior nuclear maturation, cumulus expansion, mitochondrial activity, and enhanced antioxidant markers in the spent IVM medium compared to poor-quality COCs. These results underscore the critical role of

COCs quality assessment in improving ART protocols for dromedary camels. Future research should aim to optimize culture conditions to support mitochondrial function and antioxidant defense mechanisms in poor-quality COCs, thereby enhancing the efficiency of ART in this species.

## Conflict of Interest

Authors have no conflict of interest to declare

## References


[1] Faraz, A.; Waheed, A.; Mirza, R.; Ishaq, H. and Tariq, M. (2019): Socio economic status and associated constraints of camel production in desert Thal Punjab, Pakistan. J Fish Livest Prod, 7(01): 288. DOI: 10.4172/2332-2608.1000288.

[2] Bello, A. and Bodinga, H. (2020): Common reproductive problem associated with one humped camel (Camelus dromedarius) in West Africa. Insights Vet Sci, 4(1): 1-3.

[3] Tukur, H.A.; Aljumaah, R.S.; Swelum, A.A.-A.; Alowaimer, A.N. and Saadeldin, I.M. (2020): The making of a competent oocyte–a review of oocyte development and its regulation. J Anim Reprod Biotechnol, 35(1): 2-11.

[4] Abri, M.A.A. and Faye, B. (2019): Genetic Improvement in Dromedary Camels: Challenges and Opportunities. Front Genet, 10: 167. doi: 10.3389/fgene.2019.00167. eCollection 2019.

[5] Su, Y.Q.; Sugiura, K. and Eppig, J.J. (2009): Mouse oocyte control of granulosa cell development and function: paracrine regulation of cumulus cell metabolism. Semin Reprod Med, 27(1): 32-42.

[6] Leoni, G.G. and Naitana, S. (2018): Ovine Granulosa Cells Isolation and Culture to Improve Oocyte Quality. Methods Mol Biol, 1817: 95-106.

[7] Eppig, J.J. (2018): Reproduction: Oocytes Call, Granulosa Cells Connect. Curr Biol, 28(8): R354-R6. DOI: 10.1016/j.cub.2018.03.005.

[8] Hashimoto, S. (2009): Application of in vitro maturation to assisted reproductive technology. J Reprod Dev, 55(1): 1-10.

[9] Zhang, T.; Fan, X.; Li, R.; Zhang, C. and Zhang, J. (2018): Effects of pre-incubation with C-type natriuretic peptide on nuclear maturation, mitochondrial behavior, and developmental competence of sheep oocytes. Biochem Biophys Res Commun, 497(1): 200-206.

[10] Jiang, Y.; He, Y.; Pan, X.; Wang, P.; Yuan, X. and Ma, B. (2023): Advances in Oocyte Maturation In Vivo and In Vitro in Mammals. Int J Mol Sci, 24(10): 9059. DOI: 10.3390/ijms24109059.

[11] Tukur, H.A.; Aljumaah, R.S.; Swelum, A.A.; A, N.A.; Abdelrahman, M. and Saadeldin, I.M. (2020): Effects of Short-Term Inhibition of Rho Kinase on Dromedary Camel Oocyte In Vitro Maturation. Animals (Basel), 10(5). DOI: 10.3390/ani10050750.

[12] Wang, Q. and Sun, Q.-Y. (2006): Evaluation of oocyte quality: morphological, cellular and molecular predictors. Reprod Fertil Dev, 19(1): 1-12.

[13] Duranthon, V. and Renard, J.P. (2001): The developmental competence of mammalian oocytes: a convenient but biologically fuzzy concept. Theriogenology, 55(6): 1277-89.

[14] Bahrami, M. and Cottee, P.A. (2022): Culture conditions for in vitro maturation of oocytes–A review. Reprod Breed, 2(2): 31-6.

[15] Aguila, L.; Treulen, F.; Therrien, J.; Felmer, R.; Valdivia, M. and Smith, L.C. (2020): Oocyte selection for in vitro embryo production in bovine species: noninvasive approaches for new challenges of oocyte competence. Animals, 10(12): 2196. DOI: 10.3390/ani10122196.

[16] Winterhager, E. and Kidder, G.M. (2015): Gap junction connexins in female reproductive organs: implications for women's reproductive health. Hum Reprod Update, 21(3): 340-52.

[17] Mermillod, P.; Dalbiès-Tran, R.; Uzbekova, S.; Thélie, A.; Traverso, J.M.; Perreau, C.; Papillier, P. and Monget, P. (2008): Factors affecting oocyte quality: who is driving the follicle? Reprod Domest Anim, 43: 393-400.

[18] Gilchrist, R.B.; Lane, M. and Thompson, J.G. (2008): Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum Reprod Update, 14(2): 159-77.

[19] Fatehi, A.; Zeinstra, E.; Kooij, R.; Colenbrander, B. and Bevers, M. (2002): Effect of cumulus cell removal of in vitro matured bovine oocytes prior to in vitro fertilization on subsequent cleavage rate. Theriogenology, 57(4): 1347-55.

[20] Kirillova, A.; Smitz, J.E.J.; Sukhikh, G.T. and Mazunin, I. (2021): The Role of Mitochondria in Oocyte Maturation. Cells, 10(9). DOI: 10.3390/cells10092484.

[21] Torner, H.; Brüssow, K.-P.; Alm, H.; Ratky, J.; Pöhland, R.; Tuchscherer, A. and Kanitz, W. (2004): Mitochondrial aggregation patterns and activity in porcine oocytes and apoptosis in surrounding cumulus cells depends on the stage of pre-ovulatory maturation. Theriogenology, 61(9): 1675-89.

[22] Wang, L.-y.; Wang, D.-h.; Zou, X.-y. and Xu, C.-m. (2009): Mitochondrial functions on oocytes and preimplantation embryos. J Zhejiang Univ Sci B, 10(7): 483-92.

[23] Wu, J.; Zhang, L. and Wang, X. (2000): Maturation and apoptosis of human oocytes in vitro are age-related. Fertil Steril, 74(6): 1137-41.

[24] Agarwal, A.; Said, T.M.; Bedaiwy, M.A.; Banerjee, J. and Alvarez, J.G. (2006): Oxidative stress in an assisted reproductive techniques setting. Fertil Steril, 86(3): 503-12.

[25] Lampiao, F. (2012): Free radicals generation in an in vitro fertilization setting and how to minimize them. World J Obstet Gynecol, 1(3): 29-34.

[26] Kala, M.; Shaikh, M.V. and Nivsarkar, M. (2017): Equilibrium between anti-oxidants and reactive oxygen species: a requisite for oocyte development and maturation. Reprod Med Biol, 16(1): 28-35.

[27] Kandil, O.; El-Nahla, A.; Siam, A.; Abdoon, A.; Al-Monifi, F. and Mermillod, P. (2014): Effect of media on in vitro maturation rate of dromedary camel oocytes. Glob Vet, 13(2): 159-165.

[28] Ashour, G.; El-Sayed, A.; Khalifa, M. and Ghanem, N. (2020): Effect of heat stress on developmental competence of in vitro matured oocytes of Camelus dromedaries with different qualities. World Vet J, 10(4): 658-64.

[29] Amer, H. and Moosa, A. (2009): Effect of season and culture media on the competence of dromedary camel oocyte to mature in vitro. Glob. Vet, 3(1): 1-8.

[30] Ghanem, N.; Amin, A.; Saeed, A.M.; Abdelhamid, S.M.; El-Sayed, A.; Farid, O.A.; Dessouki, S.M. and Faheem, M.S. (2020): Effects of curcumin supplementation on viability and antioxidant capacity of buffalo granulosa cells under in vitro culture conditions. World Vet J, 10(2): 146-59.

[31] Trasorras, V.; Giuliano, S. and Miragaya, M. (2013): In vitro production of embryos in South American camelids. Anim Reprod Sci, 136(3): 187-93.

[32] Moulavi, F. and Hosseini, S. (2018): Diverse patterns of cumulus cell expansion during in vitro maturation reveal heterogeneous cellular and molecular features of oocyte competence in dromedary camel. Theriogenology, 119: 259-67.

[33] Schoevers, E.; Colenbrander, B. and Roelen, B. (2007): Developmental stage of the oocyte during antral follicle growth and cumulus investment determines in vitro embryo development of sow oocytes. Theriogenology, 67(6): 1108-22.

[34] Gilchrist, R.B. (2010): Recent insights into oocyte–follicle cell interactions provide opportunities for the development of new approaches to in vitro maturation. Reprod Fertil Dev, 23(1): 23-31.

[35] Khairy, M.; Zoheir, A.; Abdon, A.; Mahrous, K.; Amer, M.; Zaher, M.; Li-Guo, Y. and El-Nahass, E. (2007): Effects of season on the quality and in vitro maturation rate of Egyptian buffalo (Bubalus bubalis) oocytes. J Cell Anim Biol, 1: 29-33.

[36] Gilchrist, R.B.; Lane, M. and Thompson, J.G. (2008): Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum Reprod Update, 14(2): 159-77.

[37] Tanghe, S.; Van Soom, A.; Nauwynck, H.; Coryn, M. and de Kruif, A. (2002): Minireview: Functions of the cumulus oophorus during oocyte maturation, ovulation, and fertilization. Mol Reprod Dev, 61(3): 414-24.

[38] May-Panloup, P.; Boguenet, M.; Hachem, H.E.; Bouet, P.E. and Reynier, P. (2021): Embryo and Its Mitochondria. Antioxidants (Basel), 10(2):139. DOI: 10.3390/antiox10020139.

[39] Kirillova, A.; Smitz, J.E.; Sukhikh, G.T. and Mazunin, I. (2021): The role of mitochondria in oocyte maturation. Cells, 10(9): 2484. DOI: 10.3390/cells10092484.

[40] Cummins, J. (1998): Mitochondrial DNA in mammalian reproduction. Rev Reprod, 3(3): 172-82.

[41] Stojkovic, M.; Machado, S.A.; Stojkovic, P.; Zakhartchenko, V.; Hutzler, P.; Goncalves, P.B. and Wolf, E. (2001): Mitochondrial distribution and adenosine triphosphate content of bovine oocytes before and after in vitro maturation: correlation with morphological criteria and developmental capacity after in vitro fertilization and culture. Biol Reprod, 64(3): 904-9.

[42] May-Panloup, P.; Chretien, M.F.; Jacques, C.; Vasseur, C.; Malthiery, Y. and Reynier, P. (2005): Low oocyte mitochondrial DNA content in ovarian insufficiency. Hum Reprod, 20(3): 593-7.

[43] Schatten, H.; Sun, Q.-Y. and Prather, R. (2014): The impact of mitochondrial function/dysfunction on IVF and new treatment possibilities for infertility. Reprod Biol Endocrinol, 12: 1-11.

[44] Van Blerkom, J.; Davis, P.W. and Lee, J. (1995): ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. Hum Reprod, 10(2): 415-24.

[45] Pasqualotto, E.B.; Agarwal, A.; Sharma, R.K.; Izzo, V.M.; Pinotti, J.A.; Joshi, N.J. and Rose, B.I. (2004): Effect of oxidative stress in follicular fluid on the outcome of assisted reproductive procedures. Fertil Steril, 81(4): 973-6.

[46] Chen, Y.; Yang, J. and Zhang, L. (2023): The Impact of Follicular Fluid Oxidative Stress Levels on the Outcomes of Assisted Reproductive Therapy. Antioxidants (Basel), 12(12): 2117. DOI: 10.3390/antiox12122117.

[47] Sakatani, M. (2017): Effects of heat stress on bovine preimplantation embryos produced in vitro. J Reprod Dev, 63(4): 347-52.

[48] Diaz, F.A.; Gutierrez-Castillo, E.J.; Foster, B.A.; Hardin, P.T.; Bondioli, K.R. and Jiang, Z. (2021): Evaluation of Seasonal Heat Stress on Transcriptomic Profiles and Global DNA

Methylation of Bovine Oocytes. Front Genet, 12: 699920. DOI: 10.3389/fgene.2021.699920.

[49] Waiz, S.A.; Raies-Ul-Haq, M.; Dhanda, S.; Kumar, A.; Goud, T.S.; Chauhan, M.S. and Upadhyay, R.C. (2016): Heat stress and antioxidant enzyme activity in bubaline (Bubalus bubalis) oocytes during in vitro maturation. Int J Biometeorol, 60(9): 1357-66.

[50] Lee, T.H.; Wu, M.Y.; Chen, M.J.; Chao, K.H.; Ho, H.N. and Yang, Y.S. (2004): Nitric oxide is associated with poor embryo quality and pregnancy outcome in in vitro fertilization cycles. Fertil Steril, 82(1): 126-31.

[51] Kazemi, A.; Ramezanzadeh, F.; Nasr-Esfahani, M.H.; Yaraghi, A.A.S. and Ahmadi, M. (2013): Does dietary fat intake influence oocyte competence and embryo quality by inducing oxidative stress in follicular fluid? Iran J Reprod Med, 11(12): 1005-12.

[52] Debbarh, H.; Louanjli, N.; Aboulmaouahib, S.; Jamil, M.; Ahbbas, L.; Kaarouch, I.; Sefrioui, O. and Cadi, R. (2021): Antioxidant activities and lipid peroxidation status in human follicular fluid: age-dependent change. Zygote, 29(6): 490-4.

**الملخص العربى**

**تأثير الدرجات المورفولوجية لمجمعات الخلايا الركاميه على معدلات الانضاج المعملى والخصائص البيوكيميائية في الجمال العربيه**

محمد عليم1 , جمال شوقي1 , ناصر غانم2 , عمر احمد فريد3 , بيشوي سامح فوزي خليل2 , ايمن مسلم1*

1 قسم التوليد والتناسل ،كليه الطب البيطري ،جامعه الزقازيق ،الزقازيق ،44519،مصر

2 قسم الانتاج الحيواني ،كليه الزراعه، جامعه القاهره ،الجيزه، مصر

3 الهيئه القومية للرقابة والبحوث الدوائية،الجيزه ،مصر

يمثل الجمل العربي قيمة اقتصادية كبيرة، لكن محدوديته الإنجابية تشكل تحديات على الخصوبة. تقدم التقنيات المساعدة علي الإنجاب (ART)، مثل الانضاج في المختبر (IVM) وإنتاج الأجنة، حلولاً محتملة لتعزيز خصوبة الإبل. هدفت هذه الدراسة إلى تقييم تأثير الخصائص المورفولوجية لمجمعات الخلايا الركامية (COCs) على معدلات إنضاج بويضات الجمل العربي والخصائص البيوكيميائية في الوسط المستهلك (IVM). تم تصنيف مجمعات الخلايا الركامية (COCs) إلى نوعية جيدة وسيئة بناءً على التقييم المورفولوجي ثم تم إنضاج البويضات في المختبر IVM لمدة 30 ساعة. تم تقييم توسع الخلايا الركامية، وإطلاق الجسم القطبي، ونشاط الميتوكوندريا وقياس العلامات البيوكيميائية بما في ذلك مضادات الأكسدة (فوق أكسيد ديسموتاز(SOD)، كاتالاز (CAT)، جلوتاثيون (GSH))، أدينوزين ثلاثي الفوسفات (ATP)، وأكسيد النيتريك (NO)، والمالونديالدهيد (MDA). أظهرت النتائج إختلافات كبيرة بين مجمعات الخلايا الركامية و البويضات الجيدة والمنخفضة الجودة في توسع الخلايا الركامية (94.8% مقابل 52.0%، P <0.0001)، وإطلاق الجسم القطبي (47.0% مقابل 22.5%، P = 0.0003)، وكثافة الميتوكوندريا (2.45 مقابل . 1.51، p<0.0001). كانت مجمعات الخلايا الركامية و البويضات ذات الجودة الجيدة لديها مستويات أعلى بكثير من SOD وCAT وGSH وATP، في حين أظهرت مجمعات الخلايا الركامية والبويضات منخفضة الجودة زيادة في مستويات NO وMDA في الوسط المستهلك IVM. تسلط هذه النتائج الضوء على الدور الحاسم لجودة مجمعات الخلايا الركامية و البويضات (COCs) في تحديد نجاح الإنضاج في الإبل العربية.