



Research Article

Quercetin improves *in vitro* maturation of bovine oocytes after a post-mortem delay

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Abstract

Objectives: Oocyte quality and maturation are critical factors determining successful fertilization and embryo development *in vitro*. However, delays in processing ovarian tissues after animal slaughter or collection can negatively impact oocyte viability and developmental potential. This study investigated the effect of Quercetin on the maturation of bovine oocytes subjected to a field-relevant post-mortem delay.

Methods: Oocytes were isolated from ovaries approximately six-hour delay post-collection, mimicking practical conditions encountered in tissue handling. Then, they were treated with hyaluronidase, mechanically denuded, and cultured with quercetin at concentration of 15 µg/mL, against a control group.

Results: The results demonstrated that quercetin improved the extrusion of polar bodies compared to the control group. Additionally, pH variations were noted among control and quercetin treated group, potentially influencing maturation outcomes.

Conclusion: These findings highlight that quercetin at 15 µg/mL significantly enhances the maturation of bovine oocytes, suggesting its potential to modulate oocyte quality *in vitro*.

Keywords: Bovine oocytes, *in vitro* maturation (IVM), polar body extrusion, post-mortem delay, quercetin

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High-quality mature oocytes are essential in both the livestock and biomedical fields [1]. *In vitro* maturation (IVM) has gained considerable interest as an alternative to *in vivo* maturation prior to fertilization, due to its practical advantages. Compared to conventional *in vitro* fertilization (IVF), IVM offers benefits such as shorter stimulation periods, reduced injection frequency, and lower overall costs associated with drugs and monitoring, making it valuable in medical treatments and livestock breeding programs [2]. This process is gaining popularity despite ongoing concerns about its inefficiency. Previous research has shown that oocytes matured under *in vitro* conditions are exposed to

various cellular stressors, which contribute to a higher incidence of loss of competence in developed embryo compared to those developed *in vivo* [3].

In many livestock, oocytes obtained from slaughterhouse-derived ovaries serve as a primary source for large-scale *in vitro* embryo production. However, the quality of these oocytes can be adversely affected when ovaries are transported over long distances from the slaughterhouse to the laboratory. Factors such as insufficient oxygen and energy supply, along with disruptions to the endogenous antioxidant systems within the isolated ovaries, may impair the viability of follicular oocytes [4].

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In the natural process of *in vivo* oocyte maturation, reactive oxygen species (ROS) are effectively neutralized by antioxidant enzymes present in the follicular fluid, ensuring a controlled balance between ROS production and elimination. In contrast, oocytes matured *in vitro* lack this enzymatic defense system, leading to an imbalance that promotes the accumulation of ROS [5]. Elevated ROS levels can interfere with proper meiotic progression and impair embryonic development, ultimately reducing the overall quality of the oocytes [1].

Over recent decades, the *in vitro* production (IVP) of bovine embryos has become an increasingly important tool in the dissemination and commercialization of high-value dairy and beef genetics. In 2021 alone, more than 1.5 million IVP-derived bovine embryos were produced worldwide [6].

The objective of this study is to investigate the effect of quercetin on the *in vitro* maturation of bovine oocytes subjected to a field-relevant post-mortem delay, thereby assessing its potential to enhance oocyte quality under suboptimal collection conditions.

Materials and Methods

Ethics committee approval

Bovine ovarian tissues were collected as byproducts of routine commercial slaughter at a local abattoir. All procedures regarding tissue collection adhered strictly to local animal welfare regulations and commercial slaughter practices. Since the tissues were collected post-mortem and did not involve any *in vivo* animal sacrifice or experimental procedure, formal review and approval by an Institutional Animal Care and Use Committee (IACUC) was not required for this study.

This preliminary and exploratory study (proof-of-concept) was conducted at the laboratories of the General Commission for Biotechnology in Damascus and the Yashfeen Center for Assisted Fertilization and IVF at the New Arab Hospital. The primary objective was to evaluate the effect of quercetin on the *in vitro* maturation of bovine oocytes under delayed processing conditions.

Our experimental procedures for oocyte isolation and *in vitro* maturation were based on previously established and validated protocols, with necessary adaptations implemented to suit the specific requirements and logistical constraints of this study [7, 8].

Ovarian tissue collection and handling

Ovaries were collected from slaughtered cows at a local abattoir. They were transported to the laboratory in a sterile physiological saline solution (0.9% NaCl) at a controlled temperature of 30–35°C. A 6-hour post-mortem delay was deliberately maintained to simulate the field-relevant conditions under which ovarian tissues are typically collected and handled.

Oocyte isolation and preparation

Cumulus–oocyte complexes (COCs) were isolated from the transported ovaries using a combined approach of slicing and aspiration, as illustrated in Figure 1.

Aspiration: An 18-gauge needle attached to a 10 mL syringe was used to aspirate oocytes from visible surface follicles with diameters ranging between 2–6 mm.

Slicing: The remaining small follicles (<2 mm) were recovered by slicing the ovarian cortex tissue.

The retrieved COCs (total of 20 suitable COCs were collected and utilized across the entire study) were washed three times in a phosphate-buffered saline (PBS) solution to remove any blood or tissue debris. Subsequently, the eight (8) morphologically best-quality COCs were selected and used for immediate culture and experimentation. Following isolation, COCs were treated with a 0.1% hyaluronidase enzyme solution at 37°C for 30 seconds to enzymatically disperse the surrounding cumulus cells. This was followed by three washes in maturation medium to remove residual enzyme. Mechanical denudation was then performed by gently pipetting the oocytes through a stripper pipette with a progressively smaller bore size (170 µm) to ensure the complete removal of all cumulus cells.

In vitro maturation and experimental groups

The experiment was performed as one biological run, with the retrieved oocytes distributed into two experimental groups for direct qualitative comparison:

- **Control group:** Four (4) denuded oocytes were cultured in the maturation medium (DMEM supplemented with 10% FBS, 2 mM Glutamine and 1% penicillin/streptomycin). The medium utilized a bicarbonate buffering system and was overlaid with a layer of mineral oil to prevent evaporation and stabilize the pH.
- **Quercetin-treated group (Q15):** Four (4) denuded oocytes were cultured in the DMEM supplemented with 10% FBS, 2 mM Glutamine and 1% penicillin/streptomycin). The medium utilized a bicarbonate buffering system and was overlaid with a layer of mineral oil to prevent evaporation and stabilize the pH. The medium utilized a bicarbonate buffering system and was overlaid with a layer of mineral oil to prevent evaporation and stabilize the pH.

The quercetin stock solution was prepared by dissolving Quercetin in DMSO; the final concentration of the solvent DMSO in the culture medium did not exceed 0.1% (v/v) to ensure non-cytotoxicity. To ensure stability and bioavailability, the concentrated Quercetin stock solution was prepared fresh prior to the experiment and was subsequently stored in light-protected containers at –20°C when not in immediate use.

The culture was carried out in a humidified atmosphere at 37°C and 6% CO₂ in air (Normoxic conditions with approximately 20% O₂) for a period of 48 hours. No medium changes were performed during the 48-hour culture period. The entire procedure was performed under a strict sterile environment.

Evaluation

After the 48-hour culture period, oocytes from both groups were evaluated under an inverted microscope to assess

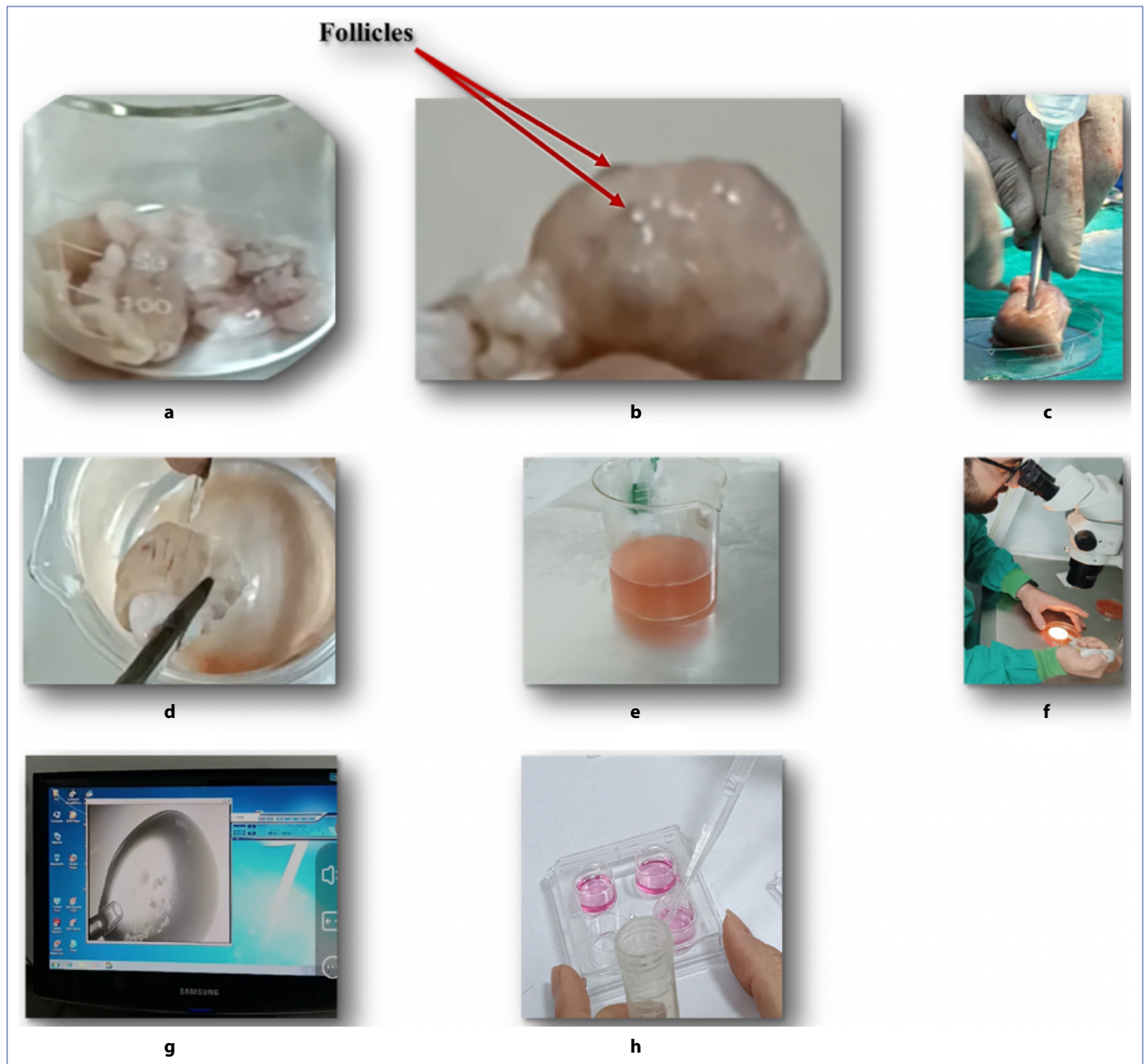


Figure 1. Bovine oocyte recovery method (Aspiration followed by slicing). (a) Collecting ovaries from the slaughterhouse, (b) bovine ovary showing visible surface follicles (indicated by arrows), (c) aspirating follicular fluid using a syringe, (d) slicing Ovaries to Recover Small Follicles and Remaining Oocytes, (e) collecting follicular fluid, (f) microscopic examination for oocytes, (g) mechanical denudation of oocytes, (h) preparing oocytes for in vitro maturation.

their maturation based on the presence and morphology of the first polar body. The pH of the maturation media was also measured.

Due to the constraints mentioned, data were analyzed as qualitative observations and comparative ratios. We acknowledge that this small sample size is insufficient for robust statistical inference. The findings are intended to establish a proof-of-concept and identify clear trends for future statistically powered studies.

Results

After 48 hours of *in vitro* culture, polar body extrusion was observed in both the control and quercetin-treated groups. In the control group, one out of four oocytes showed a polar body; however, it appeared abnormally enlarged, flat, and irregular in shape, which may indicate incomplete maturation and abnormal progression through meiosis. In contrast, in the group treated with 15 $\mu\text{g}/\text{mL}$ of quercetin, three out of four oocytes exhibited normal-sized and round (normal shaped)

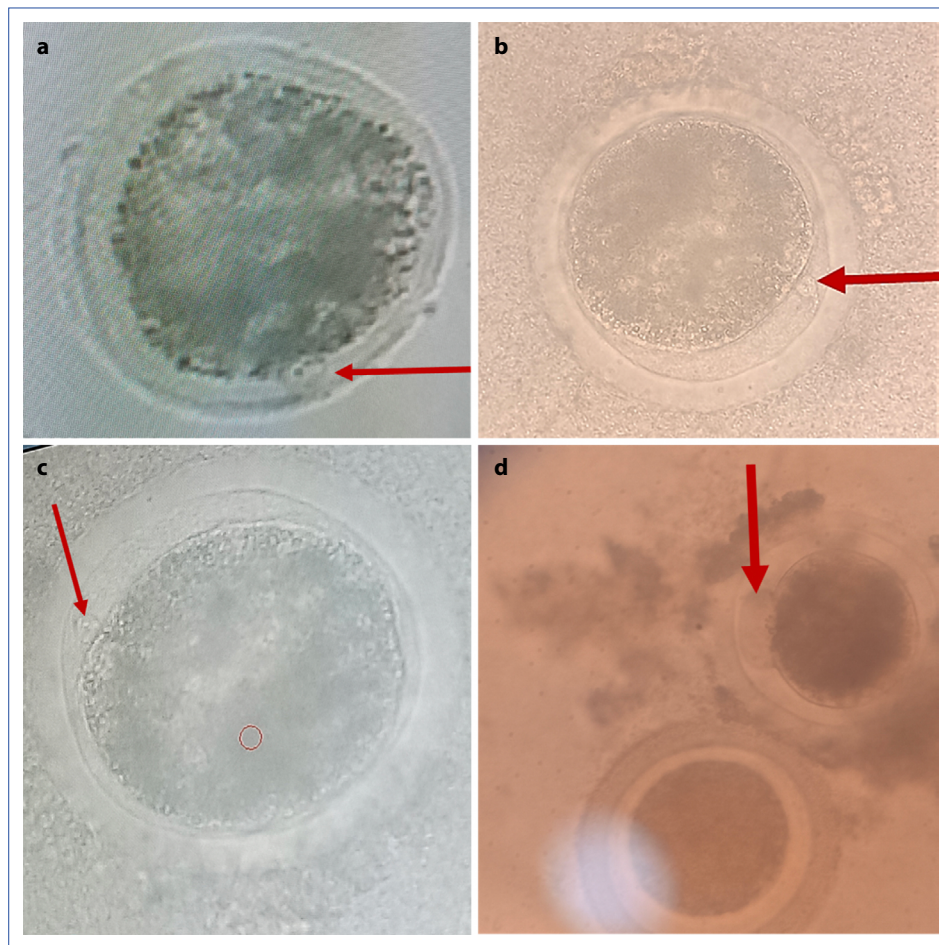


Figure 2. Bovine oocyte after 48 hours of *in vitro* culture. (a-c) Quercetin group: clearly visible first polar bodies (indicated by the arrows), suggesting completion of the first meiotic division and likely progression to the metaphase II stage. (d) Control group: A single polar body is visible (indicated by the arrow), although it appeared abnormally enlarged, flat, and irregular in shape which may reflect suboptimal maturation possibly influenced by post-mortem handling conditions.

polar bodies (Fig. 2), suggesting completion of the first meiotic division and likely progression to the metaphase II stage. These observations visually support the potential role of quercetin in enhancing oocyte maturation quality under the applied culture conditions.

The pH of the maturation media was measured after the completion of the *in vitro* culture period. The control well exhibited the highest pH value of 9.0. In comparison, the well containing quercetin at 15 $\mu\text{g}/\text{mL}$ showed a slightly lower pH of 8.3. The mildly acidic shift in the quercetin-treated wells may reflect changes in the metabolic activity during culture.

Discussion

In vitro embryo production (IVP) is gaining increasing popularity despite persistent concerns about its inefficiency. This inefficiency is likely due to the inability to perfectly replicate the complex processes of oocyte maturation, fertilization, and embryo development that occur within the *in vivo* environment [6]. Consequently, exploring factors that can improve

the success rates of this technology, particularly under suboptimal collection conditions, is of paramount importance.

This study was designed to investigate the effect of Quercetin, a potent natural flavonoid antioxidant, on the maturation of bovine oocytes subjected to a field-relevant post-mortem delay. The dose of 15 $\mu\text{g}/\text{mL}$ of quercetin was selected based on a thorough review of existing literature, which has shown this concentration to be effective in mitigating oxidative stress and improving developmental outcomes in similar *in vitro* culture systems. This dose falls within the optimal range (10–25 $\mu\text{g}/\text{mL}$) identified by previous studies as being non-toxic and beneficial for mammalian oocytes and embryos [9].

Context, methodology, and biological constraints

Our methodology intentionally created a stress-intensive, low-support environment to clearly isolate the test compound's effect. This design involved:

Severe biological stress: Simulating a 6-hour post-mortem delay, which is known to drastically compromise oocyte viability.

Low exogenous support: The intentional omission of hormonal supplements (FSH/LH/Estradiol) ensured that the oocytes relied primarily on their intrinsic competence and the protective agent (Quercetin).

Stressful atmospheric conditions: Performing the culture under normoxic conditions ($\approx 20\% \text{ O}_2$) further amplified the oxidative stress, as the *in vivo* ovarian environment is naturally hypoxic ($\approx 5\% \text{ O}_2$).

We note that the sample size ($n=4$ per group) is limited. This is a direct consequence of the biological constraint—the 6-hour delay drastically reduced the pool of suitable oocytes—and establishes the context for our qualitative data analysis in this preliminary report.

The impact of stress on meiotic progression

Our findings indicate that oocytes in both groups suffered significant stress upon entry into culture, evidenced by the fact that polar body extrusion did not occur within the standard 24-hour period, instead requiring a prolonged 48-hour culture period. This delay highlights the severity of the damage inflicted by the prolonged tissue processing interval. The extended 6-hour interval between ovary collection and the start of oocyte culture is a significant source of cellular stress. During this period, oocytes are deprived of essential oxygen and nutrients, leading to a shift toward anaerobic metabolism. This shift not only depletes vital energy reserves but also promotes the accumulation of harmful reactive oxygen species (ROS), which can disrupt critical cellular structures like the meiotic spindle and impair mitochondrial function. Furthermore, these stressful conditions can induce morphological abnormalities such as cytoplasmic granulation and abnormal polar body formation, ultimately compromising oocyte viability and developmental competence. Our observations are consistent with previous reports indicating that the yield of intact, cumulus cell-enclosed oocytes decreases as the time between animal death and ovary collection increases [10].

Quercetin's role in enhancing meiotic competence

Despite the challenging conditions, our results show a clear qualitative difference driven by Quercetin:

Morphological failure: The control group extruded a polar body that appeared abnormally enlarged, flat, and irregular in shape. This highly specific morphological defect is a strong indicator of aberrant cytoplasmic division and severe meiotic spindle dysfunction, often linked to oxidative stress.

Morphological success: In stark contrast, the Quercetin-treated oocytes produced polar bodies that were normal in size and possessed the characteristic spherical, regular shape. This confirms that Quercetin enabled the oocytes to complete the first meiotic division (Meiosis I) correctly.

This significant improvement is attributable to Quercetin's potent antioxidant properties. It successfully neutralized accumulating ROS and helped preserve the integrity of the meiotic spindle, thereby maintaining the cellular architecture essential for accurate chromosome segregation.

Metabolic health and pH analysis

The measurement of the culture medium's pH provides further evidence of Quercetin's protective function:

- **Control group (pH=9.0):** The excessively high pH suggests severely compromised cellular metabolism and the inability to produce stabilizing acidic byproducts.
- **Quercetin group (pH=8.3):** The significantly lower pH indicates that Quercetin supported a more physiologically active and balanced metabolic state. This pH stabilization confirms that Quercetin enhanced the oocyte's intrinsic metabolic resilience, allowing it to better regulate its internal environment despite the imposed stress.

Conclusion

This preliminary study provides compelling, non-random qualitative evidence that Quercetin significantly improves key indicators of oocyte quality (normal polar body morphology and metabolic stabilization) under severe stress conditions.

These strong qualitative findings form the essential foundation for a future, larger-scale study that will incorporate numerous biological replicates and power analysis to quantitatively confirm these beneficial effects and definitively validate Quercetin's role as a resilience-enhancing agent in *in vitro* maturation protocols.

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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