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# Optimization of In Vitro Maturation in Ovine Oocytes: A Comparative Study of Maturation Media

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## Abstract

**Background:** One of the most common techniques in assisted reproductive technologies (ART) is in vitro embryo production (IVEP), the success of which depends on the intrinsic quality of oocytes and the composition of the culture medium.

**Objectives:** This study investigates the effects of gonadotropin hormones (such as eCG and hCG) along with the antioxidant quercetin on the maturation of ovine oocytes, as well as the impact of different media on the expression of *Bcl2* and *Bax* genes in blastocysts.

**Methods:** After washing the oocytes, cumulus-oocyte complexes (COCs) that exhibited three or more layers of cells and homogeneous cytoplasm were selected and matured in BO-IVM, TCM (TCM-199 + 10% FBS + 10% ovine follicular fluid + 5 mg/mL FSH + 1 mg/mL estradiol-17β + 0.81 mM sodium pyruvate + 50 mg/mL gentamicin sulfate), and TCM$^{+}$ (TCM + 20 μg/mL eCG + 5 μg/mL hCG + 15 μg/mL quercetin) media for 24 hours at 38.5°C, 20% $O_2$, and 5% $CO_2$. After maturation, oocytes were fertilized with frozen ram semen, and presumed zygotes were cultured in BO-IVC under uniform conditions. Embryo development occurred at 38.5°C in a humid atmosphere with 5% $CO_2$, 5% $O_2$, and 90% $N_2$.

**Results:** The results indicated that the BO-IVM medium had a significantly higher mean percentage of zygotes, morulae, blastocysts, and hatched blastocysts compared to TCM$^{+}$ and TCM ($P < 0.05$). Moreover, blastocyst formation and hatched blastocysts were significantly more frequent in TCM$^{+}$ than in TCM ($P < 0.05$). However, the BO-IVM medium demonstrated a significant improvement compared to other maturation media. Gene expression analysis showed no significant difference in *Bcl2* expression among the groups, while *Bax* expression was significantly higher in TCM ($P < 0.05$). Overall, the BO-IVM medium was identified as the best option for optimizing oocyte maturation and producing high-quality blastocysts in ovine, with TCM$^{+}$ yielding better results than the TCM medium without hormonal and antioxidant compounds.

**Conclusions:** The results of this study showed that the addition of the antioxidant quercetin and the growth factors eCG and hCG improved the performance of the TCM medium. However, further research is needed to enhance the performance of the TCM maturation medium compared to the commercial BO-IVM medium.

*Keywords:* Oocyte Maturation, Culture Media, Gene Expression, Embryos, Ovine

## 1. Background

Assisted reproductive technologies (ART) are recognized as innovative tools for improving reproductive efficiency and accelerating genetic progress. One of the most widely used ART techniques is in vitro embryo production (IVEP) (1). The success of this process largely depends on the intrinsic quality of oocytes and the composition of in vitro culture (IVC) media. In recent decades, extensive efforts have been made to identify suitable culture media for oocyte maturation. These efforts include the development of new formulations and the enrichment of these media with various components such as follicular fluid, antioxidants, cytokines, growth factors, and hormones (2).

Oocyte maturation is one of the critical stages in the in vitro production of embryos and is considered a major challenge in this system (3, 4). This stage has significant effects on the efficiency of embryo production. In vitro conditions expose oocytes to various stress factors, including oxygen tension and physical manipulations, which can disrupt their growth and development (4). Therefore, researchers have extensively studied various aspects of oocyte maturation across different species (5).

Morphologically, oocyte maturation involves physiological changes in the nucleus and cytoplasm. The extrusion of the first polar body is considered an indicator of nuclear maturation, while the expansion of cumulus cells is regarded as a marker of cytoplasmic maturation. Under in vivo conditions, nuclear and cytoplasmic maturation occur simultaneously; however, in in vitro conditions, nuclear maturation typically precedes cytoplasmic maturation (6). Nuclear maturation refers to the transition of oocytes from the arrest of the first meiotic division at the germinal vesicle (GV) stage to the arrest of the second meiotic division at metaphase II (MII) (7). This process occurs concurrently with cytoplasmic maturation, which involves structural changes such as the distribution and redistribution of cortical granules, storage of proteins and RNA, development of calcium regulatory mechanisms, and migration of mitochondria to the peri-nuclear regions (8). These events are initiated by the binding of gonadotropins to their receptors and the activation of G-proteins, triggering a cascade of phosphorylation in cyclic AMP (cAMP)-dependent protein kinases (9).

Additionally, the supplementation of oocyte maturation media with gonadotropins has been proposed as a strategy to enhance the developmental competence of oocytes (10). Gonadotropins play a particularly important role in increasing the number of oocytes reaching the MII stage and, consequently, the overall yield of viable embryos. These hormones alter the metabolism of cumulus cells and induce the resumption of meiosis by disrupting the inhibitory state through gap junctions in oocytes (11). Gonadotropins such as equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) are used as alternatives to follicle-stimulating hormone (FSH) in the in vitro maturation (IVM) of mammalian oocytes (4, 12). The eCG, a glycoprotein extracted from the serum of pregnant mares, is recognized as an effective substitute for FSH. Due to its longer half-life and FSH-like activity, this gonadotropin has been widely applied in reproductive studies and treatments (13). The addition of eCG to oocyte culture media can increase the rate of MII oocytes in ovine (6). Similarly, hCG, which primarily mimics luteinizing hormone (LH), can alter calcium distribution in the cytoplasm and improve glutamine metabolism in oocytes when added to the maturation medium (14). Both hCG and eCG have been effectively employed in the IVM of mammalian oocytes (8). Moreover, commercially available eCG is more cost-effective compared to FSH and LH (15). Mingoti et al. demonstrated that eCG can enhance the expression of hCG receptors in cumulus cells, thereby enabling the activation of intracellular signaling pathways by hCG. Additionally, eCG has the ability to bind to both FSH and LH receptors (14).

In mammals, flavonoids exhibit a variety of biological and pharmacological effects. Research has shown that quercetin can possess anti-inflammatory and antioxidant properties, which arise from its activities in neutralizing free radicals and chelating metals. This compound also has strong antioxidant effects, but it can simultaneously induce pro-oxidant effects. There is a relationship between the free radical scavenging activity and the anti-carcinogenic and anti-inflammatory properties of quercetin (16). As a potential antioxidant, quercetin may reduce cellular apoptosis. Quercetin has been successfully used as a stimulant in the maturation of oocytes and pre-implantation and embryo development in ovine, goats, cattle, pigs, and mice in culture media (17). This compound is also capable of preventing mitochondrial dysfunction, neutralizing free radicals by removing oxidation products, and stimulating antioxidant enzymes (18). It appears that improving embryo production efficiency may be achieved through the inhibition of various systems involved in the apoptosis of oocytes (19).

## 2. Objectives

The use of an appropriate concentration of antioxidants can create optimal conditions for producing high-quality embryos. Additionally, gonadotropins exhibit significant similarities in their effects on ovarian hormones in vitro. Therefore, the aim of this study is to explore the role of gonadotropic

hormones (eCG and hCG) and quercetin, as an effective antioxidant, in enhancing the TCM-199 maturation medium. This optimized maturation medium is then compared with the commercially available BO-IVM medium. This study was conducted as a randomized trial and investigated the effects of different culture conditions on the maturation of ovine oocytes.

## 3. Methods

### 3.1. Location, Chemicals, and Media

All experiments were conducted in the Embryo Biotechnology Laboratory at the Iranian Research Organization for Science and Technology (IROST), maintaining a consistent temperature range of 27 - 31°C. Chemicals and culture media were sourced from Sigma-Aldrich (USA) and Gibco (USA), while plastic materials were obtained from Falcon (USA), unless specified otherwise. All stock solutions and media were prepared using sterile triple-distilled Milli-Q water and filtered through 0.22 μm membrane filters.

### 3.2. Oocyte Collection

Ovaries from ovine were obtained from a local slaughterhouse and transported to the laboratory within 3 hours in a phosphate-buffered saline solution containing gentamicin (50 μg/mL) at a temperature of 25 - 30°C. The ovaries were trimmed to remove surrounding adipose tissue and were subsequently washed five times with physiological saline. A surgical blade was used to slice the ovaries for oocyte retrieval. The follicular fluid and cumulus-oocyte complexes (COCs) were placed in a small petri dish containing an aspiration medium enriched with TCM-199, L-glutamine (2 mM), bovine serum albumin (BSA) (0.3%), and gentamicin sulfate (50 μg/mL). Under a stereomicroscope, COCs were collected and gently washed three times using a washing medium. This washing medium was composed of TCM-199, L-glutamine (2 mM), sodium pyruvate (0.81 mM), fetal bovine serum (FBS) (10%), and gentamicin sulfate (50 μg/mL).

### 3.3. In Vitro Maturation

Cumulus-oocyte complexes exhibiting three or more layers of cells and a homogeneous cytoplasm were selected and matured in groups of up to 15 in 100 μL droplets of BO-IVM, $TCM^+$, and TCM maturation media. The BO-IVM medium is a commercial culture medium that was considered as a control in this experiment, while the TCM medium was TCM-199 supplemented with 10% FBS, 10% ovine follicular fluid, 5 mg/mL FSH, 1 mg/mL estradiol-17β, 0.81 mM sodium pyruvate, and 50 mg/mL gentamicin sulfate. The $TCM^+$ medium included the components of the TCM medium along with the gonadotropins eCG (20 μg/mL) and hCG (5 μg/mL), as well as the antioxidant quercetin (15 μg/mL). The maturation droplets were covered with mineral oil and cultured for 24 hours at 38.5°C in an atmosphere of 20% $O_2$ and 5% $CO_2$.

### 3.4. In Vitro Fertilization

The cryopreserved semen from a ram was quickly thawed at 37°C and washed twice with 10 mL of IVF medium, which consists of Brackett and Oliphant medium containing 10 μg/mL heparin, 137.0 μg/mL sodium pyruvate, and 1.942 mg/mL caffeine sodium benzoate. The samples underwent centrifugation at 1000 rpm for 5 and 7 minutes at room temperature to wash them twice. A hemocytometer was used to determine the concentration of the spermatozoa, which was then adjusted to $1.0 \times 10^7$/mL through further dilution. A 50 μL aliquot of the sperm suspension was mixed with a 50 μL droplet of the IVF medium. The 15 matured COCs were then placed into a droplet of 100 μL IVF medium and incubated for 18 hours at 38.5°C in a humidified atmosphere containing 20% $O_2$ and 5% $CO_2$.

### 3.5. In Vitro Culture

After fertilization, the presumptive zygotes were carefully washed by pipetting to remove any remaining cumulus cells and spermatozoa. The presumptive zygotes were then cultured in the BO-IVC medium to ensure consistent conditions for all. The zygotes were cultured for 9 days in a humidified atmosphere of 5% $CO_2$, 5% $O_2$, and 90% $N_2$ at a temperature of 38.5°C.

### 3.6. Statistical Analysis

Each experimental group was subjected to three replicates. For the analysis of quantitative data, SPSS statistical software (version 16) was employed. The results are presented as mean ± standard error of the

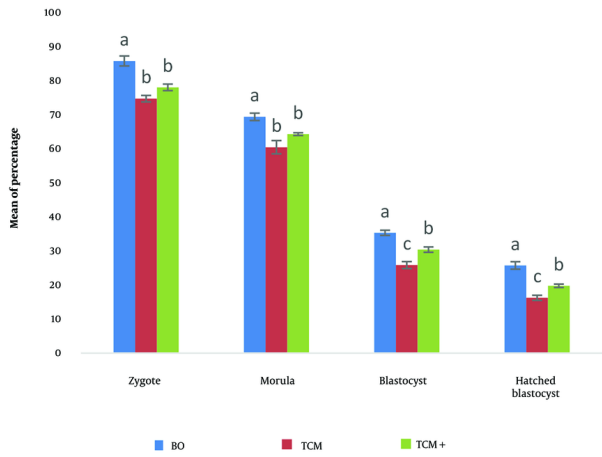


**Figure 1.** The impact of maturation media (BO-IVM, TCM and TCM$^{+}$) on primary embryo development. Different letters indicate statistically significant differences ($P \leq 0.05$).

mean. Statistical evaluation was conducted using the analysis of variance (ANOVA) method, and differences were deemed statistically significant when $P < 0.05$.

## 4. Results

### 4.1. Experiment 1: The Impact of Maturation Media on Primary Embryo Development and Their Comparison

The mean percentage of zygotes, morulae, blastocysts, and hatched blastocysts in the BO-IVM medium was significantly higher compared to the TCM$^{+}$ and TCM media ($P < 0.05$). Additionally, the mean percentage of zygotes and morulae in the TCM$^{+}$ medium was numerically higher than in the TCM medium, but no significant difference was observed between these two media. The mean percentage of blastocysts and hatched blastocysts in the TCM$^{+}$ medium was higher than in the TCM medium, with a significant difference observed ($P < 0.05$) (Figure 1).

### 4.2. Experiment 2: Examination of Gene Expression in Blastocysts Under Different Maturation Media

No significant difference was observed in the expression of the *Bcl2* anti-apoptotic gene among the BO-IVM, TCM$^{+}$, and TCM media. In the TCM medium, the expression of the *Bax* apoptotic gene was higher, with a significant difference noted compared to the other media ($P < 0.05$) (Figure 2).

## 5. Discussion

The results of this study revealed that supplementing the TCM medium with the antioxidant quercetin and the growth factors eCG and hCG not only improved blastocyst formation and hatching rates but also reduced the expression of the apoptotic gene *Bax* compared to the standard TCM medium. Gonadotropins, including eCG and hCG, are essential for oocyte maturation, influencing ovarian activity and promoting the development of viable oocytes. The eCG, which mimics FSH and LH, binds to FSH receptors and supports nuclear maturation, cumulus cell expansion, and mitochondrial activity, making it a suitable substitute for FSH in IVEP systems (8, 20). Human chorionic gonadotropin, similar to LH, binds to LHCGR in granulosa cells and is used as an LH substitute in vitro due to LH's rapid degradation (4). Studies have shown that eCG enhances cumulus cell expansion in species such as porcine, buffalo, and canine oocytes, though its effect on nuclear maturation varies (5, 6, 15). For example, Farag et al. (21) found that 10 µg/mL eCG significantly increases camel oocyte maturation rates, while the addition of 20 IU/mL eCG and hCG improves ovine oocyte maturation by enhancing cumulus expansion, polar body extrusion, and mitochondrial

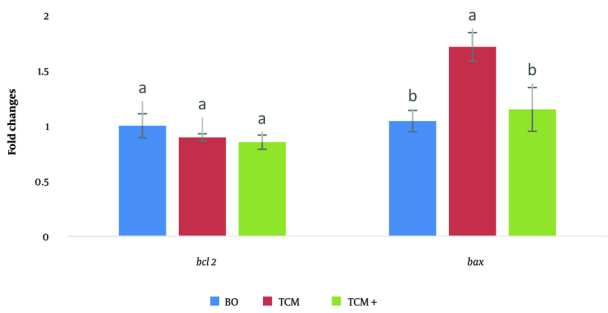


**Figure 2.** The effect of maturation media (BO-IVM, TCM and TCM$^{+}$) in regulating the expression of *Bcl2* and *Bax* genes in ovine blastocysts. Different letters indicate statistically significant differences ($p \leq 0.05$).

content (15). The inclusion of gonadotropins like eCG and FSH in maturation media boosts embryo development rates in buffalo and other species (20, 22). High concentrations of these hormones improve fertilization and embryo development, as demonstrated by Mogas et al. (23). Leisinger et al. (24) highlighted the synergistic effect of FSH and eCG on alpaca oocyte maturation, though lower concentrations reduce efficacy. Recent studies confirm that eCG enhances oocyte quality, mitochondrial activity, and gonadotropin receptor expression while reducing apoptosis (6, 8, 15). These hormones optimize nuclear and cytoplasmic maturation by increasing cAMP levels and improving cumulus-oocyte communication (20, 25). Overall, eCG and hCG play pivotal roles in improving oocyte maturation and embryo development across various species.

Kang et al. (26) reported the positive effect of quercetin on porcine oocytes, which improved embryonic development, reduced ROS production, and increased intracellular GSH levels at low concentrations; however, high concentrations were found to be detrimental. In another study, Kang et al. (16) attributed the decreased maturation rates of oocytes and blastocyst formation to the unresponsiveness of oocytes and embryos or toxicity from high levels of flavonoids. Research by Yu et al. (27) showed that quercetin enhances the quality of mouse embryos under oxidative stress conditions, leading to increased blastocyst formation and reduced apoptosis. Additionally, experiments on ovine oocytes demonstrated significantly higher cleavage and blastocyst rates, with quercetin reducing ROS accumulation and increasing overall antioxidant activity (28). Quercetin can also delay the onset of apoptosis and improve the quality of mouse oocytes (29). In goat oocytes, supplementation with quercetin resulted in decreased DNA fragmentation and increased percentages of oocytes at the MII stage (30). Studies have shown that ROS levels in quercetin-treated oocytes significantly decreased, while high doses performed poorly in reducing ROS levels (31). This compound reduces ROS levels in bovine oocytes and embryos by regulating the Nrf2-dependent oxidative stress response. Its beneficial effects on the antioxidant system and its role in reducing apoptosis in granulosa cells have also been documented (19). In their study, Davoodian et al. (19) reported that quercetin protects intestinal epithelial and endothelial cells from stress and apoptosis by modulating the expression of *Bcl2* genes. Furthermore, treatment with quercetin in cumulus cells significantly decreased the expression of the *Bax* transcript, contributing to the oocyte's defense mechanism against apoptosis. Finally, quercetin protects human granulosa cells from oxidative stress and reduces endoplasmic reticulum stress-induced apoptosis in buffalo GCs, enhancing the ovarian antioxidant capacity by increasing the expression of certain oxidative stress-related genes in mice (32-34).

The results of this study indicated that although supplementing the TCM medium with the antioxidant quercetin and growth factors eCG and hCG improved its performance, the fertilization rate, morula formation, blastocyst formation, and hatched blastocyst rates were significantly lower compared to the commercial BO-IVM

medium. The BO-IVM medium has been widely used in many studies (35-37) for embryo production. The BO-IVM medium includes essential components that enhance the maturation of oocytes in farm animals (38-41). Consistent with our results, Pryor et al. reported that specifically, embryos cultured in BO-IVC exhibited higher blastocyst rates and superior embryo quality.

### 5.1. Conclusions

The results of this study showed that the addition of the antioxidant quercetin and the growth factors eCG and hCG improved the performance of the TCM maturation medium by significantly increasing the average percentage of blastocysts and hatched blastocysts. However, the rates of fertilization, morula formation, blastocyst formation, and hatched blastocysts were significantly lower than those observed with the commercial BO-IVM medium. Therefore, further research is needed to enhance the performance of the TCM maturation medium in comparison to the commercial BO-IVM medium.

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## Footnotes

**Authors' Contribution:** A. D.: Taking samples, laboratory work, data collection, and writing; M. Z.: Supervisor, conceptualization, formal analysis, methodology, validation, writing–review and editing; A. Gh.: Methodology, writing– review and editing.

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**Data Availability:** The dataset presented in the study is available on request from the corresponding author during submission or after publication.

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## References

1. Musapoor S, Davoodian N, Kadivar A, Ahmadi E, Nazari H. Media Supplementation With Gamma-Oryzanol Improves the Outcome of Ovine Oocyte Maturation In Vitro. *Vet Med Sci*. 2025;**11**(1). e70134. [PubMed ID: 39688528]. [PubMed Central ID: PMC11651092]. https://doi.org/10.1002/vms3.70134.
2. Podda A, Dujickova L, Ariu F, Leoni GG, Izquierdo D, Paramio MT, et al. Effect of Liquid Marble 3D Culture System on In Vitro Maturation and Embryo Development of Prepubertal Goat Oocytes. *Animals (Basel)*. 2025;**15**(2). [PubMed ID: 39858188]. [PubMed Central ID: PMC11758309]. https://doi.org/10.3390/ani15020188.
3. Bahrami M, Cottee PA. Culture conditions for in vitro maturation of oocytes–A review. *Reproduct Breed*. 2022;**2**(2):31-6.
4. Bilal M, Ashraf MK, Ashraf T, Yaseen M, Husnain A, Bin Majeed MB, et al. Effect of human chorionic gonadotropin on oocyte maturation and developmental competence in buffalo. *Theriogenology*. 2025;**235**:56-63. [PubMed ID: 39787661]. https://doi.org/10.1016/j.theriogenology.2024.12.029.
5. Farag IM, Girgis SM, Khalil WKB, Hassan NHA, Sakr AAM, Abd Allah SM, et al. Effect of hormones, culture media and oocyte quality on in vitro maturation of Egyptian Sheep oocytes. *J Appl Biosci*. 2009;**24**:1520-34.
6. Wei SC, Gong ZD, Zhao HW, Liang HQ, Lai LJ, Deng YY. Equine chorionic gonadotropin influence on sheep oocyte in vitro maturation, apoptosis, and follicle-stimulating hormone receptor and luteinizing hormone receptor expression. *Genet Mol Res*. 2016;**15**(4):1-12.
7. Zhu J, Moawad AR, Wang CY, Li HF, Ren JY, Dai YF. Advances in in vitro production of sheep embryos. *Int J Vet Sci Med*. 2018;**6**(Suppl):S15-26. [PubMed ID: 30761316]. [PubMed Central ID: PMC6161858]. https://doi.org/10.1016/j.ijvsm.2018.02.003.
8. Bastos BDM, da Silva MNP, Gonçalves PR, Cândido AECM, Barberino RDS, do Monte APO, et al. Effect of different gonadotropins on in vitro maturation of sheep oocytes. *Semina: Cienc*. 2022;**43**(6).
9. Russell DL, Gilchrist RB, Brown HM, Thompson JG. Bidirectional communication between cumulus cells and the oocyte: Old hands and new players? *Theriogenology*. 2016;**86**(1):62-8. [PubMed ID: 27160446]. https://doi.org/10.1016/j.theriogenology.2016.04.019.
10. Chandra V, Sharma GT. In vitro strategies to enhance oocyte developmental competence. *Front Biosci (Schol Ed)*. 2020;**12**(1):116-36. [PubMed ID: 32114451]. https://doi.org/10.2741/S543.
11. Karami Shabankareh H, Sarsaifi K, Mehrannia T. In vitro maturation of ovine oocytes using different maturation media: effect of human menopausal serum. *J Assist Reprod Genet*. 2011;**28**(6):531-7. [PubMed ID:

21152965]. [PubMed Central ID: PMC3158248]. https://doi.org/10.1007/s10815-010-9523-3.

12. Gupta PS, Nandi S, Ravindranatha BM, Sarma PV. Effect of commercially available PMSG on maturation, fertilization and embryo development of buffalo oocytes in vitro. *Reprod Fertil Dev*. 2001;**13**(5-6):355-60. [PubMed ID: 11833930]. https://doi.org/10.1071/rd01026.
13. Di Berardino C, Peserico A, Capacchietti G, Crociati M, Monaci M, Tosi U, et al. Equine Chorionic Gonadotropin as an Effective FSH Replacement for In Vitro Ovine Follicle and Oocyte Development. *Int J Mol Sci*. 2021;**22**(22). [PubMed ID: 34830304]. [PubMed Central ID: PMC8619287]. https://doi.org/10.3390/ijms222212422.
14. Mingoti GZ, Garcia JM, Rosa-e-Silva AA. Steroidogenesis in cumulus cells of bovine cumulus-oocyte-complexes matured in vitro with BSA and different concentrations of steroids. *Anim Reprod Sci*. 2002;**69**(3-4):175-86. [PubMed ID: 11812628]. https://doi.org/10.1016/s0378-4320(01)00187-7.
15. Baiomy FM, Kamel AM, Hussein AF, Hassanin SH. The Impact of Maturation Medium Supplemented with PMSG and HCG Hormones on In vitro Maturation of Ovine Oocytes. *J Appl Veterinary Sci*. 2025;**10**(1):81-91.
16. Kang JT, Kwon DK, Park SJ, Kim SJ, Moon JH, Koo OJ, et al. Quercetin improves the in vitro development of porcine oocytes by decreasing reactive oxygen species levels. *J Vet Sci*. 2013;**14**(1):15-20. [PubMed ID: 23388446]. [PubMed Central ID: PMC3615227]. https://doi.org/10.4142/jvs.2013.14.1.15.
17. Davoodian N, Kadivar A, Ahmadi E, Nazari H, Mehrban H. Quercetin effect on the efficiency of ovine oocyte vitrification at GV stage. *Theriogenology*. 2021;**174**:53-9. [PubMed ID: 34418772]. https://doi.org/10.1016/j.theriogenology.2021.07.027.
18. Cao Y, Zhao H, Wang Z, Zhang C, Bian Y, Liu X, et al. Quercetin promotes in vitro maturation of oocytes from humans and aged mice. *Cell Death Dis*. 2020;**11**(11):965. [PubMed ID: 33177495]. [PubMed Central ID: PMC7658351]. https://doi.org/10.1038/s41419-020-03183-5.
19. Davoodian N, Kadivar A, Davoodian N, Ahmadi E, Nazari H, Mehrban H. The effect of quercetin in the maturation media on cumulus-granulosa cells and the developmental competence of bovine oocytes. *Theriogenology*. 2022;**189**:262-9. [PubMed ID: 35809360]. https://doi.org/10.1016/j.theriogenology.2022.06.026.
20. Khadr AH, El-Sherbiny AM, Abdoon ASS. Effect of Culture Media, Gonadotropins, Proteins, Growth Factors and Hyaluronic Acid on In-Vitro Maturation of Buffalo Oocytes. *J Animal Poultry Pro*. 2022;**13**(7):91-7.
21. Farag IM, Girgis SM, Zowail ME, El-Hafez MAMA. In vitro maturation of camel (Camelus dromedarius) cumulus-denuded oocytes. *World Appl Sci J*. 2013;**26**(3):352-9.
22. Hegab AO, Montasser AE, Hammam AM, El-Naga E, Zaabel SM. Improving in vitro maturation and cleavage rates of buffalo oocytes. *Animal Repro*. 2018;**6**(2):416-21.
23. Mogas T, Izquierdo D, Palomo MJ, Paramio MT. Effect of hormones, serum source and culture system on the IMV and IVF of prepubertal goat oocytes and subsequent embryo development. *Theriogenology*. 1995;(43):284.
24. Leisinger C, Coffman E, Coutinho da Silva M, Forshey B, Pinto C. Factors affecting in vitro maturation of alpaca (Lama paco) oocytes. *Anim Reprod Sci*. 2014;**150**(1-2):70-5. [PubMed ID: 25261077]. https://doi.org/10.1016/j.anireprosci.2014.08.011.
25. Quispe-Gutiérrez US, Olivera-Marocho LV, Ccopa-Ccallata J, Pahuara-Farfan LE, Barragán-Condori M, Berndtson JL. Effect of FSH and eCG on alpaca (Vicugna pacos) oocyte maturation in vitro. *J Vet Sci*. 2021;**10**(3).
26. Kang JT, Moon JH, Choi JY, Park SJ, Kim SJ, Saadeldin IM, et al. Effect of Antioxidant Flavonoids (Quercetin and Taxifolin) on In vitro Maturation of Porcine Oocytes. *Asian-Australas J Anim Sci*. 2016;**29**(3):352-8. [PubMed ID: 26950865]. [PubMed Central ID: PMC4811785]. https://doi.org/10.5713/ajas.15.0341.
27. Yu S, Long H, Lyu QF, Zhang QH, Yan ZG, Liang HX, et al. Protective effect of quercetin on the development of preimplantation mouse embryos against hydrogen peroxide-induced oxidative injury. *PLoS One*. 2014;**9**(2). e89520. [PubMed ID: 24586844]. [PubMed Central ID: PMC3931787]. https://doi.org/10.1371/journal.pone.0089520.
28. Karimian M, Zandi M, Sanjabi MR, Masoumian M, Ofoghi H. Effects of grape seed extract, quercetin and vitamin C on ovine oocyte maturation and subsequent embryonic development. *Cell Mol Biol (Noisy-le-grand)*. 2018;**64**(4):98-102. [PubMed ID: 29631690].
29. Wang H, Jo YJ, Oh JS, Kim NH. Quercetin delays postovulatory aging of mouse oocytes by regulating SIRT expression and MPF activity. *Oncotarget*. 2017;**8**(24):38631-41. [PubMed ID: 28418847]. [PubMed Central ID: PMC5503559]. https://doi.org/10.18632/oncotarget.16219.
30. Silva AAA, Silva MNP, Figueiredo LBF, Goncalves JD, Silva MJS, Loiola MLG, et al. Quercetin influences in vitro maturation, apoptosis and metabolically active mitochondria of goat oocytes. *Zygote*. 2018;**26**(6):465-70. [PubMed ID: 30767819]. https://doi.org/10.1017/S0967199418000485.
31. Banihosseini SZ, Novin MG, Nazarian H, Piryaei A, Parvardeh S, Eini F. Quercetin improves developmental competence of mouse oocytes by reducing oxidative stress during in vitro maturation. *Ann Animal Sci*. 2018;**18**(1).
32. Rashidi Z, Aleyasin A, Eslami M, Nekoonam S, Zendedel A, Bahramrezaie M, et al. Quercetin protects human granulosa cells against oxidative stress via thioredoxin system. *Reprod Biol*. 2019;**19**(3):245-54. [PubMed ID: 31383475]. https://doi.org/10.1016/j.repbio.2019.07.002.
33. Yang W, Liu R, Sun Q, Huang X, Zhang J, Huang L, et al. Quercetin Alleviates Endoplasmic Reticulum Stress-Induced Apoptosis in Buffalo Ovarian Granulosa Cells. *Animals (Basel)*. 2022;**12**(6). [PubMed ID: 35327186]. [PubMed Central ID: PMC8944572]. https://doi.org/10.3390/ani12060787.
34. Wang J, Qian X, Gao Q, Lv C, Xu J, Jin H, et al. Quercetin increases the antioxidant capacity of the ovary in menopausal rats and in ovarian granulosa cell culture in vitro. *J Ovarian Res*. 2018;**11**(1):51. [PubMed ID: 29929541]. [PubMed Central ID: PMC6013856]. https://doi.org/10.1186/s13048-018-0421-0.
35. Chelenga M, Yanagawa Y, Katagiri S, Nagano M. Pre-maturational culture promotes the developmental competence of bovine oocytes derived from an 8-day in vitro growth culture system. *J Reprod Dev*. 2023;**69**(4):214-7. [PubMed ID: 37197977]. [PubMed Central ID: PMC10435529]. https://doi.org/10.1262/jrd.2023-022.
36. Bunderson I, Liu Y, Polejaeva I. 215 Effects of serum-free maturation medium and resveratrol supplementation on ovine oocyte maturation and quality. *Repro Fertility Develop*. 2023;**36**(2):263.
37. Prochowska S, Nizanski W, Partyka A, Kochan J, Mlodawska W, Nowak A, et al. The use of human and bovine commercial media for oocyte maturation and embryo development in the domestic cat (Felis

catus). *Reprod Domest Anim*. 2019;**54**(4):719-26. [PubMed ID: 30786066]. https://doi.org/10.1111/rda.13418.

38. Pryor JH, Hasler JF, Strøbech L, Avery B, Hashem N, Menges S, et al. 86 improved bovine embryo production using novel in vitro culture systems. *Repro Fertility Develop*. 2016;**28**(2):172.
39. Ratchamak R, Authaida S, Koedkanmark T, Boonkum W, Chankitisakul V. Coenzyme Q10 Supplementation Effects on In Vitro Oocyte Maturation, Lipid Peroxidation, and Embryonic Development in Prepubertal and Aging Thai-Holstein Cows. *Animals (Basel)*. 2024;**15**(1). [PubMed ID: 39794960]. [PubMed Central ID: PMC11718854]. https://doi.org/10.3390/ani15010018.
40. Ghaedrahmati A, Mamouei M, Zandi M. Low Serum Concentration in Ovine Embryo Culture Media. *Gene, Cell and Tissue*. 2024;**11**(3).
41. Kale SD, Pawshe CH, Birade HS, Ingawale MV, Deshmukh SG, Harkal SB, et al. Effect of maturation media on early embryonic development of goat immature oocytes. *J Entomol Zool Stud*. 2020;**8**(1):1345-8.