Buletin Peternakan
Bulletin of Animal Science
Vol 50(2): 85-90, May 2026
ISSN-0126-4400/E-ISSN-2407-876X
DOI: https://doi.org/10.21059/buletinpeternak.v50i2.115278

Comparative Effects of Selenium and Gonadotropin Supplementation on In Vitro Maturation of Bligon Goat Oocytes

Arina Dinar Nurfadilla Sastranegara[1], Rona Agustina[1], Dio Fico Felsidan Diatmono[1], Fransisca Gani Padmawati[1], Pradita Iustitia Sitaresmi[2], Sigit Bintara[1] and Diah Tri Widayati[1,†]

[1]Laboratory of Animal Physiology and Reproduction, Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, 55281 Sleman, Yogyakarta, Indonesia.
[2]Research Center for Animal Husbandry, Cibinong Science Center, National Research and Innovation Agency (BRIN), 16915 Bogor, Jawa Barat, Indonesia.
[†]Corresponding Author

ABSTRACT

Selenium (Se) is a novel addition to *in vitro* maturation (IVM) media, unlike traditional gonadotropin hormones. This highlights advancements in reproductive biology and potential strategies to enhance oocyte maturation. This study was conducted to determine the effect of Se and gonadotropin hormone supplementation in maturation medium on the IVM of Bligon goat oocytes. Ovaries from Bligon goats were obtained from a slaughterhouse in the Special Region of Yogyakarta. High-quality oocytes (grades A and B) were retrieved through aspiration, washed, and cultured in a medium beneath mineral oil. Three experimental conditions were established: 50 μL unsupplemented medium (T0), 48 μL medium + 2 μL Se (T1), and 48 μL medium + 2 μL gonadotropin (T2). The culture process was conducted for 24 h at 39°C, 95% humidity, and 5% CO_2. Oocyte maturation was evaluated based on cumulus cell expansion, and the data obtained were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test. The results indicated that the inclusion of either Se (86.26 ± 7.20%) or gonadotropin (88.13 ± 7.85%) in the IVM medium significantly enhanced ($p<0.05$) the maturation rate compared to that of the unsupplemented group (50.09 ± 11.90%). However, there was no significant difference ($p>0.05$) between the two supplements. In conclusion, both Se and gonadotropin hormones proved to be effective supplements for improving the maturation rate of Bligon goat oocytes.

KEYWORDS

Bligon goat; Cumulus cell expansion; Gonadotropin; In vitro maturation; Selenium

CORRESPONDING AUTHOR

Diah Tri Widayati
widayati@ugm.ac.id

ARTICLE HISTORY

Received: January 1th, 2026
Accepted: February 19th, 2026
Available Online: May 31st, 2026

SDGs CONTRIBUTION

1. Introduction

The elevated slaughter rate of female Bligon goats has resulted in a population decline and may impede efforts to enhance their productivity. Reproductive technologies represent a strategic effort to sustain goat populations and improve their productivity (Parera et al., 2019). Assisted reproductive technologies (ART), including *in vitro* embryo production (IVEP), cryopreservation, and embryo transfer, are crucial for both species conservation and the enhancement of reproductive efficiency in livestock (Muhajir et al., 2018). Among these, IVEP, which includes the stages of oocyte collection, *in vitro* maturation (IVM), *in vitro* fertilization (IVF), and *in vitro* culture (IVC), has been extensively developed (Widayati and Pangestu, 2020). Oocytes used in the IVEP process can be obtained through ovum pick-up (OPU) from live animals or collected from slaughtered ovaries at slaughterhouses (Pranatasari et al., 2016), thereby extending the usefulness of terminated female animals and contributing to the conservation and optimization of the genetic potential of superior livestock (Widayati and Pangestu, 2020) . Among the critical stages in IVEP, IVM is notably influenced by several factors, including the composition of the culture medium, pH, temperature, hormonal supplementation, and the presence of antioxidants to reduce oxidative stress (Widayati and Pangestu, 2020; Sciorio and Rinaudo, 2023). One of the challenges faced by oocytes IVM is oxidative stress caused by the increased generation of reactive oxygen species (ROS) in the IVM medium. Excessive ROS production can ultimately result in oocyte death and embryonic loss (Rakha et al., 2022). Therefore, the addition of antioxidant substances or protective medium is crucial to address this issue.

Gonadotropin hormone is commonly supplemented into the IVM medium to support oocyte maturation. Gonadotropin play a role in stimulating follicular growth and development, as

well as in regulating the nuclear and cytoplasmic maturation of oocytes through the activation of hormonal pathways that resemble the physiological processes occurring *in vivo* (Jiang et al., 2023). However, excessive gonadotropin doses can trigger oxidative stress in oviductal tissue, resulting in cumulus cell damage and impairing proper oocyte development (Di Nisio et al., 2023). Antioxidant supplements, such as selenium (Se), have been introduced into culture media as alternatives. Se supplementation in the IVM medium has been reported to enhance the viability and maturation of heat-stressed bovine oocytes by upregulating antioxidant gene expression and downregulating apoptosis-related genes, thereby improving embryo quality and developmental competence (Toosinia et al., 2024).

Therefore, this study was conducted to evaluate the effects of Se supplementation as an antioxidant, with gonadotropin serving as a positive control, on the maturation rate of Bligon goat oocytes. It is hypothesized that Se supplementation improves IVM rate, produces outcomes comparable to those obtained with gonadotropin supplementation, and is superior to those of the unsupplemented group. This study provides insights into the potential of Se as an effective alternative antioxidant supplement for optimizing IVM in goat oocytes.

2. Materials and Methods

2.1. Study period and sample

Ovaries from Bligon goats were obtained from a local slaughterhouse in Special Region of Yogyakarta. Oocyte processing was carried out at the Laboratory of Animal Physiology and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada (UGM), Yogyakarta.

2.2. Preparation of Selenium

Se was used in the form of sodium selenite (Merck, Germany). A stock solution was prepared by dissolving sodium selenite in sterile water for injection (Erlindra et al., 2026). The solution was adjusted to the required concentration (ppm), homogenized at 600 rpm for 20 min, and diluted as needed using the following equation:

$$V_1 \times C_1 = V_2 \times C_2$$

Where V1 and C1 denote the initial volume and concentration, respectively, and V2 and C2 denote the final volume and concentration. The stock solution was subsequently diluted with maturation medium to achieve a final Se concentration of 1 ppm. The Se-supplemented medium was prepared immediately prior to use.

2.3. Oocyte collections

The ovaries were placed in a physiological saline solution (Sigma Aldrich, USA) containing penicillin and streptomycin (Meiji, Japan) at 31–34°C to reduce the risk of bacterial contamination. Subsequently, the ovaries were trimmed of excess fat tissue and processed for oocyte collection (Widayati and Pangestu, 2020). Oocyte collection was carried out using the aspiration method, in which oocytes were aspirated from follicles using a 22G, 5 mL syringe (OneMed, Indonesia). Phosphate-buffered saline (PBS; Solarbio, China) served as the aspiration medium. The quality of aspirated oocytes were evaluated using a stereomicroscope (Cole Parmer, USA), with a magnification of 10×. The oocytes used in this study were classified into two categories based on their cumulus cell morphology: grade A and B oocytes (**Figure 1**). Grade A oocytes are characterized by uniformly compact cumulus cells forming approximately five layers surrounding the oocyte, whereas grade B oocytes possess a dark cytoplasm with an intact corona radiata and are enclosed by 3–4 layers of cumulus cells (Thanaboonyawat et al., 2016). After evaluation, the oocytes were aspirated using a pipette and transferred into a tissue culture dish (TCD) 35×10 mm (Iwaki, Indonesia), where they were washed three times with PBS under a stereomicroscope to eliminate debris and residual cells.

Table 1. Components of G-IVF^{TM} Plus

Component	Composition
Buffer	Bicarbonate
Protein	Human serum albumin (HSA)
Antibiotic	Gentamicin
Carbohydrates	Glucose and fructose
pH	7.2–7.4
Usage	Ready to use after incubation at 37°C under 5% CO_2
Volume	30 and 60 mL
Storage	Store protected from light at 2–8°C

Table 2. Average maturation rate of Bligon goat oocytes cultured in different treatment media

Treatment	Number of Oocytes cultured	Mean±SD (%)
T0	85	50.09±11.90[a]
T1	81	86.26±7.20[b]
T2	84	88.13±7.85[b]
F-value		48.646
p-value		0.001

[a,b] Different superscripts in the same column show significant results (p<0.05). T0: medium without supplementation, T1: medium + 2 μL Se (1 ppm), and T2: medium + 2 μL gonadotropin (50 IU). F-value indicates the magnitude of differences among treatments, while *p*-value indicates statistical significance.

2.4. In vitro maturation (IVM)

The washed oocytes were transferred to a TCD containing G-IVF™ Plus (Vitrolife, Sweden), although primarily formulated for IVF, this medium contains balanced electrolytes, energy substrates, amino acids, and protein supplements (**Table 1**), that are also suitable for supporting oocyte viability and maturation during IVM (Widayati and Wahyuningsih, 2024). Each drop of medium contained 7–10 oocytes, which is regarded as the optimal number to provide the highest IVF outcomes in terms of embryo quality and successful fresh embryo transfer, with a reduced risk of ovarian hyperstimulation (Jamil et al., 2023). Maturation treatments consisted of supplementing G-IVF™ Plus medium with Se or gonadotropin (Gonatropin®, Japan). The treatment groups were as follows, T0: 50 μL of maturation medium without supplementation, T1: 48 μL of maturation medium supplemented with 2 μL Se (1 ppm), and T2: 48 μL of maturation medium supplemented with 2 μL gonadotropin (50 IU). Each treatment included nine replicates, with approximately 81–85 oocytes per group. Each drop of medium was overlaid with mineral oil (Sigma Aldrich, USA) to prevent evaporation and contamination. Oocytes were gently transferred using a non-heparinized hematocrite pipette (Marienfeld-Superior, Germany) to avoid damage. The oocytes were cultured in an incubator (Cole Parmer, USA) at 39°C, 95% humidity, and 5% CO_2 for 24 h.

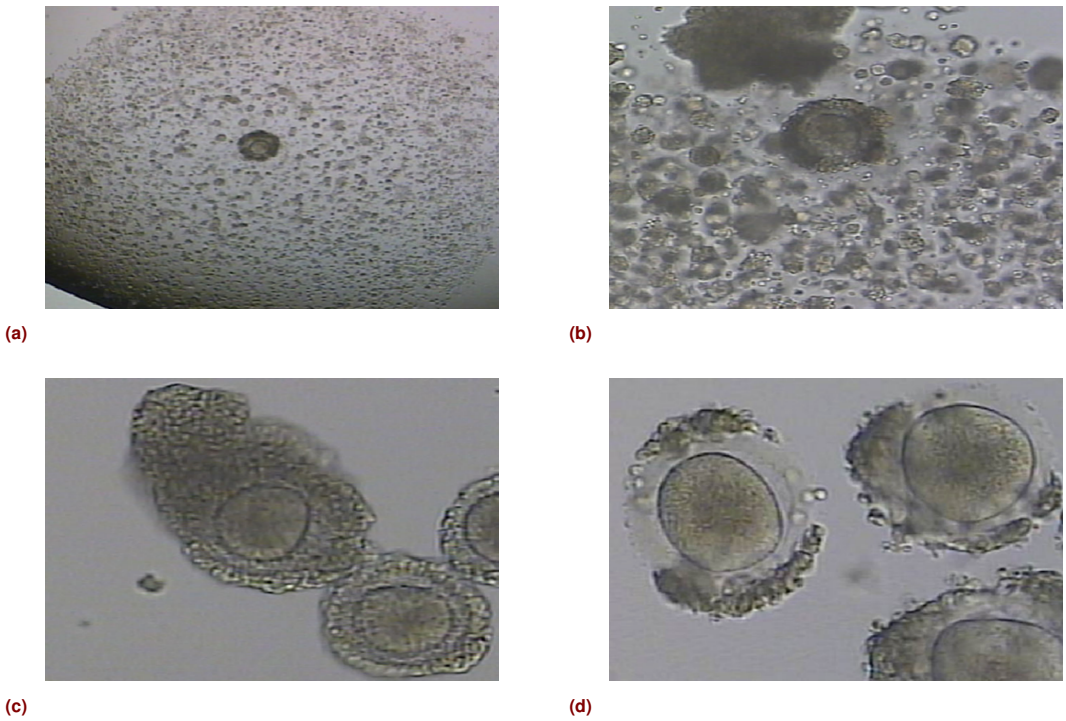

Figure 1. Immature oocyte grading according to cumulus cell morphology: a) Grade A oocyte with dispersed cumulus cells (CC) characterized by an expanded distal layer and a compact proximal layer, b) Grade B oocyte with clumped CC showing clustered distal CC and a compact proximal layer, c) Grade C oocyte with clumped CC surrounded by at least four compact CC layers, and d) Grade D oocyte with sparse CC with the cumulus-oocyte complex (COC) incompletely enclosed (Thanaboonyawat et al., 2016).

The primary parameter assessed was the number of oocytes that reached maturity per maturation session, which was evaluated according to their morphological characteristics.

2.5. Evaluation of oocyte maturation

Oocyte maturation was assessed under a light microscope (Yazumi 107 BN, Japan) after 24 h of incubation at 100× magnification. In this study, oocyte maturation was assessed based on the number of oocytes exhibiting cumulus cell expansion (Widayati et al., 2014). The percentage of mature oocytes was calculated using the following formula:

$$\text{Mature oocytes (\%)} = \frac{\text{Number of oocytes showing cumulus expansion}}{\text{Total oocytes subjected to maturation}} \times 100$$

2.6. Statistical analysis

This study was conducted using a completely randomized design (CRD) consisting of three treatments. The oocyte morphology data were descriptively analyzed based on the visual characteristics recorded during observation. The percentage of oocyte maturation was analyzed using one-way ANOVA, followed by Tukey's post hoc multiple comparisons test at a 5% (0.05) probability level. Statistical analyses were performed using SPSS version 30 with the results are presented as mean ± standard deviation (SD).

3. Results and Discussion

The results showed that supplementation of Se and gonadotropin hormones in the maturation medium affected the *in vitro* oocyte maturation rate of Bligon goats. The highest maturation percentage was shown in the T2 group (medium + 2 μL gonadotropin hormone), followed by the T1 group (medium + 2 μL Se). The lowest percentage was observed in T0 (medium without supplementation). Groups T2 and T1 showed no significant differences ($p>0.05$), whereas both were significantly different from T0 group ($p<0.05$). The percentage of goat oocyte maturation is presented in **Table 2**.

This study confirmed that Se supplementation in the maturation medium effectively supported optimal oocyte maturation compared to the medium without supplementation. Xiong et al. (2018) reported that adding Se to the IVM medium of porcine oocytes improved the culture environment and promoted oocyte maturation. Similarly, Dahlen et al. (2022) stated that Se supplementation in *in vitro* culture media across various animal species, including cattle, dog, pig, and yak, has shown positive effects on embryo development and viability by reducing ROS formation and minimizing deoxyribonucleic acid (DNA) damage. Moreover, Guseva et al. (2024) found that antioxidant supplementation during IVM, including Se, significantly improved oocyte competence and subsequent embryo development by mitigating oxidative stress. The morphology of the immature oocytes used in this study is presented in the **Figure 2**.

Cumulus cell expansion is a representative morphological indicator of oocyte maturation. This process facilitates sperm migration during IVF. Pranatasari et al. (2016) reported that intact cumulus cells surrounding the oocyte play a crucial role in providing essential regulatory factors during maturation, maintaining meiotic progression, and supporting cytoplasmic maturation. According to Negrón-Pérez et al. (2025), cumulus cell expansion is a key indicator of optimal oocyte maturation. This process reflects the physiological interactions between the oocyte and its surrounding cumulus cells, which are

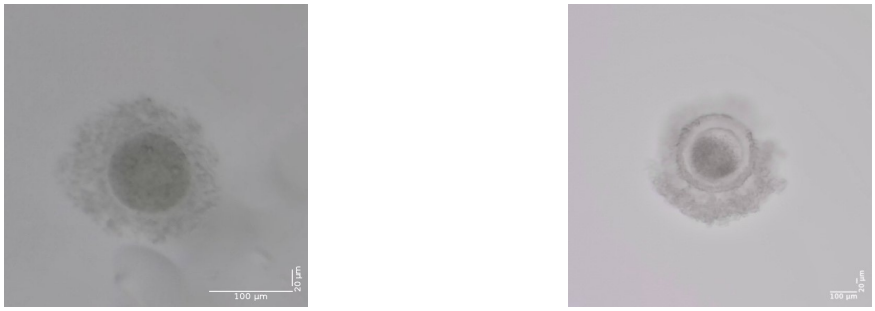

Figure 2. Immature oocytes of grades A and B were used in this study with a magnification of 100×: (a) Grade A oocytes with a cumulus cell area of 131.83 μm and (b) Grade B oocytes with a cumulus cell area of 80.06 μm.

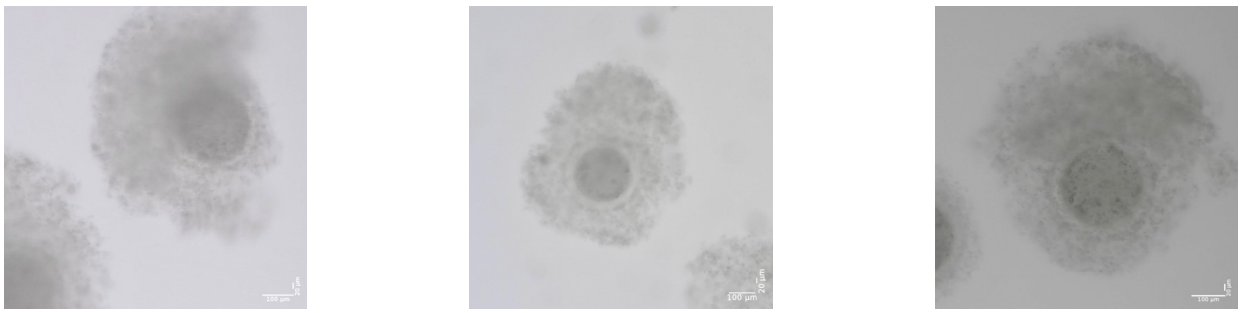

Figure 3. Cumulus cell expansion after IVM in several treatment groups with a magnification of 100×: (a) mature oocyte without supplementation, showing cumulus cell expansion of 153.79 μm, (b) mature oocyte after Se supplementation, showing cumulus cell expansion of 178.08 μm, and (c) mature oocyte after gonadotropin supplementation, showing cumulus cell expansion of 183.42 μm.

essential for the transfer of signalling molecules and maturation-supporting factors. Moreover, cumulus expansion plays a significant role in improving oocyte competence for successful fertilization. Chang et al. (2023) further supported this hypothesis by showing that oocyte maturation involves both nuclear and cytoplasmic maturation. Nuclear maturation is characterized by the completion of meiotic division and extrusion of first polar body (PB1). Baldini et al. (2024) stated that synchronization between nuclear and cytoplasmic maturation is crucial for ensuring successful fertilization and subsequent embryonic development. Cytoplasmic maturation involves the coordinated redistribution of organelles and accumulation of energy reserves, enzymes, and proteins. These processes form the fundamental basis for fertilization and early embryo development, ultimately determining the optimal size and functional competence of oocytes.

Morphologically, oocytes matured with Se and gonadotropin supplementation exhibited wider cumulus cell expansion than the unsupplemented group (**Figure 3**). Se supplementation in the maturation medium produced oocytes with well-expanded cumulus cells, indicating that Se contributes not only to the enhancement of maturation rate but also to the preservation of oocyte morphological quality. Pamungkas et al. (2012) reported that Se in cell culture systems protected cells from oxidative damage, free radicals, and lipid peroxide accumulation. According to Tripathi et al. (2023), the structural preservation of cumulus cells is vital for sustaining reciprocal communication between the oocyte and its companion cells, thereby supporting cytoplasmic development and optimizing oocyte metabolic competence. Furthermore, Guo et al. (2026) stated that Se supplementation enhances mitochondrial activity and optimizes adenosine triphosphate (ATP) synthesis, thereby supporting oocyte developmental competence by maintaining adequate energy availability for meiotic progression.

The increased maturation rate observed in oocytes supplemented with Se is consistent with the findings of Xiong et al. (2018), who reported that Se is an essential trace element that plays a critical role in reproduction, immune function, antioxidant defense, embryonic growth, and various physiological processes. Pei et al. (2023) added that, as a key component of several mammalian enzymes, including glutathione peroxidase (GPx), Se regulates multiple enzymatic systems such as metalloenzymes, lipid metabolism, iron transport, DNA synthesis and transport, glucose utilization, and free radical metabolism. According to Ferro et al. (2021), these enzymes catalyze the reduction of peroxides and repair oxidized proteins, directly lowering ROS burden. GPx, an endogenous antioxidant derived from Se, is crucial for maintaining cellular function. It increases GPx activity and total antioxidant capacity (TAC) and helps maintain reduced glutathione (GSH) levels, shifting the intracellular redox balance toward a less oxidative state. Song et al. (2024) reported that by reducing ROS, Se preserves mitochondrial membrane potential and reduces DNA fragmentation in oocytes and surrounding cumulus cells, improving oocyte quality and developmental competence.

Furthermore, Se has a limited therapeutic range, being beneficial at low concentrations but becoming toxic at higher concentrations, where it can induce oxidative stress or apoptosis and inhibit maturation. Optimal dose depends on species, source of oocytes including cumulus oocyte complex (COC)

vs denuded, baseline medium composition, and presence of other antioxidants. Yuan et al. (2024) stated that through these mechanisms, Se contributes directly and indirectly to embryonic development. Variations in Se content and bioavailability across tissues highlight the need for Se supplementation in specific areas to prevent reproductive impairments.

In addition to Se, gonadotropin hormones have been proven to be effective in maintaining oocyte quality during maturation. According to Lunenfeld et al. (2019), gonadotropins secreted by the pituitary gland play a central role in regulating reproductive function through two main components, such as follicle stimulating hormone (FSH) and luteinizing hormone (LH). The FSH is involved in follicular maturation and spermatogenesis, whereas LH regulates ovulation and corpus luteum (CL) formation. Both hormones are widely used to enhance reproductive efficiency in livestock and infertility therapy in humans. Furthermore, Ashibe et al. (2021) reported that FSH directly contributes to oocyte maturation by modulating cumulus cell activity surrounding the oocyte. The FSH stimulation of COC induces cumulus expansion, characterized by increased hyaluronic acid synthesis via hyaluronan synthase 2 (HAS2) activation. The hyaluronic acid subsequently interacts with inter-alpha trypsin inhibitor (ITI), tumor necrosis factor-stimulated gene 6 (TSG6), and pentraxin 3 (PTX3) to form a hyaluronan-rich extracellular matrix. This matrix maintains COC integrity, protects oocytes from mechanical and enzymatic damage during ovulation, and facilitates intercellular communication essential for optimal oocyte maturation.

4. Conclusion

Based on this study, both Se and gonadotropin supplementation effectively enhanced Bligon goat oocyte maturation, performing significantly better than unsupplemented media. These findings indicate that Se may be considered a suitable alternative to gonadotropin hormones for use in the IVM medium. Although ROS were not directly assessed in this study and the effectiveness of Se was evaluated solely based on the number of mature oocytes, the improved oocyte maturation observed with Se supplementation may indicate a more favorable intracellular environment during IVM.

5. Conflict of interest

The authors declare that there are no potential conflicts of interest related to this article.

6. Funding statement

This study was supported by UGM, through *Rekognisi Tugas Akhir*/Final Project Recognition (RTA) Program 2025 (No. 4193/UN1.P1/Dit-Lit/PT.01.03/2025).

7. Acknowledgement

The authors are grateful to UGM for the financial support provided for this study. Furthermore, the authors thank the Faculty of Animal Science, UGM, for the provision of facilities and technical support during the study.

8. Author's contribution

The authors declare that their contributions to this manuscript are as follows: DTW, ADNS, and SB designed the study. ADNS and RA collected the experiments data. ADNS, RA, DFFD, FGP, and PIS conducted the statistical analysis, data interpretation, and literature search. ADNS, DFFD, FGP, and PIS drafted the original manuscript. DTW and ADNS performed final editing manuscript. DTW and SB directed and supervised of the study.

9. Ethics approval

This study was conducted using slaughterhouse by-products. Therefore, no live animals were directly involved. While the ovaries were verified to be from the Bligon goat breed, specific data regarding the reason for slaughter, animal age, and prior husbandry management were unavailable. Consequently, formal institutional ethical approval was not required.

REFERENCES

Ashibe, S., Irisawa, K., Yokawa, K., and Nagao, Y. (2021). Mechanism of the adverse effect of hyaluronidase used for oocyte denudation on early development of bovine embryos. *Zygote*, 29(5):337–341.

Baldini, G. M., Lot, D., Malvasi, A., Laganà, A. S., Vimercati, A., Dellino, M., Cicinelli, E., Baldini, D., and Trojano, G. (2024). Abnormalities of oocyte maturation: Mechanisms and implications. *International Journal of Molecular Sciences*, 25(22):12197.

Chang, T., Zhao, J., Li, Q., Meng, A., Xia, Q., Li, Y., Xiang, W., and Yao, Z. (2023). Nuclear-cytoplasmic asynchrony in oocyte maturation caused by tubb8 variants via impairing microtubule function: a novel pathogenic mechanism. *Reproductive Biology and Endocrinology*, 21(1):109.

Dahlen, C. R., Reynolds, L. P., and Caton, J. S. (2022). Selenium supplementation and pregnancy outcomes. *Frontiers in Nutrition*, 9:1011850.

Di Nisio, V., Antonouli, S., Colafarina, S., Zarivi, O., Rossi, G., Cecconi, S., and Poma, A. M. G. (2023). Repeated rounds of gonadotropin stimulation induce imbalance in the antioxidant machinery and activation of pro-survival proteins in mouse oviducts. *International Journal of Molecular Sciences*, 24(11):9294.

Erlindra, C. P., Diatmono, D. F. F., Padmawati, F. G., Sastranegara, A. D. N., Sitaresmi, P. I., Bintara, S., and Widayati, D. T. (2026). Regulation of reactive oxygen species by sodium selenite in AndroMed® extender enhances post-thaw saanen buck semen quality. *Caraka Tani: Journal of Sustainable Agriculture*, 41(1):32–45.

Ferro, C., Florindo, H. F., and Santos, H. A. (2021). Selenium nanoparticles for biomedical applications: From development and characterization to therapeutics. *Advanced Healthcare Materials*, 10:2100598.

Guo, H., Yang, T., Xia, C., Ni, Z., Lou, Y., Deng, H., Zuo, Z., Zhu, Y., Wang, X., and Fang, J. (2026). Glutathione selenium nanoparticles alleviate oxidative stress-induced reproductive dysfunction by regulating mitochondrial dynamics. *Free Radical Biology and Medicine*, 242:108–119.

Guseva, O., Kan, N., Chekmareva, V., Kokorev, D., and Ilyasov, P. (2024). The impact of antioxidant supplements on oocytes and preimplantation embryos of humans and mammals, and their potential application for mitigating the consequences of oxidative stress *in vitro*: A review. *Reproductive and Developmental Medicine*, 8(4):252–263.

Jamil, M., Debbarh, H., Kabit, A., Ennaji, M., Zarqaoui, M., Senhaji, W. R., Hissane, M., Saadani, B., Louanjli, N., and Cadi,

R. (2023). Impact of the number of retrieved oocytes on IVF outcomes: Oocyte maturation, fertilization, embryo quality and implantation rate. *Zygote*, 31(1):91–96.

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. *International Journal of Molecular Sciences*, 24(10):9059.

Lunenfeld, B., Bilger, W., Longobardi, S., Alam, V., D'Hooghe, T., and Sunkara, S. K. (2019). The development of gonadotropins for clinical use in the treatment of infertility. *Frontiers in Endocrinology*, 10:429.

Muhajir, M., Karja, N. W. K., Setiadi, M. A., and Adnyane, I. K. M. (2018). *in vitro* maturation competence of oocyte and histology observation of follicle after storage of sheep ovary at 4°c. *Acta Veterinaria Indonesiana*, 6(2):16–23.

Negrón-Pérez, V. M., Al Naib, A., Zezeski, A. L., McCracken-Harlow, V. L., Perry, G. A., Ealy, A. D., and Rhoads, M. L. (2025). Cumulus cell expansion, nuclear maturation and embryonic development of bovine cumulus-oocyte complexes matured in varying concentrations of follicular fluid. *PLoS ONE*, 20(2):e0318376.

Pamungkas, F. A., Setiadi, M. A., and Karja, N. W. K. (2012). Characteristics and *in vitro* fertilization ability of ram spermatozoa: Comparison of epididymal and ejaculated spermatozoa. *Media Peternakan*, 35(1):38–44.

Parera, H., Lenda, V., Lusi, R. M., and Manoh, O. A. (2019). Kualitas oosit kambing Kacang dari status reproduksi yang berbeda. *PARTNER*, 24(1):860–866.

Pei, J., Pan, X., Wei, G., and Hua, Y. (2023). Research progress of glutathione peroxidase family (gpx) in redoxidation. *Frontiers in Pharmacology*, 14:1147414.

Pranatasari, D., Kustono, K., and Widayati, D. T. (2016). Supplementation of gonadotropin hormone into *in vitro* maturation medium to increase 4 cell stadium embryo development of bligon goat. *Buletin Peternakan*, 40(2):83.

Rakha, S. I., Elmetwally, M. A., El-Sheikh Ali, H., Balboula, A., Mahmoud, A. M., and Zaabel, S. M. (2022). Importance of antioxidant supplementation during *in vitro* maturation of mammalian oocytes. *Veterinary Sciences*, 9(8):439.

Sciorio, R. and Rinaudo, P. (2023). Culture conditions in the IVF laboratory: State of the art and possible new directions. *Journal of Assisted Reproduction and Genetics*, 40(11):2591–2607.

Song, J., Xiao, L., Zhang, Z., Wang, Y., Kouis, P., Rasmussen, L. J., and Dai, F. (2024). Effects of reactive oxygen species and mitochondrial dysfunction on reproductive aging. *Frontiers in Cell and Developmental Biology*, 12:1347286.

Thanaboonyawat, I., Makemaharn, O., Petyim, S., Laokirkkiat, P., and Choavaratana, R. (2016). The correlation of cumulus mucification patterns with oocyte maturation rate *in vitro* in FSH + LH-primed IVM cycles: A prospective study. *Archives of Gynecology and Obstetrics*, 293(3):681–686.

Toosinia, S., Davoodian, N., Arabi, M., and Kadivar, A. (2024). Ameliorating effect of sodium selenite on developmental and molecular response of bovine cumulus-oocyte complexes matured *in vitro* under heat stress condition. *Biological Trace Element Research*, 202:161–174.

Tripathi, S. K., Nandi, S., Gupta, P. S. P., and Mondal, S. (2023). Antioxidants supplementation improves the quality of *in vitro* produced ovine embryos with amendments in key development gene expressions. *Theriogenology*, 201(1):41–52.

Widayati, D. T., Fatmawati, D. H., Ariesta, N., and K., K. (2014). The influence of follicular fluid on *in vitro* developmental competence media of Bligon goat oocytes. *Jurnal Kedokteran Hewan*, 8(1):64–67.

Widayati, D. T. and Pangestu, M. (2020). Effect of follicle-stimulating hormone on Bligon goat oocyte maturation and embryonic development post *in vitro* fertilization. *Veterinary World*, 13(11):2443–2446.

Widayati, D. T. and Wahyuningsih (2024). *Panduan Praktikum Fertilisasi in vitro*. Fakultas Peternakan UGM, Yogyakarta.

Xiong, X., Lan, D., Li, J., Lin, Y., and Li, M. (2018). Selenium supplementation during *in vitro* maturation enhances meiosis and developmental capacity of yak oocytes. *Animal Science Journal*, 89(2):298–306.

Yuan, S., Zhang, Y., Dong, P.-Y., Chen, Y.-M., Liu, J., Zhang, B.-Q., Chen, M.-M., Zhang, S.-E., and Zhang, X.-F. (2024). A comprehensive review on potential role of selenium, selenoproteins and selenium nanoparticles in male fertility. *Heliyon*, 10(15):e34975.