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Original Research

Optimal Allogeneic Platelet-Rich Plasma Concentration to Improve *In Vitro* Maturation of Germinal Vesicle Oocytes From Women With Polyendocrine Metabolic Ovarian Syndrome: A Prospective Cohort Study

Tiara Kusumaningtyas[1,2,*], Ita Fauzia Hanoum[3], Rr Vicki Nuri Prijayanti[3], Diany Ayu Suryaningtyas[3], Annisa Nur Islahi[2], Naila Nabila Rahmani[2], Intan Kusumaningtyas[2], Berli Kusuma[2], Anto Sawarno[2], Dewi Kartikawati Paramita[4], Djaswadi Dasuki[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, 55281 Yogyakarta, Indonesia
[2]Department of Obstetrics and Gynecology, Permata hati Hospital, 34124 Metro, Lampung, Indonesia
[3]Permata Hati Infertility Clinic, Reproductive Health Installation, Dr. Sardjito General Hospital, 55281 Yogyakarta, Indonesia
[4]Department of Histology and Cell Biology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, 55281 Yogyakarta, Indonesia
*Correspondence: dr.tiarakusumaningtyas@gmail.com (Tiara Kusumaningtyas)

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Abstract

Background: *In vitro* maturation is a long-established technique used to obtain mature oocytes outside the human body. As an alternative to assisted reproductive technology (ART) protocols involving controlled ovarian stimulation (COS) followed by in vitro fertilization (IVF), IVM may facilitate oocyte maturation with less severe side effect and more cost effective in patients with polyendocrine metabolic ovarian syndrome (PMOS). Culture media supplemented with platelet-rich plasma (PRP) have previously shown promise in improving oocyte maturation. This study examined the potential utility of allogeneic PRP supplementation in enhancing the maturation of germinal vesicle (GV)-stage oocytes collected from patients with PMOS who underwent controlled ovarian stimulation. **Methods:** A prospective cohort study was conducted to determine the optimal concentration of allogeneic PRP for oocyte maturation. Subsequently, 28 GV-stage oocytes were cultured for 24 h in media supplemented with either 0% PRP (n = 14) or 5% PRP (n = 14). Oocyte maturation, total oocyte score (TOS), and fertilization rate were then assessed. **Results:** Supplementation with 5% PRP yielded the highest rate of oocyte maturation (66.7%) among the concentrations tested, and significantly improved maturation compared with 0% PRP (71.4% vs. 21.4%, respectively; $p = 0.021$). Morphological quality, as reflected by the TOS (3.9 ± 1.37), and fertilization rate (80%) were also improved following supplementation with 5% PRP, indicating support of oocyte maturation. **Conclusions:** Supplementation with 5% PRP was found to support oocyte maturation under controlled ovarian stimulation. Further studies are required to optimize the use of PRP without controlled ovarian stimulation in clinical *in vitro* maturation (IVM) protocols that may benefit patients with PMOS. **Study Registration:** This clinical study has been registered at https://clinicaltrials.gov/study/NCT07514234 (registration number: NCT07514234) and https://ina-crr.kemkes.go.id/en/studi/1632 (registration number: INA-F814749).

Keywords: platelet-rich plasma; oocyte maturation; *in vitro* maturation

1. Introduction

Assisted reproductive technology (ART) involving controlled ovarian stimulation (COS) followed by *in vitro* fertilization (IVF) is a well-established method for increasing fertility rates in women. However, the hyperstimulation required to induce oocyte maturation can lead to ovarian hyperstimulation syndrome (OHSS). The risk of OHSS is particularly significant for infertility patients with conditions such as polyendocrine metabolic ovarian syndrome (PMOS) [1]. To address this issue, *in vitro* maturation (IVM) has been developed as an alternative method to facilitate oocyte maturation with lower potential morbidity and less severe side effects, while simultaneously offering a more accessible option for patients with limited finances [2]. Research on human oocyte IVM began in 1965, culminating in the first live birth from an IVM-derived embryo in 1991. Although studies have shown that IVM can achieve an oocyte maturation rate of approximately 33.84%, the fertilization rate of *in vitro*-matured oocytes remains lower than that of oocytes matured *in vivo* [3]. Due to inconsistent fertility and pregnancy rates, coupled with limited clinical adoption, interest in IVM has waned in recent years [4].

A primary challenge with IVM is that while oocytes may reach the metaphase II (MII) stage, their quality is often compromised by suboptimal culture conditions. Inadequate culture environments adversely affect meiotic competence and early embryonic development, thereby reducing

overall fertility outcomes [5]. Optimizing the culture environment by supplementing the culture medium with various growth factors [6] may enhance nuclear maturation and improve embryo quality. Several supplementation strategies have been explored, including the addition of follicle-stimulating hormone (FSH), estrogen, progesterone, and follicular fluid [7], as well as co-culture with stem cells [8]. A previous study reported that culture supplementation with mesenchymal stem cells increased MII oocyte yield 6.9-fold in patients with PMOS [9].

Platelet-rich plasma (PRP) is a concentrated plasma fraction with a high platelet count. It is used extensively in regenerative medicine and is a potential supplementation agent for the induction of oocyte maturation. PRP is rich in growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor, and fibroblast growth factor (FGF). These bioactive molecules promote collagen synthesis, suppress inflammatory cytokines, and stimulate cell proliferation, thereby facilitating tissue regeneration and repair [10,11]. In reproductive medicine, PRP has been investigated for its potential to improve fertility outcomes. Endometrial studies have shown that PRP can enhance endometrial receptivity and implantation rates [12]. Furthermore, intraovarian PRP injection can stimulate folliculogenesis and improve oocyte retrieval. In perimenopausal women, ovarian PRP infusion has been associated with the restoration of menstrual cycles and spontaneous pregnancies [13]. This is likely mediated by increased levels of anti-Müllerian hormone and FSH, which collectively promote follicular development.

These promising findings indicate that PRP holds potential for further optimizing IVM culture conditions by providing a source of supportive growth factors for oocyte maturation [10,11,12,13]. However, IVM has not been used routinely in IVF clinics and laboratories as an alternative to COS, particularly in Indonesia. Therefore, the present study included germinal vesicle (GV)-stage oocytes collected from PMOS patients who underwent COS. This preliminary investigation was conducted to assess the potential for allogeneic PRP supplementation to increase oocyte maturation. Several parameters were assessed in this research, including oocyte maturation outcomes, oocyte morphological quality assessed via the total oocyte score (TOS), and subsequent fertilization potential after PRP supplementation.

2. Materials and Methods

2.1 Study Design

This study was designed as a prospective cohort study and has been registered at https://clinicaltrials.gov/study/NCT07514234 (NCT07514234) and https://ina-crr.kemkes.go.id/en/studi/1632 (INA-F814749). A total of 37 GV-stage oocytes with normal morphology were obtained using the ovum pick up (OPU) procedure on 15 PMOS patients who underwent IVF treatment at the Permata Hati Infertility Clinic from 2023–2024. The preliminary study was conducted using three different concentrations of PRP supplementation (5%, 25%, and 50%), with three GV-stage oocytes in each group. The optimal concentration of PRP was decided based on the maturation rate and the quality and stability of the culture media.

Once the best concentration was chosen, the remaining 28 GV-stage oocytes were randomly divided into the control group (PRP 0%, n = 14) and the PRP supplementation group (PRP 5%, n = 14). Before assessment, cumulus-oocyte complexes (COCs) were enzymatically denuded using hyaluronidase (Vitrolife, Gothenburg, Sweden) and mechanically cleaned with 170 µm and 140 µm micropipettes. This step removed cumulus cells (CCs) from GV-stage oocytes, thereby facilitating observation of the effects of PRP supplementation in oocytes. Each GV-stage oocyte was cultured for 24 h at 37 °C in a humidified incubator containing 6% CO_2, in either control media (G-IVF™ PLUS) or one of three treatment media (G-IVF™ PLUS + PRP at 5%, 25%, or 50%).

The primary outcome was oocyte maturation, defined as the transition from GV to MII stage and evaluated as the presence of the first polar body after 24 h of culture. Secondary outcomes included TOS and fertilization outcomes. TOS is a semi-quantitative, morphological tool used to evaluate oocyte morphology. The method used in this research was based on the scoring system developed by Lazzaroni-Tealdi et al. [14], which evaluates six key morphological parameters: oocyte shape, oocyte size, ooplasm characteristics, perivitelline space structure, zona pellucida thickness, and polar body morphology. Each parameter is scored using a standardized three-point scale (+1, 0, −1). The raw dataset and the scoring criteria for TOS have been uploaded to Mendeley Data (see the availability of data and materials). Fertilization was assessed approximately 17 ± 1 h post-injection, consistent with the standard window for pronuclear evaluation of intracytoplasmic sperm injection (ICSI)-derived zygotes. Assessment criteria for the fertilized oocytes included the presence of two polar bodies and two pronuclei (2 PN) under the microscope [15].

2.2 PRP and Culture Media Preparation

To minimize potential confounding factors, this study used allogeneic PRP. A healthy female donor aged 20–30 years with a history of spontaneous pregnancy and no reproductive disorder was selected. A total of 35 mL of blood was collected and centrifuged at 103 ×g (2400 rpm) for 10 min, followed by 230 ×g (3600 rpm) for 15 min. This process yielded approximately 12 mL of PRP, which was aliquoted into 1.5 mL microtubes and stored at –80 °C until use. The protocol for PRP preparation was carried out according to routine clinical practice, although details regarding PRP activation, including quantitative platelet,

leukocyte counts, and growth factor components were not recorded.

G-IVF™ PLUS (Vitrolife, Gothenburg, Sweden) was used as the oocyte maturation medium. PRP stock solutions were prepared at concentrations of 5%, 25%, and 50%. Prior to use, frozen PRP aliquots were thawed at room temperature for 10 min and then mixed with G-IVF™ PLUS culture medium. The prepared stock solutions were stored in a freezer until required for the culture procedure.

2.3 Statistical Analysis

Statistical analysis for maturation outcomes was conducted using Fisher's exact test in IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA), with significance set at $p < 0.05$. This test was chosen because of its suitability for comparing small sample sizes with similar variance distributions. TOS was subjected to statistical analysis using the Mann–Whitney U test and the Hodges–Lehmann estimator.

3. Results

3.1 Optimal PRP Concentration

From 2023 to 2024, 15 ovum pick-up (OPU) procedures were performed in polyendocrine metabolic ovarian syndrome (PMOS) patients and 37 GV were obtained. A preliminary study was conducted in three groups of GV using three different PRP concentrations.

A concentration of 5% PRP was deemed optimal (Table 1). Allogeneic PRP concentrations of 25% and 50% were considered suboptimal as they caused cells to clump, making evaluation difficult. Higher PRP concentrations were also associated with morphological abnormalities, such as cytoplasmic vacuolization, irregular perivitelline space, and abnormal polar bodies (Fig. 1), limiting further evaluation in this study. The TOS peaked at 5% PRP supplementation, followed by a noticeable decline at PRP concentrations of 25% and 50%.

Table 1. Maturation rate after 24 h IVM in different PRP supplementation media.

Group	Total oocytes (n)	MII (n)	Percentage (%)
PRP 5%	3	2	66.7%
PRP 25%	3	*	*
PRP 50%	3	*	*

*Could not be evaluated. IVM, *in vitro* maturation; PRP, platelet-rich plasma; MII, metaphase II.

3.2 Oocyte Maturation Rate

Based on the optimization results from a limited exploratory experiment, this study subsequently used 5% PRP supplementation to evaluate oocyte morphology. Oocytes were assessed microscopically based on nuclear and cytoplasmic maturation criteria. GV oocytes collected during OPU were allocated to either the PRP 5% group or the PRP 0% (control) group.

Oocyte maturation was evaluated after 24 h of culture. Although all GV-stage oocytes progressed towards the metaphase I (MI) stage, only those reaching MII were classified as mature. Oocytes that lacked a polar body and remained in MI were considered immature. In contrast, the presence of a polar body in MII oocytes indicated the completion of meiosis and readiness for fertilization. As shown in Table 2, a total of 13 oocytes successfully matured following 24 h in culture. The 5% PRP supplementation group showed a significantly higher rate of oocyte maturation compared to the control group ($p = 0.021$).

Table 2. Maturation rate after 24 h in the control and treatment groups.

Group	Total oocytes (n)	MII (n)	Percentage (%)	*p*-value*
PRP 0%	14	3	21.4%	0.021
PRP 5%	14	10	71.4%	
Total	28	13		

*Statistical comparison was performed using Fisher's exact test.

3.3 Oocyte Morphological Quality Following PRP Supplementation

Morphological evaluation was conducted using the total oocyte score (TOS). This system was applied to mature oocytes (MII stage) in order to evaluate their structural quality prior to fertilization.

A total of 13 mature oocytes (3 from the PRP 0% group and 10 from the PRP 5% group) were assessed using the TOS. Statistical analysis was conducted using the Mann-Whitney U test. As shown in Table 3, the PRP 5% group had a higher average TOS than the control group, although the difference was not statistically significant ($p = 0.217$). The Hodges-Lehmann estimator indicated a median difference of –2.0 (95% CI: –2.0 to 0.0), implying a potentially beneficial role of PRP supplementation. In support of this finding, the PRP 5% group displayed superior morphological quality compared to the PRP 0% group, with most oocytes exhibiting a uniform zona pellucida, clear cytoplasm, normal perivitelline space, and visible polar body.

Table 3. Average total oocyte score (TOS).

Group	MII (n)	Average TOS	*p*-value*
PRP 0%	3	2.67 ± 1.15	0.217
PRP 5%	10	3.90 ± 1.37	

*Statistical comparison was performed using the Mann-Whitney test (*p*-value = 0.217).
Each of the six oocyte morphology parameters was scored as +1 (good), 0 (moderate), or –1 (poor), giving a possible total range of –6 to +6.

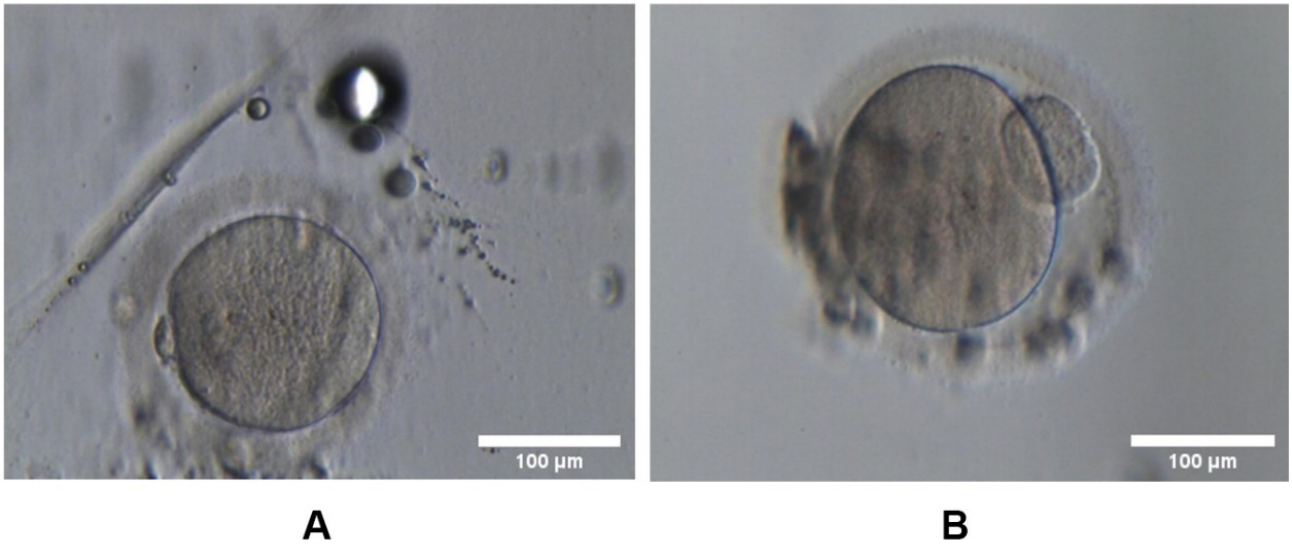

Fig. 1. Oocyte maturation in different PRP concentrations. (A) 25% PRP-supplemented medium. (B) 50% PRP-supplemented medium. Scale bar: 100 µm. Magnification: 200×.

3.4 Fertilization Rate

Following morphological assessment, intracytoplasmic sperm injection (ICSI) was conducted on all 13 mature oocytes. Successful fertilization of the MII oocytes was achieved in 8 cases, all of which were in the PRP 5% group (Table 4). The fertilization rate in the PRP 5% group was 80% (8/10). Due to the presence of a zero-event group, statistical comparison and estimation of confidence intervals were not conducted. The results are presented in descriptive form only.

Table 4. Fertilization rate.

Group	MII oocytes (n)	Fertilized (n)	Fertilization rate (%)
PRP 0%	3	0	0%
PRP 5%	10	8	80%

4. Discussion

IVM has been proposed as an alternative method for overcoming infertility in women with PMOS [1,16]. However, previous studies have reported inconsistent fertilization rates and pregnancy outcomes [3,4]. In view of this, the present study explored a potential strategy to increase fertility outcomes by improving IVM culture media. Various efforts have been made to improve IVM culture media, including PRP. PRP is an important source of growth factors and could potentially support the maturation of GV oocytes that fail to mature during the standard IVF cycle.

Based on the findings of our study, supplementation with PRP at a concentration of 5% yielded the best results and the highest maturation rate (66.7%). PRP supplementation at a concentration of 50% increased the medium viscosity, resulting in morphological abnormalities and the failure of oocyte maturation and fertilization. PRP supplementation at a concentration of 5% significantly improved the maturation rate from 21.4% to 71.4% compared to PRP 0% (p = 0.021). This level of PRP supplementation may also be associated with improved oocyte morphological quality, as observed by a higher average TOS (3.9 ± 1.37 vs. 2.67 ± 1.15, respectively) and a clear cytoplasm, uniform zona pellucida, normal perivitelline space, and polar body. Furthermore, PRP supplementation yielded an excellent fertilization rate of 80% compared to 0% in the control group, suggesting that PRP may support or even enhance the structural and functional maturation of oocytes.

Our findings align with previous reports on the regenerative potential of PRP, demonstrating its role in promoting tissue growth and angiogenesis. While these earlier studies were focused primarily on ovarian tissue implantation, the present work provides further preliminary phenotypic evidence that PRP supplementation is associated with improved oocyte maturation outcomes, even in the absence of natural somatic support from CCs [17]. CCs play a critical role in the immediate microenvironment of the oocyte, providing metabolic and regulatory support through gap junctions and paracrine signaling, including growth factors that regulate nuclear and cytoplasmic maturation [18]. A previous study reported improved IVM outcomes with PRP supplementation, particularly in cultures containing intact CCs and using a PRP supplementation concentration of 5% [19]. In the present reductionist model using denuded oocytes, PRP supplementation was associated with the progression of a proportion of oocytes to the MII stage. However, the absence of a comparison group with intact cumulus–oocyte complexes prevents any definitive conclusions regarding the ability of PRP to functionally re-

place CC-derived support. The current observations should therefore be interpreted as exploratory findings.

Several intracellular signaling pathways, including the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (p38), and transforming growth factor-β (TGF-β)/mothers against decapentaplegic homolog (SMAD) pathways, are known to be dysregulated during oocyte maturation in women with PMOS, contributing to impaired folliculogenesis and increased oocyte apoptosis. Several growth factors present in PRP, such as insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), and transforming growth factor beta-1 (TGF-β1) [20], may restore these dysregulated signaling pathways. In the absence of CCs, PRP may serve as a functional replacement by providing a rich mixture of growth factors and cytokines that support cell proliferation, angiogenesis, and collagen synthesis, while protecting against cellular stress [21]. PRP could therefore mimic the supportive role of CCs [22], and PRP supplementation may be associated with favorable morphological characteristics and fertilization outcomes.

IGF-1 activates the PI3K/Akt signaling pathway, enhancing mitochondrial function and maintaining cytoplasmic integrity, which are essential for proper oocyte maturation [23]. The coordinated effect of PRP growth factors may underlie the improved nuclear and cytoplasmic maturation, oocyte morphology, and fertilization rates observed in the current study. Insulin-like growth factor 1 receptor (IGF-1R) activation can also trigger recruitment of the adaptor protein Shc, linking IGF signaling to the MAPK/ERK pathway, and thus contributing to the regulation of intracellular functions that support oocyte maturation. The presence of IGF-1 in PRP has the potential to compensate for the loss of CCs support and to restore these dysregulated signaling pathways, thereby enhancing the competence of denuded oocytes and supporting the resumption of meiosis [24,25].

In denuded oocytes, EGF from PRP is associated with oocyte maturation through the activation of epidermal growth factor receptor (EGFR) expressed on oocytes, even in the absence of paracrine signals from CCs. Intracellular ERK activation in denuded oocytes contributes to the regulation of the maturation-promoting factor (MPF); cyclin-dependent kinase 1 (CDK1)–Cyclin B, spindle stability, and cytoplasmic quality during *in vitro* maturation. The presence of EGF in PRP may restore MAPK/ERK pathway activation and improve oocyte maturation competence in the absence of CCs [26]. The TGF-β superfamily may also support oocyte maturation in the absence of CCs. TGF-β in PRP can compensate for the lost paracrine signals by directly activating the TGF-β/SMAD pathway and regulating the expression of genes important for maintaining the stability of oocyte cytoplasm and granulosa cells. This improves the oocyte microenvironment, reduces stress and apoptotic signaling, and enhances oocyte readiness for the completion of *in vitro* maturation [27].

Nevertheless, not all oocytes respond equally to PRP. While PRP supports a favorable microenvironment, its effects are limited by the intrinsic quality of the oocyte itself. Factors such as chromosomal abnormalities or cytoplasmic defects may impair maturation, even under optimal conditions [28]. Previous studies have shown that extending IVM culture beyond 8 h may lead to chromosomal damage, thereby limiting their developmental potential [29]. Although PRP supplementation significantly improved maturation, a small number of oocytes in the control group still matured in the absence of PRP and CCs. GV-stage oocytes may continue to develop if their requirements are met, even in the absence of additional supplementation. In the present study, G-IVF™ PLUS was used as the base medium, providing essential factors for oocyte development. Consistent with this, some studies have reported successful IVM using only commercial media without additional supplements [30]. Other studies have found no significant differences in GV and MI maturation rates between commercial IVF and culture media [31].

Study Limitations

Overall, our statistical analysis indicated that PRP supplementation had a significant effect on oocyte maturation and fertilization. Additional data on factors such as embryo quality may provide valuable insights for expanding these findings. Further research with larger sample sizes and molecular-level assessments will be necessary to validate the current results and determine the optimal PRP concentration for oocyte culture. Moreover, this study used GV oocytes retrieved from COS, and hence the exposure to hormone stimulation may confound oocyte quality and maturation dynamics. Consequently, our results cannot be directly extrapolated to unstimulated IVM cycles in a real-world setting, and further studies using unstimulated IVM models are required to validate the findings. Despite these limitations, the current findings highlight the potential of low-dose PRP as a supportive factor for the enhancement of both oocyte morphology and fertilization potential.

5. Conclusions

Overall, the present study found that supplementation with 5% PRP has significant potential for supporting oocyte maturation under controlled ovarian stimulation. Further investigations with larger and balanced sample sizes, robust methodologies, and the use of PRP without controlled ovarian stimulation are needed to refine clinical IVM for PMOS patients, along with embryo quality testing and molecular-level assessments.

Abbreviations

IVF, *in vitro* fertilization; IVM, *in vitro* maturation; ICSI, intracytoplasmic sperm injection; PRP, platelet-rich plasma; PMOS, polyendocrine metabolic ovarian syndrome; MII, metaphase II; MI, metaphase I; PDGF, platelet-derived growth factor; PI3K/Akt, phosphoinositide 3-kinase/protein kinase B; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; p38, p38 mitogen-activated protein kinase; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor 1 receptor; EGFR, epidermal growth factor receptor; OPU, ovum pick-up; COC, cumulus-oocyte complexes; GV, germinal vesicle; FSH, follicle-stimulating hormone; CCs, cumulus cells.

Availability of Data and Materials

The study dataset has been deposited at https://data.mendeley.com (DOI: 10.17632/6y2hftfjzd.1; link: https://data.mendeley.com/datasets/6y2hftfjzd/1).

Author Contributions

TK, DD, DKP designed the project. TK, NNR, IFH RVNP, DAS, ANI collected the data and performed the research. TK, AS, IK, BK, DD, and DKP analyzed the data. TK, NNR, IK, BK, AS wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Medicine, Public Health, and Nursing at Universitas Gadjah Mada (Approval No. KE/FK/0022/EC/2023). All the participants provided signed informed consent.

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Conflicts of Interest

The authors declare no conflicts of interest.

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