Journal of Reproduction & Infertility
Volume 26, Issue no. 3
https://doi.org/10.18502/jri.v26i3.20179

Original Article

Check for updates

The Effect of hCG Supplementation on Embryo Quality after Rescue *In Vitro* Maturation (r-IVM)

Novita Prasetiawati [1], Ayu Mulia Sundari [2], Agus Supriyadi [3], Hadi Sjarbaini [3], Tarigan Sudirmanto [3], Gde Suardana [3], Mohammad Haekal [3], Gangsar Pariyanti [3], Deana Rosaria Indah [3], Mulyoto Pangestu [4], Anom Bowolaksono [1, 2*]

1- Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, Indonesia
2- Cellular and Molecular Mechanisms in Biological System (CEMBIOS) Research Group, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, Indonesia
3- Melati Clinic, Harapan Kita Women and Children Hospital, Jakarta, Indonesia
4- Department of Obstetrics and Gynecology, School of Clinical Sciences, Monash University, Victoria, Australia

Abstract

Background: The supplementation of human chorionic gonadotropin (hCG) into culture medium in rescue *in vitro* maturation (r-IVM) has been reported to improve the maturation rates of immature oocytes derived from stimulated cycles. However, the impact of this enrichment on embryo quality is yet to be understood. The purpose of the current study was to investigate the effect of hCG on the embryo quality following r-IVM.

Methods: A total of 152 immature oocytes consisting of germinal vesicles (GV) and metaphase I (MI) were collected and classified into 1) control group (n=73; GV=22, MI=51) cultured in culture medium only, and 2) experimental group (n=79; GV= 23, MI=56) cultured in culture medium supplemented with 0.5 *IU* hCG. Study parameters were analyzed using Student's T-test or Kruskal Wallis and chi-square at a 95% confidence level.

Results: After 24 *hr*, the maturation rate of the control and experimental groups was comparable (57% *vs*. 70%, p=0.58). Following intracytoplasmic sperm injection (ICSI), the fertilization rate was significantly higher in the experimental group than in the control group (49% *vs*. 36%; p=0.03). However, the number of good-quality embryos was similar in the groups (16% *vs*. 6%).

Conclusion: Our study suggests that hCG supplementation into the culture medium during r-IVM does not affect embryo quality but improves the fertilization rate. Further research is needed to scrutinize the role of hCG in fertilization.

Keywords: Embryo, Human chorionic gonadotropin, Oocytes, Rescue IVM.
To cite this article: Prasetiawati N, Sundari AM, Supriyadi A, Sjarbaini H, Sudirmanto T, Suardana G, et al. The Effect of hCG Supplementation on Embryo Quality after Rescue *In Vitro* Maturation (r-IVM). J Reprod Infertil. 2025;26(3):152-158. https://doi.org/10.18502/jri.v26i3.20179.

* *Corresponding Author:*
Anom Bowolaksono, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, Indonesia
E-mail: alaksono@sci.ui.ac.id

Received: 25, Mar. 2025
Accepted: 25, Aug. 2025

Introduction

Maturation failure after controlled ovarian stimulation (COS) is one restriction to the success of in vitro fertilization (IVF). Approximately 30% of the oocytes retrieved during ovum pick-up (OPU) are immature, thereby reducing the availability of mature oocytes and limiting the number of potential embryos (1–3). There is still no solid explanation on why some oocytes remain immature after stimulation (2). It has been proposed that immature oocytes may originate from non-dominant small follicles where the luteinizing hormone (LH)-signaling pathway is not fully developed or from large preovulatory follicles that do not appropriately respond to hCG (2, 4, 5). Ovarian hyper-response, an adverse response of the ovary to injectable gonadotropin, has also been implicated, as a higher proportion of immature oocytes is usually found among high responders (6). Moreover, a break in double-stranded DNA is another reported factor contributing to the presence of immature oocytes (7).

r-IVM is a viable alternative protocol for IVF patients with a high number of immature oocytes after COS. This technique allows for the maturation of COS-derived immature oocytes across various developmental stages in a laboratory setting, thus potentially increasing the number of mature oocytes available for fertilization and improving the chances of successful pregnancy (8). Studies suggest that immature oocytes undergoing r-IVM are capable of completing meiosis and fertilization at similar rates to sibling metaphase II (MII) oocytes. This technique is even superior in women with diminished ovarian reserve, as evidenced by a better maturation rate, fertilization rate, and embryo quality compared to women with normal ovarian reserve (9, 10). However, while r-IVM offers promise to improve IVF outcome, its clinical efficacy is still limited by suboptimal maturation rates and poor developmental potential. As such, improving the protocol to better mimic the *in vivo* environment is crucial for realizing the full potential of r-IVM.

Tantitham et al. reported that supplementation of hCG into the culture medium increased the maturation of immature oocytes derived from stimulated cycles. Following 24 *hr* of culture, it was revealed that the maturation rate of immature oocytes cultured in the hCG-supplemented medium was higher compared to those cultured in culture medium only (48% *vs*. 39.2%). An analysis of the glucose-6-phosphate dehydrogenase (G6PD) activity, a rate-limiting enzyme of the pentose phosphate pathway involved in meiosis resumption, demonstrated that the proportion of BCB-positive oocytes was significantly higher in the hCG-treated group, supporting the potential role of hCG in promoting oocyte maturation (11). A study on animals similarly showed that the proportion of cumulus-oocyte complexes (COCs) with cumulus expansion and the proportion of metaphase II oocytes were the highest in media containing hCG (12). Moreover, several studies have demonstrated that hCG priming before oocyte retrieval results in a higher percentage of metaphase II oocytes at the time of retrieval (13, 14). A higher fraction of mature oocytes after extending the hCG priming duration from 35 to more than 35 *hr* was also reported (15, 16).

Oocyte maturation is driven by LH through the activation of maturation-promoting factor (MPF), a protein complex consisting of Cdc2 and cyclin B, leading to the breakdown of the germinal vesicle and the condensation of chromosomes, marking the start of meiosis (17). Nevertheless, reports suggest that hCG can trigger the resumption of meiosis via binding to the LH receptor due to structural homology (18, 19). hCG has been shown to have a higher affinity for the LH receptor compared to LH itself and to be five-fold more effective in promoting cAMP activity than equimolar concentrations of LH (20). Hence, hCG has been used as a surrogate for the LH surge during ovarian stimulation (21). However, although the effect of hCG on oocyte maturation of immature oocytes obtained from unstimulated or stimulated cycles has been well recognized, its effect on the embryo quality following r-IVM is unknown. The purpose of the current study was to investigate the effect of hCG supplementation on the embryo quality of immature oocytes after r-IVM.

Methods

Study population: This experimental study was conducted at Melati Clinic, Harapan Kita Women and Children Hospital, Jakarta, Indonesia, from February 2018 to August 2021. A total of 44 infertile women who had undergone IVF due to primary or secondary infertility were included in the study. Women aged ≥35 years, those with male factor infertility, and poor responders were excluded from the study. The sample size was calculated using a formula for a randomized controlled design with 80% power and a 5% significance level. According to the calculation, a total of 74 samples (37 samples per group) were required. Only oocytes with normal appearance and morphology were used in each group. Oocytes with abnormalities in shape, cytoplasm, granularity, or perivitelline space were excluded from the study. Total population sampling was conducted due to the small size of the study population. A total of 152 immature oocytes consisting of GV and MI oocytes were retrieved and randomly classified into 1) control group (n=73; GV=22, MI=51) cultured in culture medium only, and 2) experimental group (n=79; GV=23, MI=56) cultured in culture medium with 0.5 *IU* hCG supplementation. The hCG dose was selected based on its extensive use in human oocyte IVM, despite being non-physiological (11, 22). The randomization of oocytes into groups was performed using a computergenerated random number table. This study was approved by the Ethics Committee of Harapan Kita Women and Children Hospital (IRB/22/06/ETIK/2021).

Ovarian stimulation and oocyte culture: All study participants received an antagonist ovarian stimulation protocol. Briefly, 150-225 *IU* rFSH (Follitropin alfa, Gonal F; Merck Serono, Germany) per day was administered starting from the second day of the menstrual cycle. GnRH antagonist (Cetrotide; Asta Medica, Germany) was later given on the fifth day of the menstrual cycle. When there were at least three ≥18 *mm* follicles, hCG 10.000 *IU* was administered, and OPU was performed in the next 34-36 *hr*. COC was collected and cultured in a fertilization medium (G IVF Plus; Vitrolife, Sweden) for 2 *hr* under 6% CO^2. The remaining cumulus cells were removed by hyaluronidase (Hyase; Vitrolife, Sweden).

Maturation, fertilization, and embryo quality assessment: Following 24 *hr* of culture, nuclear maturation was assessed by the presence of the first polar body in the perivitelline space. Oocyte maturation rate was calculated as the percentage of MII oocytes relative to the total number of cultured oocytes. MII oocytes were then inseminated by ICSI; meanwhile, GV and MI oocytes were classified as immature oocytes and were divided into a control and experimental group as described in the study population. Fertilization was evaluated in the following 18 *hr* by the presence of two polar bodies. Fertilization rate was calculated by dividing the total number of fertilized oocytes at the pronuclear stage by the total number of MII-inseminated oocytes, multiplied by 100. Fertilized oocytes were cultured for the next two days to observe their embryonic development. Embryos were morphologically graded into good, moderate, and poor according to Alpha Scientists in Reproductive Medicine and ESHRE recommendations (23).

Statistical analysis: Descriptive statistics were used to summarize the characteristics of the study participants. Categorical and numerical variables were presented as the number of subjects and percentage (%), mean±standard deviation, or median (minimum–maximum) if applicable. Quantitative and qualitative data were analyzed using Student's T-test or Kruskal Wallis and chi-square at a 95% confidence level. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 24 (IBM, USA).

Results

Characteristics of the studied participants are summarized in table 1. The mean age of participants in the control and experimental group was 34.43±3.23 and 33.55±4.04 years, respectively (p=0.43), with normal BMI (22.82±1.68 *vs.* 23.22±2.84, p=0.06). Among 23 women in the control group, 5 (21.7%) participants had tubal factor infertility, 15 (65.2%) participants had en-

Table 1. Characteristics of study participants

Characteristics	Overall (n=44)	Control group (n=23)	Experimental group (n=21)	p-value
Age (years)	33.73±3.73	34.43±3.23	33.55±4.04	0.43
BMI (*kg/m²*)	23.12±5.24	22.82±1.68	23.22±2.84	0.06
Etiology of infertility				
Tubal factor	11 (25.0)	5 (21.7)	6 (28.6)	
Endometrial factor	26 (59.1)	15 (65.2)	11 (52.4)	-
PCO	2 (4.5)	1 (4.3)	1 (4.8)	
Other factors	5 (11.4)	2 (8.7)	3 (14.3)	
Antral follicle count	10.29±5.76	10.41±5.60	10.14±6.16	0.54
Stimulation duration (days)	10.72±1.40	10.23±1.16	10.62±1.51	0.67
Oocyte yield				
GV	1 (0–7)	2 (0–5)	1 (0–7)	
MI	1 (0–5)	1 (0–5)	1 (0–3)	0.49
MII	9 (5–13)	9 (6–11)	9 (5–13)	
Gonadotropin dosage (*IU*)	2509.45±792.46	2550.16±680.92	2510.84±761.39	0.51

Data are presented as mean±standard deviation (SD) for age, BMI, antral follicle count, stimulation duration, and gonadotropin dosage; median (min–max) for oocyte yields and number of subjects (percentage) for etiology of infertility. Student's T-test or Kruskal–Wallis tests were used for the numerical variables. Chi-square tests were used for the categorical variables. BMI=Body Mass Index, GV=Germinal Vesicle, MI=Metaphase I, MII=Metaphase II dometrial factor infertility, 1 (4.3%) participant had PCO, and 2 (8.7%) participants had infertility due to other factors. In the experimental group, the number of women with tubal factor infertility, endometrial factor infertility, PCO, and other factors was 6 (28.6%), 11 (52.4%), 1 (4.8%), and 3 (14.3%), respectively. No significant differences were found in terms of the etiology of infertility between groups. The antral follicle count, stimulation duration, and total gonadotropin dosage were similar between the control and the experimental group (10.41±5.60 *vs*. 10.14±6.16, p=0.54; 10.23±1.16 *vs*. 10.62±1.51, p=0.67; and 2550.16±680.92 *vs*. 2510.84±761.39, p=0.51).

The median of retrieved oocytes was 1, 1, and 9 for GV, MI, and MII oocytes, respectively (p=0.49). After 24 *hr* of culture, immature oocytes in the experimental group had a similar maturation rate to that of the control group (70% *vs*. 57%; p=0.58). Specifically, the number of GV oocytes that developed into MII oocytes and MI oocytes that developed into MII oocytes were 14 *vs*. 8 and 42 *vs*. 34, respectively (p=0.36; Table 2).

After ICSI, fertilization rates of the experimental group were significantly higher than the control group (49% *vs*. 36%, p=0.03; Table 3). However, embryo quality was comparable between the control and experimental groups (p=0.06; Table 3). The proportion of good, moderate, and poor- quality embryos in the control and experimental groups was 6% *vs*. 16%, 18 *vs*. 34%, and 76% *vs*. 50%, respectively.

Discussion

In the present study, hCG supplementation into the culture medium did not improve embryo quality during r-IVM, but it significantly increased fertilization rate. There are two types of IVM, based on the origin of the cultured immature oocyte, namely IVM and r-IVM. IVM refers to the maturation of oocytes retrieved from unstimulated or minimally stimulated cycles, whereas r-IVM involves the maturation of oocytes retrieved from stimulated IVF cycles that were initially intended for fertilization but were found to be immature (24). The clinical use of r-IVM remains a topic of debate, with studies showing both benefits and drawbacks. Nonetheless, although r-IVM represents a valuable tool in preserving mature oocytes, its ability to support subsequent embryo development remains a subject of ongoing research (8, 25).

In vivo, oocyte maturation is a tightly controlled process involving intricate interactions among hormones, somatic cells, and transcription factors. In contrast, oocytes matured *in vitro* lack these regulatory mechanisms due to removal from their follicular environment, leading to a decrease in intra-oocyte cAMP concentration and spontaneous maturation, which potentially compromises the quality of the resulting oocytes and embryos (24). Achieving effective r-IVM is particularly challenging because oocytes are cultured only after cumulus cell removal, which deprives them of essential support. Hence, various strategies

Table 2. Maturation rate

Characteristics	Control group	Experimental group	p-value
Immature	73	79	-
GV	22	23	-
MI	51	56	-
Maturation rate	42 (57)	56 (70)	0.58
GV to MII	8 (36)	14 (60)	-
MI to MII	34 (67)	42 (75)	-

Data are presented as numbers and percentages. Chi-square tests were performed with a 95% confidence level. GV=Germinal Vesicle, MI=Metaphase I, MII=Metaphase II

Table 3. Fertilization rate and embryo quality

Characteristics	Control group	Experimental group	p-value
Embryo quality			
Good	1 (6)	4 (16)	
Moderate	3 (18)	8 (34)	
Poor	13 (76)	12 (50)	
Fertilization	16 (36)	24 (49)	0.03

Data are presented as number (percentage). Chi-square tests were used for statistical analysis at 95% confidence level have been explored to improve the protocol, involving supplementation of culture media with hormones and growth factors (24, 26, 27).

In 2020, Tantitham et al. investigated for the possible benefit of hCG on *in vitro* maturity of oocytes obtained from ovarian stimulation cycles for IVF (11). hCG is a glycoprotein secreted by the trophoblast after conception (28). In the traditional view, it biologically functions to stimulate progesterone release from the corpus luteum, making it essential for the maintenance of pregnancy (29). However, some studies have suggested that hCG also plays an important role in oocyte maturation as it mimics LH action (16, 22). In the present study, the maturation rate of oocytes cultured in hCG-supplemented medium was higher compared to those cultured in hCG-free medium, but this difference was not statistically significant. Although not statistically significant, our study has illuminated the potential link between hCG and meiosis resumption.

The role of hCG in meiosis resumption is mediated through its binding to the LH receptor. Due to structural homology, hCG and LH share the same alpha subunit and about 85% of the beta subunit amino acid sequence, allowing hCG to mimic LH and activate the LH receptor (18, 19).

Studies suggest that LH receptor is highly expressed in the mural granulosa cells, and its expression in cumulus cells gradually decreased from MII oocytes to GV oocytes (30). In the present study, the oocytes were denuded, potentially resulting in the loss of LH receptors. However, immature oocytes in this study retained some cumulus and granulosa cells, presumably due to factors like the presence of more gap junctions and a more compact arrangement of cumulus cells. This explains why hCG still functions on oocytes after denudation.

Following ICSI, our study demonstrated that the fertilization rate is significantly higher in oocytes cultured in hCG-supplemented medium. There is limited data regarding the effect of hCG on fertilization. However, Drakakis et al. in 2009 reported that hCG addition to rFSH during ovarian stimulation resulted in a higher fertilization and pregnancy rate, with a trend toward improved implantation rates (31). Fertilization is the insertion of paternal chromosome to oocyte by which only a completely mature oocyte will be recognized and fertilized by a penetrating sperm (32). hCG is reported to have five-fold greater potency than LH on activating cAMP pathway and its production, whereas cAMP is known to regulate sperm motility and the acrosome reaction, both of which support sperm entry into the oocyte (33, 34). It seems that hCG supplementation in the experimental group of the present study enhanced cAMP production, thus enhancing the fertilization rate.

In terms of embryo development, to the best of our knowledge, this is the first study to investigate the effect of hCG supplementation on embryo quality. Therefore, no corresponding data are currently available. Nevertheless, Ramu et al. (2011) found that hCG was detected in 93 out of 102 of day-two embryo culture media using enzyme-linked immunosorbent assay. Correlation analysis demonstrated that the abundance of hCG was independent of embryo developmental status (35). Based on these findings, hCG appears to be naturally re-leased by the embryo during the culture process. Therefore, it is plausible that the comparable embryo quality observed in this present study could be due to the presence of hCG in the control group, suggesting that the addition of 0.5 *IU* hCG to the culture medium in the experimental group was insufficient. A limitation of this study is the small sample size due to the limited availability of immature oocytes. Future animal experiments are needed to validate these results.

Conclusion

Our study indicated that the addition of hCG into the culture medium of immature oocytes (GV, MI) does not affect embryo quality after r-IVM. However, it significantly improves the fertilization rate. Further research is required to investigate the role of hCG in fertilization.

Acknowledgement

This research was funded by the Directorate of Research and Community Service, University of Indonesia through Hibah PUTI Pascasarjana 2022 Grant (NKB-271/UN2.RST/HKP.05.00/2022).

Conflict of Interest

None.

References

1. Handayani N, Sundari AM, Aprilliana T, Boediono A, Polim AA, Wiweko B, et al. Immature oocyte proportion in a cohort led to poor embryo development but did not reduce clinical pregnancy rate. Middle East Fertil Soc J. 2024;29(1):23.
2. Lee HJ, Jee BC, Suh CS, Kim SH, Moon SY. Oocyte maturity in relation to woman's age in in

vitro fertilization cycles stimulated by single regimen. Yonsei Med J. 2012;53(1):181-5.

3. Rienzi L, Ubaldi F, Anniballo R, Cerulo G, Greco E. Preincubation of human oocytes may improve fertilization and embryo quality after intracytoplasmic sperm injection. Hum Reprod. 1998;13(4): 1014-9.
4. Astbury P, Subramanian GN, Greaney J, Roling C, Irving J, Homer HA. The presence of immature GV–stage oocytes during IVF/ICSI is a marker of poor oocyte quality: a pilot study. Med Sci (Basel). 2020;8(1):4.
5. Teramoto S, Osada H, Sato Y, Shozu M. Nondominant small follicles are a promising source of mature oocytes in modified natural cycle in vitro fertilization and embryo transfer. Fertil Steril. 2016; 106(1):113-8.
6. Kok JD, Looman CWN, Weima SM, Te Velde ER. A high number of oocytes obtained after ovarian hyperstimulation for in vitro fertilization or intracytoplasmic sperm injection is not associated with decreased pregnancy outcome. Fertil Steril. 2006;85 (4):918-24.
7. Coticchio G, Dal Canto M, Guglielmo MC, Albertini DF, Mignini Renzini M, Merola M, et al. Double-strand DNA breaks and repair response in human immature oocytes and their relevance to meiotic resumption. J Assist Reprod Genet. 2015;32 (10):1509-16.
8. Wei J, Luo Z, Dong X, Jin H, Zhu L, Ai J. Cut-off point of mature oocyte for routine clinical application of rescue IVM: a retrospective cohort study. J Ovarian Res. 2023;16(1):226.
9. Shani AK, Haham LM, Balakier H, Kuznyetsova I, Bashar S, Day EN, et al. The developmental potential of mature oocytes derived from rescue in vitro maturation. Fertil Steril. 2023;120(4):860-9.
10. Ahmad MF, Mohd Nor NY, Mohammad Ramadneh MM, Roseli NI, Elias MH, Mat Jin N, et al. Comparative analysis of rescue-in vitro-maturation (r-IVM) outcomes in women with diminished ovarian reserve (DOR) versus normal ovarian reserve (NOR). Biomedicines. 2025;13(5):1084.
11. Tantitham C, Panunumpa S, Satirapod C. The effect of human chorionic gonadotropin on the in vitro development of immature to mature human oocytes: a randomized controlled study. J Hum Reprod Sci. 2020;13(2):133-7.
12. De Los Reyes M, De Lange J, Miranda P, Palominos J, Barros C. Effect of human chorionic gonadotrophin supplementation during different culture periods on in vitro maturation of canine oocytes. Theriogenology. 2005;64(1):1-11.
13. Son WY, Yoon SH, Lim JH. Effect of gonadotrophin priming on in-vitro maturation of oocytes collected from women at risk of OHSS. Reprod Biomed Online. 2006;13(3):340-8.
14. Zheng X, Wang L, Zhen X, Lian Y, Liu P, Qiao J. Effect of hCG priming on embryonic development of immature oocytes collected from unstimulated women with polycystic ovarian syndrome. Reprod Biol Endocrinol. 2012;10:40.
15. Skvirsky S, Blais I, Lahav-Baratz S, Koifman M, Wiener-Megnazi Z, Dirnfeld M. Time interval between hCG administration and oocyte pick up: analysis of oocyte maturation, embryonic morphology, morphokinetics, and IVF outcome. Clin Exp Obstet Gynecol. 2021;48(2):402-9.
16. Son WY, Chung JT, Chian RC, Herrero B, Demirtas E, Elizur S, et al. A 38 h interval between hCG priming and oocyte retrieval increases in vivo and in vitro oocyte maturation rate in programmed IVM cycles. Hum Reprod. 2008;23(9):2010-6.
17. Arroyo A, Kim B, Yeh J. Luteinizing hormone action in human oocyte maturation and quality: signaling pathways, regulation, and clinical impact. Reprod Sci. 2020;27(6):1223-52.
18. Abbara A, Clarke SA, Dhillo WS. Novel concepts for inducing final oocyte maturation in in vitro fertilization treatment. Endocr Rev. 2018;39(5):593-628.
19. European IVF-monitoring consortium (EIM); European society of human reproduction and embryology (ESHRE), Kupka MS, D'hooghe T, Ferraretti AP, de Mouzon J, et al. Assisted reproductive technology in Europe, 2011: results generated from European registers by ESHRE. Hum Reprod. 2016;31(2):233-48.
20. Casarini L, Lispi M, Longobardi S, Milosa F, la Marca A, Tagliasacchi D, et al. LH and hCG action on the same receptor results in quantitatively and qualitatively different intracellular signalling. PLoS One. 2012;7(10):e46682.
21. Castillo JC, Humaidan P, Bernabéu R. Pharmaceutical options for triggering of final oocyte maturation in ART. Biomed Res Int. 2014;2014: 580171.
22. Ge HS, Huang XF, Zhang W, Zhao JZ, Lin JJ, Zhou W. Exposure to human chorionic gonadotropin during in vitro maturation does not improve the maturation rate and developmental potential of immature oocytes from patients with polycystic ovary syndrome. Fertil Steril. 2008;89(1):98-103.
23. Alpha scientists in reproductive medicine and ESHRE special interest group of embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011;26(6):1270-83.

24. Das M, Son WY. In vitro maturation (IVM) of human immature oocytes: is it still relevant? Reprod Biol Endocrinol. 2023;21(1):110.

25. Soler N, Cimadomo D, Escrich L, Grau N, Gal An A, Alam P, et al. Rescue in vitro maturation of germinal vesicle oocytes after ovarian stimulation: the importance of the culture media. Hum Reprod. 2025;40(8):1504-15.

26. Coticchio G, Cimadomo D, De Vos M, Ebner T, Esbert M, Escribá MJ, et al. To rescue or not to rescue immature oocytes: prospects and challenges. Fertil Steril. 2025;123(5):749-58.

27. Procházka R, Bartková A, Němcová L, Murín M, Gad A, Marcollová K, et al. The role of MAPK3/1 and AKT in the acquisition of high meiotic and developmental competence of porcine oocytes cultured in vitro in FLI medium. Int J Mol Sci. 2021; 22(20):11148.

28. Choi J, Smitz J. Luteinizing hormone and human chorionic gonadotropin: origins of difference. Mol Cell Endocrinol. 2014;383(1-2):203-13.

29. Nwabuobi C, Arlier S, Schatz F, Guzeloglu-Kayisli O, Lockwood CJ, Kayisli UA. hCG: biological functions and clinical applications. Int J Mol Sci. 2017;18(10):2037.

30. Maman E, Yung Y, Kedem A, Yerushalmi GM, Konopnicki S, Cohen B, et al. High expression of luteinizing hormone receptors messenger RNA by human cumulus granulosa cells is in correlation with decreased fertilization. Fertil Steri. 2025;97 (3):592-8.

31. Drakakis P, Loutradis D, Beloukas A, Sypsa V, Anastasiadou V, Kalofolias G, et al. Early hCG addition to rFSH for ovarian stimulation in IVF provides better results and the cDNA copies of the hCG receptor may be an indicator of successful stimulation. Reprod Biol Endocrinol. 2009;7:110.

32. Siu KK, Serrão VHB, Ziyyat A, Lee JE. The cell biology of fertilization: Gamete attachment and fusion. J Cell Biol. 2021;220(10):e202102146.

33. Riccetti L, De Pascali F, Gilioli L, Potì F, Giva LB, Marino M, et al. Human LH and hCG stimulate differently the early signalling pathways but result in equal testosterone synthesis in mouse leydig cells in vitro. Reprod Biol Endocrinol. 2017;15(1): 2.

34. Tesarik J, Mendoza-Tesarik R. Cyclic adenosine monophosphate: a central player in gamete development and fertilization, and possible target for infertility therapies. Int J Mol Sci. 2022;23(23): 15068.

35. Ramu S, Acacio B, Adamowicz M, Parrett S, Jeyendran RS. Human chorionic gonadotropin from day 2 spent embryo culture media and its relationship to embryo development. Fertil Steril. 2011;96 (3):615-7.