In vitro maturation of human oocytes: Its role in infertility treatment and new possibilities

Eun Mi Chang¹, Hang Seok Song², Dong Ryul Lee², Woo Sik Lee¹, Tae Ki Yoon¹

¹Department of Obstetrics and Gynecology, Fertility Center of CHA Gangnam Medical Center, CHA University, Seoul; ²Department of Biomedical Science, College of Life Science, CHA University, Seoul, Korea

IVM refers to the maturation of immature oocytes in culture after their recovery from small antral follicles at the stage prior to selection and dominance. IVM requires little or no FSH in vivo and has been proposed as an alternative to conventional IVF, since it reduces the primary adverse effects caused by controlled ovarian stimulation, including the ovarian hyperstimulation syndrome. Moreover, IVM is a promising option for cases for which no standard protocol is suitable, such as FSH resistance, contraindications for ovarian stimulatory drugs, and the need for urgent fertility preservation. Recently, IVM has been used in women with regular cycles and normal ovaries. However, the pregnancy rate following IVM is suboptimal compared with that of conventional IVF, indicating that further studies to optimize the protocol and the culture conditions are warranted.

Keywords: Fertility preservation; Follicle culture; Infertility; In vitro maturation; Oocyte

Introduction

Since Pincus and Enzmann [1] described IVM in rabbit oocytes in 1935, it has been the primary method for producing offspring in agriculturally valuable species through IVF. IVM of human oocytes was first reported in 1965, followed by successful pregnancy and delivery reported in 1989 [2]. IVM has been suggested as an alternative to conventional IVF for minimizing the risk of the ovarian hyperstimulation syndrome (OHSS) in patients with the polycystic ovarian syndrome (PCOS). Moreover, the procedure costs less than IVF because it does not involve expensive gonadotropin injections [3,4]. Recently, IVM has been proposed as the method of choice for patients undergoing anticancer treatment, particularly for women who require rapid fertility preservation or face the risk of estrogen-sensitive cancer recurrence [5,6]. However, despite these advantages, the maturation rate and the developmental potential of embryos derived from IVM oocytes are significantly lower than those of oocytes matured in vivo. Moreover, the pregnancy rate with IVM is low compared with IVF. These findings indicate that IVM clinical protocols and culture technology need improvement [7].

Indications for IVM

The indications for IVM include women at risk of OHSS, those with PCOS or PCO-like ovaries, those with estrogen-sensitive cancers, and those who require rapid fertility preservation before beginning potentially gonadotoxic treatments. Nonetheless, IVM has not been widely accepted in infertility clinics worldwide, because pregnancy rates with IVM are lower than those achieved with conventional IVF. Moreover, recent developments in controlled ovarian stimulation protocols, which prevent OHSS in patients with PCOS, such as the use of a GnRH antagonist to prevent premature luteinization or a GnRH agonist, rather than hCG, to trigger ovulation, have removed the risk of ovarian hyperstimulation. However, in addition to decreasing the major side effects of controlled ovarian stimulation, such as OHSS, the IVM procedure eliminates the need for frequent sonographic...
monitoring and costs less than IVF; consequently, a few experimental studies have performed IVM in women with regular cycles and normal ovaries and in situations where no suitable standard protocol exists, such as oocyte donation, FSH resistance, and other contraindications for ovarian stimulatory drugs. Furthermore, IVM is a promising technique for fertility preservation. Recent improvements in oncological treatment have markedly increased cancer survival rates; however, the risk of losing ovarian function remains. In this regard, IVM, which can be performed rapidly without hormonal stimulation before cancer treatment, may be an attractive option for these patients.

**IVM for fertility preservation**

Currently, the clinical options for female fertility preservation are surgical intervention and cryopreservation of the embryo, oocyte, or ovarian tissue. A recent report proposes using a GnRH agonist during chemotherapy, suggesting that it may lessen ovarian function loss; however, currently, its effect has not been established [8]. Thus, the only viable option with proven effectiveness for fertility preservation is embryo cryopreservation. Nevertheless, embryo cryopreservation may not be suitable for women without a male partner. Additionally, even though embryo cryopreservation for breast cancer patients has been successfully used for fertility preservation without specific side effects, the theoretical risk for increasing cancer recurrence exists [9]. Ovarian tissue can be cryopreserved and orthotopically transplanted, this option remains experimental and few successful births have been reported [10,11]. Additionally, ovarian tissue removal and transplantation requires repeated operations, which may be a significant physical and financial burden for patients. Oocyte cryopreservation can overcome these shortcomings and is currently regarded as the most promising method for female fertility preservation. To date, more than 500 live births have resulted from oocyte cryopreservation; however, only a few have been reported following the implantation of cryopreserved IVM oocytes [12]. It should be noted that IVM can be performed urgently irrespective of the phase of the menstrual cycle without affecting the quantity and maturation rate of the oocytes. Therefore, IVM may be a useful option for fertility preservation in cancer patients without ovarian stimulation and with no delay in the cancer treatment. Recently, one successful pregnancy resulting from cryopreserved embryos obtained from IVM oocytes after oophorectomy in an ovarian cancer patient was reported [13]. One of the technical problems yet to be resolved is the time at which to freeze the immature oocytes. Cryopreservation at the germinal vesicle stage may reduce damage to the oocyte; however, recent evidence indicates improved survival with cryopreservation before immature oocyte vitrification [14,15].

**Clinical regimens in IVM**

The clinical protocol for IVM has not changed substantially since the technique was developed. Until recently, three clinical regimens have been used. IVM without priming is the original and most rigorous protocol. Without gonadotropin administration, oocyte retrieval was scheduled when the larger follicles were 10 to 12 mm, a stage at which dominance has not been established. Recently, this protocol has been used for suitable normo-ovulatory women in addition to patients with PCOS [4,16].

An alternative protocol that used a small amount of FSH to increase the oocyte yield and the maturation rate was introduced between 1999 and 2000. The results of FSH priming in IVM are controversial, and recent evidence has not shown a clear advantage for the technique [17-19]. The third technique, IVM with hCG priming, is used as an alternative to FSH priming in order to facilitate the resumption of meiosis in vivo before full maturation [20,21]. A recent statement by the American Society for Reproductive Medicine Committee defined IVM as “maturation in culture of immature oocytes after their recovery from follicles which may or may not have been exposed to FSH, but were not exposed to either LH or hCG prior to retrieval to induce meiotic resumption.” The first two protocols meet this definition; however, hCG priming is the most commonly used protocol because it significantly increases the pregnancy rate. According to studies that compared the different protocols of IVM in women with PCOS, hCG priming, which was not dose-dependent, raised the maturation rate from 69% to 84%, fertilization rate from 45% to 80%, pregnancy rate from 31% to 38.5%, and live birth rate to 33%. FSH priming increased the pregnancy rate from 0% to 29% [22].

In the Fertility Center of the CHA Gangnam Medical Center, hCG priming has been significantly more successful than no priming for implantation (26.4% vs. 22.6%) and for the pregnancy rate (61.9% vs. 45.7%) [23]. Determining the optimal timing for oocyte collection is the most significant dilemma for IVM. A previous study suggested that the selection of a dominant follicle may induce endocrine changes in the remaining cohort that are detrimental to their subsequent fertilization and embryonic development. However, the selection of the dominant follicle can take place while postponing oocyte pickup during endometrial growth. Several studies investigating the size of the follicle at the time of oocyte retrieval have shown that follicle sizes up to 12 mm did not compromise the outcome [16]. Moreover, the luteal phase oocyte retrieval did not differ from the follicular phase retrieval in terms of maturation and fertilization rate [24]. These findings support the usefulness of IVM for urgent fertility preservation. Recently, several protocols using oral contraceptives or estrogen have been developed to reduce the complexity of immature oocyte retrieval timing [25].
Implantation environment

To prepare the endometrium for IVM, patients are administered estradiol valerate commencing the day of oocyte retrieval and vaginal progesterone for luteal support commencing the day of fertilization. However, endometrial development may be insufficient compared with that of natural or stimulated cycles. In this regard, a recent study showed that embryos generated from IVM can be successfully cryopreserved and transferred during the next hormone replacement cycle with high implantation and pregnancy rates [26]. Furthermore, the underlying health conditions in IVM candidates may contribute to low implantation and pregnancy rates following the procedure. The most common indication for IVM is PCOS, which is often accompanied by the metabolic syndrome and insulin resistance. A recent study found that the embryo and oocyte quality was comparable in insulin-resistant and non-insulin-resistant patients; however, implantation (11.6% vs. 28.7%) and pregnancy rates (23.5% vs. 53.1%) were lower in the insulin-resistant patients following IVM [27]. These findings highlight the importance of the implantation environment for successful IVM implantation. However, few studies have investigated the optimal endometrial conditions for IVM, and further studies in this field are needed.

Pregnancy outcome after IVM

Although promising data on the IVM technique have been published, unfortunately, there is still no evidence from randomized clinical trials upon which to base any practice recommendations regarding IVM before IVF or ICSI for women with PCOS [21]. Observational studies showed a high maturation rate of the oocytes of up to 80.3%, fertilization rates from 10% to 76.5%, clinical pregnancy rates from 21.5% to 50% per cycle, implantation rates of around 18%, and live birth rates from 15.9% per retrieval to 33% per cycle [21,28-30]. Retrospective studies reported similar results with oocyte maturation rates of up to 84%, fertilization rates from 43% to 70%, and pregnancy rates from 22% to 56.6%, while rates of miscarriage, ectopic pregnancy, and late fetal loss were similar for IVM and IVF or ICSI groups of women with PCOS [21,31-33].

Births following IVM/IVF are estimated to be more than 2,500; however, because most of the deliveries following IVM have not been reported and published appropriately, accurate data are limited. Until recently, approximately 400 births were reported from six centers, and the results suggest no increase in the anomaly rate after IVM compared with conventional IVF (Table 1) [34-40]. The largest recent IVM study, which included 200 births, found no increase in adverse outcomes and a 178-g increase in birth weight [38]. These findings suggest that IVM reduced the risk of epigenetic disease with the added benefit of avoiding ovarian hyperstimulation or that IVM may increase the risk of imprinting disorders. Because the imprinting process involves de novo methylation in the developing germ cells, genome-wide demethylation in early embryos, and safe propagation of blastocysts and somatic cells, prolonging in vitro culture may increase the risk of defects. However, current evidence regarding the effects of oocyte culture and the risks of imprinting disorders is reassuring compared with the effects of preimplantation embryo culture and the risks of imprinting defects [41]. Furthermore, previous findings of a higher KvDMR1 methylation status in germinal vesicle- and metaphase I-arrested oocytes in non-stimulated patients with PCOS [21,31-33].

Table 1. Reported birth outcomes after IVM

| Citation (authors, ref no.) | Year | Number of included births | Obstetric and perinatal outcomes |
|-----------------------------|------|---------------------------|---------------------------------|
| Cha et al. [35]             | 2005 | 20 Singleton, 4 twin live births after IVM | 3 Congenital anomalies (5.3%)  |
|                             |      |                           | 2 Major (omphalocele: miscarriage, hydrops fetalis: termination, normal chromosome) |
| Mikkelsen [39]             | 2005 | 47 Births after IVM       | No specific abnormalities related to the IVM procedure |
|                             |      |                           | One 46 XX with CCNH gene variation, inherited paternally (no clinical significance) |
|                             |      |                           | One IUFD, induction failure, and asphyxia |
| Soderstrom-Anttila et al. [40] | 2006 | 40 Singletons, 3 sets of twins | 8 (19%) Minor developmental problems expressed |
|                             |      |                           | One optical glioma |
|                             |      |                           | Neuropsychological development within the normal range at 2 yr of age |
| Shu-Chi et al. [37]         | 2006 | 21 IVM births             | Growth and developmental scale comparison with non IVM, no developmental delay |
| Bucketti et al. [36]        | 2007 | 55 IVM, 217 IVF, and 160 ICSI babies compared | Risk of congenital anomalies (odd ratios) 1.42, 1.21, and 1.69, respectively |
| Fadini et al. [38]          | 2012 | 200 Babies born following IVM | No detected major congenital abnormalities |

IVM, in vitro maturation; ref no., references number; IUFD, intrauterine fetal death; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.
[42,43]. Thus, at present, we cannot draw any conclusions as to whether IVM is superior to or worse than conventional IVF.

**Efforts to improve IVM outcomes**

To improve IVM efficiency, the optimal culture conditions for IVM must be determined, and the various molecular mechanisms underlying oocyte maturation must be identified. Over the last 20 years, advances in culture conditions have continuously improved IVM’s efficacy. Nonetheless, IVM does not support all nuclear and cytoplasmic changes that occur physiologically as a result of ovulatory stimulus in vivo. Thus, biological studies of ovaries and oocytes have focused on the basal molecular mechanisms underlying oocyte maturation and the molecules regulating oocyte maturation. These studies investigated the paracrine factors participating in cell-to-cell communication in oocyte maturation, the molecular basis for oocyte meiotic arrest, and the resumption and mechanism of oocyte maturation and ovulation after the LH surge [44]. Recent findings have shown the importance of epidermal growth factor (EGF) signaling during oocyte maturation after LH stimulation [45]. Adding EGF family molecules, such as amphiregulin and epiregulin, to the culture media increases the IVM rate of immature human oocytes [46]. Additionally, brain-derived neurotrophic factor (BDNF) and glial-cell-derived neurotrophic factor (GDNF), which are expressed in granulosa cells via LH/hCG signaling, have been recently reported to increase maturation rates in human oocytes [47]. Delaying the maturation process has been suggested as an option to combine with cytoplasmic maturation, and recent reports have revealed that the addition of dibutyl cyclic adenosine 3’5’-monophosphate (cAMP) was effective for preventing germinal vesicle breakdown in mouse oocytes, reflecting the critical role of cAMP in arresting meiosis in vivo [48]. Furthermore, the inhibition of protein synthesis and the use of kinase inhibitors have been found to inhibit germinal vesicle breakdown [49].

**Conclusion**

IVM technology has continued to improve since the first IVM-induced pregnancy in 1989. Recent investigations into oocyte development and the interaction between oocytes and the surrounding cells may further improve the culture conditions for oocyte IVM. Although the pregnancy rate following IVM is slightly lower than that of conventional IVF, several recent reports regarding the pregnancy rates following improvements in clinical protocols and culture conditions are promising. Further improvements in IVM efficiency that advance the basic understanding of oocyte maturation may help broaden the use of IVM for fertility preservation and in patients who are infertile.

**Conflict of interest**

No potential conflict of interest relevant to this article was reported.

**References**

1. Pincus G, Enzmann EV. The comparative behavior of mammalian eggs in vivo and in vitro: I. the activation of ovarian eggs. J Exp Med 1935;62:665-75.
2. Cha KY, Koo JJ, Ko JJ, Choi DH, Han SY, Yoon TK. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. Fertil Steril 1991;55:109-13.
3. Cha KY, Chian RC. Maturation in vitro of immature human oocytes for clinical use. Hum Reprod Update 1998;4:103-20.
4. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril 1994;62:353-62.
5. Oktay K, Buyuk E, Rodriguez-Wallberg KA, Sahin G. In vitro maturation improves oocyte or embryo cryopreservation outcome in breast cancer patients undergoing ovarian stimulation for fertility preservation. Reprod Biomed Online 2010;20:634-8.
6. Fadini R, Dal Canto M, Mignini Renzini M, Millani R, Fruscio R, Cantu MG, et al. Embryo transfer following in vitro maturation and cryopreservation of oocytes recovered from antral follicles during conservaive surgery for ovarian cancer. J Assist Reprod Genet 2012;29:777-81.
7. Practice Committees of the American Society for Reproductive Medicine the Society for Assisted Reproductive Technology. In vitro maturation: a committee opinion. Fertil Steril 2013;99:663-6.
8. Yang B, Shi W, Yang J, Liu H, Zhao H, Li X, et al. Concurrent treatment with gonadotropin-releasing hormone agonists for chemotherapy-induced ovarian damage in premenopausal women with breast cancer: a meta-analysis of randomized controlled trials. Breast 2013;22:150-7.
9. Turan V, Bedoschi G, Moy F, Oktay K. Safety and feasibility of performing two consecutive ovarian stimulation cycles with the use of letrozole-gonadotropin protocol for fertility preservation in breast cancer patients. Fertil Steril 2013;100:1681-5.e1.
10. Donnez J, Silber S, Andersen CY, Demeestere I, Piver P, Meirion D, et al. Children born after autotransplantation of cryopreserved ovarian tissue: a review of 13 live births. Ann Med 2011;43:437-50.
11. Anderson RA, Wallace WH. Fertility preservation in girls and young women. Clin Endocrinol (Oxf) 2011;75:409-19.
12. Chian RC, Uzelac PS, Nargund G. In vitro maturation of human...
immature oocytes for fertility preservation. Fertil Steril 2013;99:1173-81.

13. Prasath EB, Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M, et al. First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient. Hum Reprod 2014;29:276-8.

14. Cao YX, Chian RC. Fertility preservation with immature and in vitro matured oocytes. Semin Reprod Med 2009;27:456-64.

15. Borini A, Bianchi V. Cryopreservation of mature and immature oocytes. Clin Obstet Gynecol 2010;53:763-74.

16. Fadini R, Dal Canto MB, Renzini MM, Brambillasca F, Comi R, Fumagalli D, et al. Predictive factors in in-vitro maturation in unstimulated women with normal ovaries. Reprod Biomed Online 2009;18:251-61.

17. Suikkari AM, Tulppala M, Tuuri T, Hovatta O, Barnes F. Luteal phase start of low-dose FSH priming of follicles results in an efficient recovery, maturation and fertilization of immature human oocytes. Hum Reprod 2000;15:747-51.

18. Mikkelsen AL, Lindenberg S. Benefit of FSH priming of women with PCOS to the in vitro maturation procedure and the outcome: a randomized prospective study. Reproduction 2001;122:587-92.

19. Fadini R, Dal Canto MB, Mignini Renzini M, Brambillasca F, Comi R, Fumagalli D, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study. Reprod Biomed Online 2009;19:343-51.

20. Chian RC, Gulekli B, Bucket WM, Tan SL. Priming with human chorionic gonadotrophin before retrieval of immature oocytes in women with infertility due to the polycystic ovary syndrome. N Engl J Med 1999;341:1624, 6.

21. Bucket WM, Chian RC, Tan SL. Human chorionic gonadotrophin for in vitro oocyte maturation: does it improve the endometrium or implantation? J Reprod Med 2004;49:93-8.

22. Siristatidis CS, Vrachnis N, Creatsa M, Maheshwari A, Bhattacharya S. In vitro maturation in subfertile women with polycystic ovarian syndrome undergoing assisted reproduction. Cochrane Database Syst Rev 2013;10:CD006606.

23. Kim MK, Park EA, Kim HJ, Choi WY, Cho JH, Lee WS, et al. Does supplementation of in-vitro culture medium with melatonin improve IVF outcome in PCOS? Reprod Biomed Online 2013;26:222-9.

24. Maman E, Meirov D, Brengauz M, Raanani H, Dor J, Hourvitz A. Luteal phase oocyte retrieval and in vitro maturation is an optional procedure for urgent fertility preservation. Fertil Steril 2011;95:64-7.

25. Vitek WS, Witmyer J, Carson SA, Robins JC. Estrogen-suppressed in vitro maturation: a novel approach to in vitro maturation. Fertil Steril 2013;99:1886-90.

26. Ortega-Hrepich C, Stoop D, Guzman L, Van Landuyt L, Tournaye H, Smitz J, et al. A “freeze-all” embryo strategy after in vitro maturation: a novel approach in women with polycystic ovary syndrome? Fertil Steril 2013;100:1002-7.

27. Chang EM, Han JE, Seok HH, Lee DR, Yoon TK, Lee WS. Insulin resistance does not affect early embryo development but lowers implantation rate in in vitro maturation-in vitro fertilization-embryo transfer cycle. Clin Endocrinol (Oxf) 2013;79:93-9.

28. Child TJ, Abdul-Jalil AK, Gulekli B, Tan SL. In vitro maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovarian syndrome. Fertil Steril 2001;76:936-42.

29. Beckers NG, Pieters MH, Ramos L, Zeilmaker GH, Fauser BC, Braat DD. Retrieval, maturation, and fertilization of immature oocytes obtained from unstimulated patients with polycystic ovary syndrome. Hum Reprod 2000;15:165-70.

30. Soderstrom-Anttila V, Makinen S, Tuuri T, Suikkari AM. Favourable pregnancy results with insemination of in vitro matured oocytes from unstimulated patients. Hum Reprod 2005;20:1534-40.

31. Le Du A, Kadoch IJ, Bourcigaux N, Doumerc S, Bourrier MC, Chevalier N, et al. In vitro oocyte maturation for the treatment of infertility associated with polycystic ovarian syndrome: the French experience. Hum Reprod 2005;20:420-4.

32. Mikkelsen AL, Lindenberg S. Benefit of FSH priming of women with PCOS to the in vitro maturation procedure and the outcome: a randomized prospective study. Reproduction 2001;122:587-92.

33. Buckett WM, Chian RC, Holzer H, Dean NL, Sylvestre C, Holzer HE, Tan SL. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. Hum Reprod 2000;15:165-70.

34. Beckers NG, Pieters MH, Ramos L, Zeilmaker GH, Fauser BC, Braat DD. Retrieval, maturation, and fertilization of immature oocytes obtained from unstimulated patients with polycystic ovary syndrome. Hum Reprod 2000;15:165-70.

35. Soderstrom-Anttila V, Makinen S, Tuuri T, Suikkari AM. Favorable pregnancy results with insemination of in vitro matured oocytes from unstimulated patients. Hum Reprod 2005;20:1534-40.

36. Le Du A, Kadoch IJ, Bourcigaux N, Doumerc S, Bourrier MC, Chevalier N, et al. In vitro oocyte maturation for the treatment of infertility associated with polycystic ovarian syndrome: the French experience. Hum Reprod 2005;20:420-4.

37. De Vos M, Ortega-Hrepich C, Albuaz FK, Guzman L, Polyzos NP, Smitz J, et al. Clinical outcome of non-hCG-primed oocyte in vitro maturation treatment in patients with polycystic ovaries and polycystic ovarian syndrome. Fertil Steril 2011;96:860-4.

38. Cha KY, Chung HM, Lee DR, Kwon H, Chung MK, Park LS, et al. Obstetric outcome of patients with polycystic ovarian syndrome treated by in vitro maturation and in vitro fertilization-embryo transfer. Fertil Steril 2005;83:1461-5.

39. Bucket WM, Chian RC, Holzer H, Dean N, Usher R, Tan SL. Obstetric outcomes and congenital abnormalities after in vitro maturation, in vitro fertilization, and intracytoplasmic sperm injection. Obstet Gynecol 2007;110:885-91.

40. Shu-Chi M, Jiann-Loung H, Yu-Hung L, Tseng-Chen S, Ming IL, Tsu-Fuh Y. Growth and development of children conceived by in vitro maturation.
in-vitro maturation of human oocytes. Early Hum Dev 2006;82:677-82.
38. Fadini R, Mignini Renzini M, Guarneri T, Dal Canto M, De Ponti E, Sutcliffe A, et al. Comparison of the obstetric and perinatal outcomes of children conceived from in vitro or in vivo matured oocytes in in vitro maturation treatments with births from conventional ICSI cycles. Hum Reprod 2012;27:3601-8.
39. Mikkelsen AL. Strategies in human in-vitro maturation and their clinical outcome. Reprod Biomed Online 2005;10:593-9.
40. Soderstrom-Anttila V, Salokorpi T, Pihlaja M, Serenius-Sirve S, Suikkari AM. Obstetric and perinatal outcome and preliminary results of development of children born after in vitro maturation of oocytes. Hum Reprod 2006;21:1508-13.
41. Anckaert E, De Rycke M, Smits J. Culture of oocytes and risk of imprinting defects. Hum Reprod Update 2013;19:52-66.
42. Khoueiry R, Ibala-Rhomdane S, Mery L, Blachere T, Guerin JF, Lornage J, et al. Dynamic CpG methylation of the KCNQ1OT1 gene during maturation of human oocytes. J Med Genet 2008;45:583-8.
43. Market-Velker BA, Zhang L, Magri LS, Bonvissuto AC, Mann MR. Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. Hum Mol Genet 2010;19:36-51.
44. Li R, Albertini DF. The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. Nat Rev Mol Cell Biol 2013;14:141-52.
45. Park JY, Su YQ, Ariga M, Law E, Jin SL, Conti M. EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science 2004;303:682-4.
46. Ben-Ami I, Kornsky A, Bern O, Kasterstein E, Komarovsky D, Ron-El R. In vitro maturation of human germinal vesicle-stage oocytes: role of epidermal growth factor-like growth factors in the culture medium. Hum Reprod 2011;26:76-81.
47. Zhao P, Qiao J, Huang S, Zhang Y, Liu S, Yan LY, et al. Gonadotrophin-induced paracrine regulation of human oocyte maturation by BDNF and GDNF secreted by granulosa cells. Hum Reprod 2011;26:695-702.
48. Chen J, Hudson E, Chi MM, Chang AS, Moley KH, Hardie DG, et al. AMPK regulation of mouse oocyte meiotic resumption in vitro. Dev Biol 2006;291:227-38.
49. Anderiesz C, Fong CY, Bongso A, Trounson AO. Regulation of human and mouse oocyte maturation in vitro with 6-dimethylaminopurine. Hum Reprod 2000;15:379-88.