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

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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].

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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].

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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 andnon-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 55.6%, 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, compared with the same stage-stimulated oocytes, suggest that ovarian stimulation may interfere with the establishment of imprinting

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
Buckett 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 dibutryl 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 ofprotein 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.

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