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Clinical Application of In Vitro Maturation of Oocytes

Xiaolin La, Jing Zhao and Zhihui Wang

Abstract

In vitro maturation (IVM) is a technique used to induce immature oocytes collected in different periods of embryonic growth. The rates vary for immature oocytes collected from different clinical sources to potentially develop into embryos and achieve live birth. As an effective treatment method, IVM can be used to treat patients with polycystic ovary syndrome (PCOS), ovarian hyperresponsiveness, and hyporesponsiveness, as well as to preserve the fertility of cancer patients. This technology has been used worldwide for the birth of thousands of healthy babies. The improvement in clinical IVM technology mainly focuses on the IVM medium and the optimization of the culture environment and operation process. At present, with the improvement in the in vitro fertilization (IVF) efficiency and culture systems, a natural cycle or mild stimulation may be more suitable for women receiving IVF treatments. A new treatment option was proposed to combine natural cycle/mild stimulation IVF with IVM. In particular, the combination of mild stimulation IVF and IVM is not only expected to become a viable alternative to current standard treatments but may also become a potential option of first-line treatment.

Keywords: in vitro maturation (IVM), assisted reproductive technologies (ARTs), cytoplasmic maturation, antral follicles, granulosa cells

1. Introduction

In the 1960s, major milestones were achieved in in vitro maturation (IVM) of human oocytes, and in vitro fertilization (IVF) of IVM oocytes was also established. Therefore, modern assisted reproductive technologies (ARTs) are based on IVM. Currently, the clinical application of IVM may be extended to treat patients with polycystic ovary syndrome (PCOS), ovarian hyperresponsiveness, and hyporesponsiveness, as well as to preserve the fertility of cancer patients [1]. In 2013, the practice committees of the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART) stated that the clinical pregnancy rate of IVM was still lower than that of conventional IVF, and hence IVM could not yet be considered the first treatment choice for all cases of female infertility [2].

The current standard protocol for ovulation induction in clinical practice involves intense stimulation with nonphysiological doses of gonadotropins to obtain an average of 10–15 or even dozens of mature oocytes per woman. Although the regimen of high-dose gonadotropin treatment may enable the retrieval of a larger number of oocytes, this approach can exert several short- and long-term adverse effects, including the risk of ovarian hyperstimulation syndrome (OHSS).

At present, with the improvement in the IVF efficiency and culture systems, a natural cycle or mild stimulation may be more suitable for women receiving IVF treatments. A previous study showed that natural cycle or mild stimulation IVF is more effective than conventional stimulation protocols in patients with a low functional ovarian reserve [3]. In contrast to the standard stimulation protocol, the mild stimulation protocol is a safer and more rational regimen that helps reduce the hormone dosage, lower treatment risks, and retrieve a small number of high-quality oocytes. Despite these theoretical advantages, the mild stimulation protocol has yet to become a mainstream treatment modality in the United States. With the development of IVM technology, a modified protocol able to increase the success rates of natural cycle or mild stimulation IVF has been established. In this protocol, in addition to the retrieval of mature oocytes in naturally or mildly stimulated cycles, immature oocytes from small follicles are also retrieved for IVM, thereby increasing the total number of retrieved oocytes in a single treatment cycle and the clinical pregnancy rate. Data from previous clinical studies has shown that the combined use of natural cycle or mild stimulation IVF with IVM can expand the applicable scope of IVM technology to the treatment of various types of female infertility and has resulted in satisfactory clinical pregnancy rates and live birth rates [4, 5].

2. Mechanism of oocyte maturation

Cyclic adenosine monophosphate (cAMP) plays an important role in regulating the maturation of oocytes. The mural granulosa cells (MGC) located on the follicular wall contain natriuretic peptide precursor C (NPPC), while the cumulus cells around oocytes express natriuretic peptide receptor 2 (NPR2). Oocyte-derived paracrine factors can promote the activation of NPR2 in cumulus cells, while the NPPC in mural granulosa cells can bind to NPR2 receptors in cumulus cells to produce cyclic guanosine monophosphate (cGMP), which then enters into oocytes through gap junctions to inhibit the activity of phosphodiesterase (PDE3A), thereby maintaining a high level of cAMP in oocytes and the arrest of oocytes in the meiosis cycle. The activation of PDE3A by luteinizing hormone (LH) downregulates the level of cAMP in oocytes and induces the maturation of oocytes, thereby relieving the immature oocytes in the germinal vesicle (GV) stage or first meiotic metaphase (MII) from cell cycle arrest, so that they can complete the first meiosis and enter the second MII to develop into mature oocytes [6]. Zhang et al. [7] reported that estradiol can promote and maintain the expression of NPR2 in cumulus cells and participate in NPPC-mediated meiotic arrest of oocytes *in vitro*. These studies have opened up a new field of molecular mechanistic research on resuming the meiosis of oocytes, providing a theoretical basis for revealing the molecular mechanisms underlying the maturation of oocytes.

Studies have found that small molecule ribonucleotides (microRNAs) are also important for oocyte maturation. A certain number of dynamic and stable microRNAs were found in both mature oocytes and early-stage embryos, presumably contributing to the maturation of oocytes. Kim et al. [8, 9] reported that microRNAs may affect oocyte maturation by altering the gene expression and function of cumulus cells through cumulus cell interaction and paracrine secretion. Let-7 is one of the most abundant microRNAs in the ovary. Upregulation of Let-7c can increase the rate of oocyte maturation, suggesting that Let-7c may be involved in the information exchange between oocytes and surrounding mural granulosa cells. In addition, maturation-promoting factor (MPF), cytostatic factor (CSF), oocyte maturation inhibitor (OMI), and mitogen-activated protein kinase (MAPK) are involved in oocyte maturation and division [7]. The mechanisms underlying oocyte maturation awaits further studies.

3. Definition of oocyte IVM

The biological definition of oocyte IVM is to remove immature oocytes in the GV stage from antral follicles and culture them in a suitable culture system, so that these immature oocytes can mature to MII stage in vitro. However, the clinical definition of IVM technology for immature human oocytes is completely different from its biological definition. The differences include the different sources of immature oocytes, the different protocols used to induce ovulation, and the different time of oocyte retrieval. These factors may lead to the situation where the immature oocytes retrieved clinically are not in the GV stage. The use of human chorionic gonadotropin (hCG) to induce ovulation prior to clinical retrieval of oocytes may lead to the initiation of endogenous oocyte maturation, and hence some of the retrieved immature oocytes may have undergone germinal vesicle breakdown (GVBD) or entered the MI stage. Although immature oocytes in the MI stage have initiated the process of in vivo maturation, they still need to participate in the procedure of in vitro culture and maturation. Therefore, the definition of clinical IVM treatment should include the in vitro culture of immature oocytes in the GV and MI stages.

A recent point of view proposed to give the clinical definition of IVM of immature oocytes based on the diameter of follicles when the oocytes are retrieved [10]. However, this definition is not completely scientific, since the meiotic state of oocytes cannot be completely determined according to the size of follicles during the stimulation cycle [11, 12]. In addition, for immature oocytes collected from different clinical sources, their maturation rate and the rates to potentially develop into embryos and achieve live birth are different. Therefore, for clinical definition and research of IVM, attention should be paid to the effect of different sources of immature oocytes on the efficiency of IVM.

4. Factors affecting the in vitro maturation of oocytes

4.1 Effect of culture time on in vitro maturation of oocytes

Maturation of oocytes includes the nuclear and cytoplasmic maturation of oocytes. Nuclear maturation refers to the rupture of the germinal vesicle, separation of homologous chromosomes, appearance of the perivitelline space, and discharge of the first polar body. Cytoplasmic maturation refers to the completion of protein phosphorylation and dephosphorylation as well as the rearrangement of organelles in oocytes. Only the oocytes whose nucleus and cytoplasm are matured simultaneously can have adequate fertility and the potential for embryo development. Studies have found that most oocytes cultured in vitro can reach maturity within 24–48 h. The length of in vitro culture can affect the developmental potential of the embryo. The rate of nuclear maturation in oocytes cultured for 48 h in vitro is significantly higher than that for 24 h, but the rate of cytoplasmic maturation in oocytes cultured for 48 h is not significantly different from that for 24 h. Excessive culture time leads to the aging of oocytes and an increased level of associated genetic risks. When the culture time is too short, the maturation of cytoplasm and nucleus is not synchronized and will affect the subsequent development potential of the embryo. Wrenzycki et al. [13] found that oocytes only possess the ability to mature in the final stage of development. Therefore, adequate extension of IVM time can promote the necessary process of oocyte maturation, increase the rate of nuclear maturation in immature oocytes, and significantly improve in vitro developmental potential of oocytes and the rate of high-quality embryos.

4.2 Effects of hormones on in vitro maturation of oocytes

Gonadotropin can promote the expansion of cumulus cells and stimulate the maturation of the nucleus and cytoplasm of oocytes, thus facilitating the formation of embryos and blastocysts in the cleavage stage and playing an important role in follicular development [14]. The addition of follicle-stimulating hormone to the culture medium for oocyte maturation can promote the cytoplasmic maturation of oocytes. Some scholars believe that the effect of follicle-stimulating hormone is related to its concentration. When the concentration is 5 g/mL, a relatively high cleavage rate (79.1%) and blastocyst rate (16.1%) can be obtained [15]. The addition of LH or human chorionic gonadotropin to the IVM culture medium may promote protein synthesis, enhance oocyte metabolism, and facilitate oocyte maturation. The concentration of estradiol (E2) in the human body increases with an increasing volume of follicles. In addition, estradiol is involved in maintaining the meiotic arrest of oocytes and can promote the cytoplasmic maturation of oocytes. During in vitro culture of oocytes, nuclear maturation is faster than cytoplasmic maturation. Therefore, the addition of E2 to the culture medium helps synchronize the development of the nucleus and cytoplasm in oocytes.

4.3 Effect of antioxidant addition on in vitro maturation of oocytes

As a hydrophobic activator, forskolin (FSK) can increase the activity of adenylate cyclase in mammalian cells and the level of intracellular cAMP. By adding FSK to an IVM culture medium, Ezoë et al. [16] significantly improved the developmental capacity of oocytes in the GV stage. By adding FSK to the culture medium, Zeng et al. [17] promoted the synchronization of nuclear and cytoplasmic maturation and increased the rates of maturation, cleavage, and high-quality embryos. During IVM, the presence of oxidative stress may block oocyte maturation, lead to abnormal gene expression, and impair the cytoplasmic and nuclear development of oocytes, thereby resulting in the failure to obtain high-quality oocytes and decreasing the fertility and developmental capacity. The addition of antioxidants to the culture medium can reduce the damage caused by oxidative stress. By adding α -lipoic acid to the culture medium, Zavareh et al. [18] reduced the content of active oxygen, increased the total antioxidation capacity, and promoted the nuclear and cytoplasmic maturation of oocytes in vitro. The results of Mokhber et al. [19, 20] showed that an appropriate concentration of a natural antioxidant, crocin (100 g/mL), and aqueous extract of saffron (40 g/mL) can increase the concentration of glutathione (GSH), protect oocytes, and significantly increase IVM rate and fertility rate.

4.4 Effect of co-culture with mural granulosa cells on in vitro maturation of oocytes

Together with cumulus cells and follicular fluid, MGC form an in vivo environment for oocyte maturation. Co-culture with MGC can increase the rate of nuclear and cytoplasmic maturation of immature oocytes. Studies have shown that co-culture with parietal MGC can improve the nuclear maturation of naked oocytes, slow down the nuclear maturation of naked oocytes, increase the content of glutathione in naked oocytes, reduce the activity of glucose-6-phosphate dehydrogenase in naked oocytes, increase the rate of cytoplasmic maturation, and facilitate the simultaneous development of the nucleus and cytoplasm of oocytes [21]. Although immature oocytes detached of MGC can still mature, they cannot undergo normal

fertilization and development because the cytoplasm is not synchronously matured [22]. The addition of a certain amount of MGC to the culture medium can delay the nuclear maturation of oocytes, so that the maturation of the nucleus and cytoplasm becomes more synchronized. However, there is currently no uniform standard for the amount of MGC addition. Choi et al. [23] significantly increased the development potential of embryos by co-culturing the oocyte-corona-cumulus complex with naked oocytes at a 1:5 ratio.

4.5 Effect of co-culture with oviductal epithelial cells on in vitro maturation of oocytes

Some scholars have pointed out that the maturation of oocytes is completed in the fallopian tube, and hence some components of the fallopian tube may affect the maturation process of oocytes. Human tubal fluid (HTF) has been used to culture oocytes. Shirazi et al. [24] co-cultured ovine oocytes with oviductal epithelial cells (OECs) and conducted IVF, resulting in higher cleavage and blastocyst rates.

4.6 Effect of co-culture with mesenchymal stem cells on in vitro maturation of oocytes

In addition to the potential of self-renewal and multidirectional differentiation, mesenchymal stem cells (MSCs) can also secrete a variety of cytokines and growth factors, and some biologically active factors can enhance the in vitro maturation of oocytes and subsequent developmental potential of embryos. By adding MSCs to a culture medium, Ling et al. [25] significantly increased the maturation rate and rate of blastocyst formation of immature murine oocytes. It can be seen that the co-culture system with MSCs can promote the simultaneous development of the nucleus and cytoplasm of murine oocytes.

5. Sources of immature oocytes

5.1 Oocyte retrieval from cesarean section or gynecological surgery

Immature oocytes retrieved from the ovarian cortex during cesarean section can be cultured in vitro to achieve maturation, fertilization, and healthy progeny. The mature oocytes cultured in this way are expected to be used as the source of oocytes to preserve female fertility [26]. At present, few studies have investigated the approach to obtain immature oocytes during cesarean section for in vitro culture, and hence more studies are needed to prove the safety and effectiveness of immature oocytes obtained from cesarean section.

In addition to cesarean section, immature oocytes can also be obtained via gynecological surgery in the follicular phase or luteal phase. The number of retrieved oocytes is mainly related to the age, pathological status, and stage of the menstrual cycle of the patient. Clinical studies have confirmed that oocyte retrieval carried out at different stages of the menstrual cycle does not affect the rate of in vitro maturation and the rate of fertilization of oocytes, suggesting that IVM technique can be used to preserve fertility in cancer patients during the follicular phase or luteal phase [27]. Therefore, for cancer patients who lack sufficient time for treatment and are unable to use hormone to induce ovulation, immature oocytes can be retrieved before chemotherapy to carry out IVM and vitrification to maximize the preservation of fertility.

5.2 PCOS patients

A large number of antral follicles are present in the ovary of infertile women with anovulatory PCOS. These antral follicles are more sensitive to gonadotropins, and hence the risk of OHSS is increased when hormones are used to induce ovulation. Therefore, for PCOS patients, immature oocytes can be retrieved from antral follicle for in vitro maturation [28]. The use of hCG at 36 h before oocyte retrieval in PCOS patients can promote the resumption of meiosis of immature oocytes and their in vitro maturation, improving the rate of pregnancy and clinical outcomes [29]. The use of small doses of gonadotropin before the retrieval of immature oocytes from PCOS patients is also beneficial to improve the maturation potential of oocytes, increasing the rate of embryo implantation and clinical pregnancy. In addition, IVM techniques can also be considered for some PCOS patients with no or low response to hormones [30].

5.3 Women with normal ovaries and menstrual cycles

Based on the advantages of low hormone dosage, low cost, and simple treatment process, IVM has been gradually applied to the treatment of infertile women with normal ovaries and regular menstrual cycles. However, it remains controversial as whether the use of hCG is required in the IVM treatment of this type of patient prior to oocyte retrieval. It should be noted that the hCG trigger exerts different effects on normal ovaries and PCOS patients. In the IVM treatment cycle of PCOS patients, dominant follicles are barely visible in the ovary, but MI-stage oocytes can be retrieved from small follicles after hCG-induced ovulation. However, after the hCG trigger is used in the normal ovary during the follicular phase, most oocytes retrieved from small follicles are oocytes in the GV stage. There is currently no evidence suggesting that the hCG trigger exerts a significant effect on the pregnancy rate, live birth rate, or abortion rate in the IVM of immature oocytes obtained from normal ovaries [31]. However, the accuracy of these findings is limited by the small number of samples. Therefore, a well-designed, randomized, and controlled clinical trial is needed to further confirm the optimal dosage and timing of hCG administration.

6. IVM culture system

6.1 Improvement of IVM media

The in vitro maturation of oocytes is mainly affected by culture conditions. At present, the common media used for the IVM of immature human oocytes include TCM-199 medium, Ham's F10 medium, and Chang's medium. In addition, serum, gonadotropin [follicle-stimulating hormone and luteinizing hormone], growth factors, and steroids can be added in a basal medium to produce a complex medium. At present, commercial IVM media have been widely used. However, no breakthrough has been made in the research on improving the quality of oocytes by improving the IVM medium. In recent years, research and application of antioxidants and growth-promoting factors have promoted the advancement of this technology to some extent. In addition, using cell cycle regulators or inhibitors of mitotic spindle formation, the synchronization of nuclear and cytoplasmic maturation of immature oocytes can be achieved by inhibiting GVBD, thereby increasing the blastocyst rate and live birth rate in animal models [32]. However, the safety and efficacy of this method in human oocytes should be further verified.

6.2 Optimization of IVM culture environment and process

The culture environment, equipment, and related operations in the IVM system may affect the in vitro maturation and embryo development of immature oocytes. Therefore, the optimization of the embryo culture environment and process of in vitro operation will help to maintain the potential of embryonic development [33, 34]. The three-dimensional culture system can support the development of follicles by using biological materials to maintain cell-to-cell information exchange. In addition, mature oocytes can be obtained by using a three-dimensional culture system in the in vitro culture of anterior follicles of nonhuman primates [35], although no reports are available regarding the use of a three-dimensional culture system in the in vitro culture of immature human oocytes. A past study has used microreactors to form three-dimensional bioreactors to support the growth of different types of cells [36]. Consisting of a drop of liquid encapsulated by hydrophobic powder particles, this system can provide a suitable microenvironment for in vitro maturation of oocytes. In addition, the development of microfluidic technology will exert an important impact in the field of human gametes and preimplantation embryo development and will have potential applications in the field of ART. This technology enables the creation of microfluidic models mimicking the “menstrual cycle of women” [37]. These models include interconnected 3D models of different tissues, such as 3D models of the ovaries, fallopian tubes, uterus, cervix, and vagina, in the female reproductive system and the endocrine cycle between various organ modules. The mechanical and biochemical properties of microfluidic systems still require intensive research before these systems can be applied to clinical applications in areas such as IVM of immature human oocytes.

7. Clinical application and safety of IVM

At present, the in vitro maturation rate of immature human oocytes can reach 70%, but the developmental potential of mature oocytes obtained in vitro is still lower than that of mature oocytes obtained in vivo. In addition, the rate of blastocyst development and the rate of implantation are relatively low after the fertilization of IVM oocytes. The main reason of such discrepancy may be related to non-synchronized nuclear and cytoplasmic maturation during IVM. With the further development in the basic and clinical research of IVM, the in-depth study on the mechanisms of oocyte maturation and mastery of key factors involved in oocyte maturation will contribute to the improvement and optimization of clinical IVM technology.

The results of current research showed that human oocytes matured in vivo or in vitro display no significant differences in terms of their spindle morphology, organelle distribution, cortical particle distribution, and mitochondrial morphology [38, 39]. By observing embryos dynamically using time-lapse videos, it was confirmed that oocytes matured in vivo or in vitro showed no significant differences in terms of the morphological dynamics observed during the early development of embryos derived from these oocytes [40]. Another study has also shown that the oocytes matured in vitro and in vivo are different in terms of their organelle function, distribution, and gene expression [41]. The different experimental conclusions mentioned above may be caused by different sources and quality of oocytes used in these studies. Therefore, attention should be paid to clarify the IVM efficiency of oocytes retrieved from different sources, so as to reasonably evaluate the safety of IVM. In terms of epigenetics, a study has reported that IVM exerts no significant effect on the methylation level of maternal imprinted genes, such as

LIT1, SNRPN, PEG3, and GTL2, in human oocytes [42]. After an imprinted gene examination was carried out for infant chorionic cells and cord blood obtained from IVM and a standard stimulation protocol, no significant difference was observed between the two methods [43, 44]. Currently, the follow-up of IVM-aided pregnancies shows that the IVM technique does not increase the risk of pregnancy, the rate of maternal complications, and the rate of neonatal abnormalities [45, 46]. However, due to a small sample size and the lack of in-depth study on epigenetics, the clinical application and safety of IVM still require investigations of large sample sizes to reach a definitive conclusion regarding the safety of IVM in terms of epigenetics.

At present, more than 5000 IVM babies have been born worldwide, and the rate of clinical pregnancy among PCOS patients undergoing IVM treatment can reach about 35–40% [47]. IVM has been extended from the basic research to the treatment of patients with PCOS, ovarian hyperresponsiveness and hyporesponsiveness, as well as cancer patients to preserve the fertility. Therefore, IVM has a prospect of broad applications.

8. Conclusion

At present, the application scope of IVM technology can be extended to patients with various causes of infertility. In addition, the IVM technology is associated with acceptable pregnancy and live birth rates. Although IVM has been used as an effective treatment and achieved significant outcomes with thousands of healthy IVM babies having been delivered, IVM is still considered as an experimental technique by the society. With the development of IVM technology, the combination of natural cycle IVF with the IVM of immature oocytes can be used as an attractive regimen to promote IVM treatment. More infertile women can benefit from such approaches if the treatment process is simplified by mild stimulation, especially when the difficulty to obtain immature oocytes is reduced. Therefore, the combination of mild stimulation IVF and IVM treatment can become a viable alternative to current standard treatments. With the accumulation of more experience and results, it will be further demonstrated that the combination of mild stimulation IVF and IVM is not only a viable alternative to current standard treatments but may also become a potential option of first-line treatment.

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