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# *In vitro* maturation: Clinical applications

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Oocyte *in vitro* maturation (IVM) is an assisted reproductive technology in which oocytes are retrieved from the antral follicles of unstimulated or minimally stimulated ovaries. IVM of human oocytes has emerged as a promising procedure. This new technology has advantages over controlled ovarian stimulation such as reduction of costs, simplicity, and elimination of ovarian hyperstimulation syndrome. By elimination or reduction of gonadotropin stimulation, IVM offers eligible infertile couples a safe and convenient form of treatment, and IVM outcomes are currently comparable in safety and efficacy to those of conventional *in vitro* fertilization. IVM has been applied mainly in patients with polycystic ovary syndrome or ultrasound-only polycystic ovaries, but with time, the indications for IVM have expanded to other uncommon situations such as fertility preservation, as well as to normal responders. In this review, the current clinical experiences with IVM will be described.

**Keywords:** *In vitro* maturation; Ovarian hyperstimulation syndrome; Infertility

## Introduction

Since its introduction in the 1990s, *in vitro* maturation (IVM) has emerged as an attractive infertility treatment.

Early experience with IVM yielded limited success, but advances in IVM protocols and improvements in maturation methods as well as culture media have led to satisfactory pregnancy rates in appropriately selected patient groups [1,2].

IVM has the potential to substitute for, or at least be an adjuvant to, standard *in vitro* fertilization (IVF) protocols for a number of reasons. Requiring no or very little gonadotropin supplementation *in vivo*, IVM has been proposed as an alternative assisted reproduction technology (ART) approach to reduce important drawbacks of controlled ovarian stimulation (COS), such as the cost and inconvenience of injectable gonadotropin therapy.

For patients undergoing IVM, the risk of ovarian hyperstimulation syndrome (OHSS) is virtually eliminated.

In 1935, Pincus and Enzmann reported that immature rabbit oocytes removed from their natural ovarian environment were capable of undergoing spontaneous maturation and fertilization *in vitro*.

Initial work with IVM in human beings was reported in the 1960s by Edwards [3].

Cha et al. [4] reported the first baby using immature oocytes collected from nonstimulated cycles in a donor oocyte program, while Trounson et al. [1] reported the first successful pregnancy and birth from untreated polycystic patients using the mother's own oocytes in 1994.

The first attempts at using IVM oocytes collected from nonstimulated ovaries dates back to the beginning of the 1990s, but a systematic development of IVM occurred only in the second half of that decade.

IVM is especially useful for women with polycystic ovaries (PCO) who are at risk of developing ovarian hyperstimulation syndrome (OHSS). However, with time, the indications for IVM have expanded remarkably and include normal responders, poor responders, and fertility preservation.

The present review describes clinical experiences with IVM and some uncommon indications of IVM based on clinical reports.

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## Clinical aspects of IVM

### 1. Patient selection

The most appropriate candidates for IVM are PCO/PCOS patients. By not using COS, the risk of OHSS is diminished or even eliminated [5]. IVM also can be applied in normo-ovulatory women. Along with the decreased risk of OHSS, reduced cost, reduced drug-related side effects, and decreased psychological stress are advantages of IVM. Antral follicle count (AFC) is important in patient selection for IVM in normo-ovulatory patients. If the number of follicles available for recovery is too low, the cycle outcome would be unsatisfactory. At minimum, an AFC more than 5 is required. Suggested parameters for selection of women candidates for IVM treatment are as follows [6]: age $\leq$36 years, body mass index $<$30 kg/m$^2$, FSH $<$10 mIU/mL, estradiol $<$250 pmol/mL, AFC $>$5, and antimüllerian hormone (AMH) $>$1.3 ng/mL.

### 2. Methods

A baseline ultrasound scan is performed between days 2 and 5 of the menstrual cycle. It is possible to do this in an induced menstrual cycle if the patient is amenorrheic. The AFC is important for making a decision on whether to do IVM or not. An AFC of 7 or more is required for a comparable success rate. If eligible for IVM, a second scan is performed on days 7 to 9. An ultrasound scan is performed every 2 to 3 days thereafter. Priming with human chorionic gonadotropin (hCG) improves the maturation rate of oocytes *in vitro* [7]. 10,000 IU of hCG is administered when the endometrial thickness has reached $\geq$6 mm. It is recommended that oocyte retrieval be conducted before a dominant follicle is apparent. A previous study showed detrimental effects of the presence of a dominant follicle $>$14 mm on the day of oocyte retrieval [8]. Others have suggested the oocyte retrieval timing is appropriate when the follicular size is 8 to 12 mm [7].

One of the major advantages of IVM over standard IVF is that IVM does not require ovarian stimulation. However, several studies have found that FSH priming (mild ovarian stimulation) improves the oocyte maturation rate and endometrial development in PCOS patients, while no benefit has been observed in normo-ovulatory women. Until more comparative studies are available, the concept of follicular priming of IVM has been a topic of debate.

Oocyte retrieval is performed 36 hours after hCG administration with intravenous sedation or local anesthesia. Because the size of the follicles is smaller, it is recommended that the depth of the transvaginal ultrasound be adjusted to produce images 2 to 3 times larger than conventional IVF. Retrieval is conducted under ultrasound guidance with a 19-G, single-lumen aspiration needle with aspiration pressure less than 100 mm Hg [9]. Without COS, the ovarian surface is harder than in conventional IVF. Puncture with a wrist snap is necessary to enter the ovaries in IVM. Multiple curettage of the follicle wall is recommended due to the tightly packed cumulus oocyte complex around the immature oocytes.

Immature oocytes are cultured for a day for IVM and fertilized after maturation. Because it takes a day for maturation of oocytes, day 3 embryos are transferred 4 days after oocyte retrieval.

The ability to retrieve immature oocytes successfully and the capacity to identify and handle immature oocytes in the laboratory, as with any technical procedure, involve a learning curve.

Adequate endometrial development is essential in any ART procedure, but even more so in IVM because a dominant follicle or a corpus luteum is not routinely formed, thus possibly compromising both the follicular and luteal sex steroid contribution to the development of the endometrium.

For endometrial preparation, estradiol valerate (6 mg, Progynova, Schering, Seoul, Korea) is administered daily starting on the day of oocyte retrieval. Luteal phase support with daily muscular injection of progesterone in oil is started on the next day of oocyte retrieval. An additional injection of 10,000 IU of hCG is administered on the same day for luteal phase support. Estradiol and progesterone supplementation is continued until the 10th week of pregnancy.

### 3. Outcomes

In a previous study, the clinical pregnancy rate and implantation rate were comparable in IVM and conventional IVF cycles [9]. However, the miscarriage rate was significantly higher in the IVM group. In another retrospective study, the miscarriage rate was higher in the IVM group than the IVF group [10]. However, they suggest that the difference between the IVM and IVF groups is related to polycystic ovary syndrome rather than to the IVM procedure. Others have shown comparable perinatal outcomes, including major and minor malformations [11,12].

In IVM cycles, implantation and pregnancy rates are lower compared with traditional IVF cycles, but accurate patient selection can improve IVM clinical outcomes.

To date, it is estimated that approximately 400 children have been born from IVM. Several studies have reported information about obstetric outcomes, perinatal outcomes, and incidence of congenital malformation of children born after IVM. It will require many more years of IVM data collection for a meaningful analysis; however, the data on the safety of IVM appears to be reassuring.

Our unit has been performing IVM treatment cycles for patients with polycystic ovary syndrome (PCOS) since 2001. Based on our data, implantation rates ranged from 10% to 15% and clinical pregnancy rates ranged from 35% to 40%.



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## Natural cycle IVF/M

Several approaches have been taken to overcome the complications of controlled ovarian hyperstimulation in IVF. First, unstimulated natural cycle IVF has been used for its convenience and reduced side effects. It was reported that after four cycles, the cumulative pregnancy rate was 46% and live birth rate was 32% [13]. However, natural cycle IVF carries risks of premature LH surge, frequently resulting in failure of oocyte collection and fertilization. Another alternative to the controlled ovarian hyperstimulation IVF cycle is IVM, as explained in the previous section, although its candidates have been limited mainly to PCOS patients with an antral follicle count over 7. It was traditionally believed that the dominant follicle would cause the development of the small follicles to deteriorate and induce them to fall into programmed cell death. However, Chian et al. [14,15] suggested that the maturational and developmental competence of immature oocytes were not harmed by the presence of a dominant follicle or phase of folliculogenesis. Therefore, IVM combined with natural cycle IVF, also known as natural cycle IVF/M was developed to greatly augment the success rate of natural IVF through immature oocyte retrieval and maturation of small follicles [16]. In addition, natural cycle IVF/M plays a vital role in the expansion of the candidates for IVM from PCOS patients to ovulatory non-PCOS patients and in avoiding cancellation of cycles if a dominant follicle develops [15].

### 1. Methods

The baseline ultrasound scan is performed on day 3 of the menstrual cycle to ensure that more than seven small antral follicles were present in both ovaries. Transvaginal ultrasound scans are repeated from day 7-9 at 1-3 day intervals until the leading follicle has reached 12-14 mm in diameter and the endometrial thickness is over 6 mm, an optimal time for patients to receive HCG administration, as mature oocytes can be retrieved from follicles as small as 11.5 mm in diameter and the risk of premature ovulation increases when the size of leading follicles reaches >16 mm in diameter [16]. The following steps are performed in the same manner as the IVM cycle [9].

### 2. Outcome

Natural cycle IVF/M was successful in more than 50% of women aged <35 years, achieving a 40.4% of pregnancy rate and 17.8% implantation rate, a result similar to IVM alone and controlled hyperstimulation cycles [9]. It was also reported that the live birth rate was higher when the mature oocytes were retrieved at egg retrieval although the clinical pregnancy rates and implantation rates were similar [17].

Our unit has been performing natural cycle IVF/M treatment cycles for normal responders since 2005. Based on our data, the clinical pregnancy rate was about 50%.

## IVM as an alternative for over-responders

Some women are extremely sensitive to stimulation with exogenous gonadotropins and are at increased risk of developing OHSS; sometimes, it is a potentially life-threatening complication [18]. Several preventive methods have been proposed to reduce the incidence and severity of OHSS, including cancellation of the treatment cycle, cryopreservation of all embryos, or intravenous administration of albumin or other plasma-expanding agents [19-21]. However, these methods are not efficient in preventing OHSS. Another popular strategy is withholding gonadotropin stimulation, the 'coasting' method. The advantage of coasting is that the treatment cycle is not necessarily cancelled and that no additional procedure is needed [22]. However, coasting cannot be applied when the signs and symptoms predictive of OHSS are observed early in the stimulation phase of the cycle, because premature withholding of gonadotropin may result in the arrest of follicular growth and atresia of oocytes. In addition, with coasting, frequent estimations of the serum estradiol level and ultrasound scans are needed in order to determine the time of hCG administration. Furthermore, the crucial timing of hCG administration has not been well defined.

Recently, it has been reported that mature oocytes were collected from infertile women with PCOS when the leading follicle reached a mean diameter of 12-14 mm following administration of hCG. This finding suggests that limited ovarian stimulation can result in retrieval of mature oocytes and may prevent the recurrence of severe forms of OHSS without reducing the clinical pregnancy rate from that of conventional IVF treatment [23,24].

Lim et al. [25] showed the benefit of interruption of controlled ovarian hyperstimulation (COH) for IVF because of the risk of OHSS. A woman is considered an 'over-responder' when there are more than 20 follicles with a mean diameter >10 mm in both ovaries following gonadotropin stimulation for at least 5 days. When the leading follicle reached 12 to 14 mm in diameter, 10,000 IU of hCG was administered, and oocyte collection was performed 36 hours later. The investigators reported retrieving 628 immature oocytes, of which 76% matured *in vitro*, 82% of those fertilized, and 94% of embryos cleaved, resulting in an overall pregnancy rate of 46% per transfer [25].

Mature and immature oocyte retrieval followed by IVM is an efficient method for the prevention of OHSS during ovarian stimulation without compromising the pregnancy outcome for IVF treatment cycles in women with PCOS. The important criteria for this alternative is to stop gonadotropin stimulation when there are ultrasonographic signs of OHSS risk, where there are more than 20 growing follicles with a mean diameter >10 mm, and to administer hCG when the leading follicles reach 12-14 mm in diameter.

This study illustrates the potential role of IVM as an alternative to

cancellation of IVF in high responders.

## IVM of oocytes: uncommon indications

### 1. Ovarian resistance to FSH

Ovarian resistance to FSH is a rare condition characterized by hypergonadotrophic hypogonadism [26]. These patients are misdiagnosed with premature ovarian failure (POF), but they have a normal antral follicle count and normal anti-Müllerian hormone level. Many patients with the ovarian resistance syndrome have a defective response both to endogenous and exogenous FSH [27].

Grynberg et al. [28] recently reported on two pregnancies achieved using IVM in two women whose ovaries were resistant to FSH. The success of IVM proved that oocytes in this clinical situation are healthy, and IVM may be an alternative to egg donation for women suffering from gonadotropin resistance.

### 2. Fertility preservation

Over the last few decades, the incidence of cancer in females has increased even as mortality rates have declined due to progress in cancer treatments. Advances in chemotherapy and radiation therapy have significantly improved cure rates for many teenage girls and young women. As a consequence, the number of long-term survivors is increasing and their future quality of life has become a major concern. However, chemotherapy and radiation therapy with a field that includes the pelvis have an adverse effect on ovarian reserve, which may lead to premature ovarian failure (POF) and infertility [29]. A number of options have been developed to enable these women to preserve their fertility, such as surgical interventions and cryopreservation cells/tissues. Cryopreservation of oocytes and/or embryos after COH is the most established fertility preservation method. However, gonadotropin administration is contraindicated in estrogen-sensitive diseases. IVM of oocytes has been proposed as a promising fertility preservation option for these women [30].

Huang et al. [31] were the first to suggest the application of IVM as a fertility preservation method in Patients exposed to a risk of premature ovarian failure. These investigators have reported that for oocyte vitrification, the average number of oocytes retrieved was 11.4, the IVM rate was 64.2%, and an average of 7.9 mature oocytes were vitrified per patient.

These data seem to suggest that IVM may be a possible option for fertility preservation in women suffering from cancer.

## Conclusion

Recent published studies show that IVM technology is emerging in the clinical field of ART, with improving success rates. Although IVM may be not ready to offer to all types of infertility patients, IVM is clearly a safer alternative than IVF in patients sensitive to gonadotropins. In addition, IVM in a natural cycle can be used as an option with an acceptable pregnancy rate in a selected group of patients.

IVM may be the procedure of choice not only for infertile couples but also for obtaining oocytes for preservation of fertility. IVM is a good alternative in fertilization preservation in cancer patients. IVM can be used safely without concern for the high estradiol level in fertility preservation of hormone sensitive cancer patients. Because there is no delay for COS, IVM could be the method of choice when cancer therapy should be started as soon as possible.

There are many concerns about IVM infants with regard to obstetric and perinatal outcomes as well as long-term development. At the present, the clinical safety of IVM cannot be completely assessed, but several studies have reported that infants conceived after IVM were not associated with increased adverse outcomes compared with children conceived *in vivo*.

## Conflict of interest

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

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