In vitro maturation: a committee opinion

The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology

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The initial results of in vitro maturation suggest the potential for clinical application. However, at this time in vitro maturation should be performed only as an experimental procedure evaluating both efficacy and safety in carefully selected patients. (Fertil Steril® 2013;99:663–6. ©2013 by American Society for Reproductive Medicine.)
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In vitro fertilization usually involves ovarian stimulation with exogenous gonadotropins to maintain the development of gonadotropin-sensitive follicles and inhibit the atresia of the non-dominant follicles (1, 2). The term in vitro maturation (IVM) refers to the maturation in culture of immature oocytes after their recovery from follicles that may or may not have been exposed to exogenous follicle-stimulating hormone (FSH) but were not exposed to either exogenous luteinizing hormone (LH) or human chorionic gonadotropin (hCG) prior to retrieval to induce meiotic resumption. During IVM, such immature oocytes progress from prophase I stage (i.e., from the germinal vesicle [GV]) through meiosis I to reach metaphase II (MII). The use of exogenous LH or hCG prior to retrieval ("follicular priming") has been introduced into clinical applications in order to increase the likelihood of obtaining usable oocytes (3–5). While this practice is not IVM in the strictest sense, outcomes with these methods will be included in this document.

The human oocyte reaches its full size (approximately 100 μm to 120 μm) at the antral stage, during which time the follicular diameter is only a fraction of its final ovulatory diameter. The ability of an oocyte to resume and complete meiosis is linked closely to follicular diameter (6). In humans, the kinetics of oocyte maturation have been established with oocytes obtained from oophorectomy specimens from nonmalignant gynecological disorders and then cultured to undergo IVM (Fig. 1) (3, 4, 7, 8).

It should be noted that even if an immature oocyte progresses to the MII stage, complete oocyte competence is not necessarily obtained. In many cases, oocyte maturation in vitro is accomplished morphologically but without acquiring developmental competence following in vitro culture. In order for a mature oocyte to undergo successful fertilization and subsequent development, synchronization of nuclear and cytoplasmic maturation must occur. Nuclear maturation consists of germinal vesicle breakdown induced by the LH surge followed by resumption of

meiosis and extrusion of the first polar body (MII). Cytoplasmic maturation is more difficult to assess microscopically but refers to an accumulation of factors that prepare the cytoplasm for fertilization and embryonic development (9). Epigenetic processes are a component of nuclear and cytoplasmic oocyte maturation, influencing development after fertilization (10, 11). The potential for abnormal methylation of maternally expressed genes needs careful evaluation in oocytes matured in vitro.

POTENTIAL APPLICATIONS OF IVM

Potential indications for IVM include patients at risk for ovarian hyperstimulation syndrome (OHSS), those with limited time for ovarian stimulation, and those with a contraindication to sustained elevations of estradiol (E2).

Since IVM either omits gonadotropin stimulation or uses a short course of gonadotropins, there is a reduction in the number of days of follicular monitoring, utilization of gonadotropins, and the risk of OHSS. Women with polycystic ovary syndrome (PCOS) or PCO-like ovaries may benefit from IVM. These women have the highest number of antral follicles for potential retrieval and are at highest risk of developing OHSS under traditional stimulation protocols.
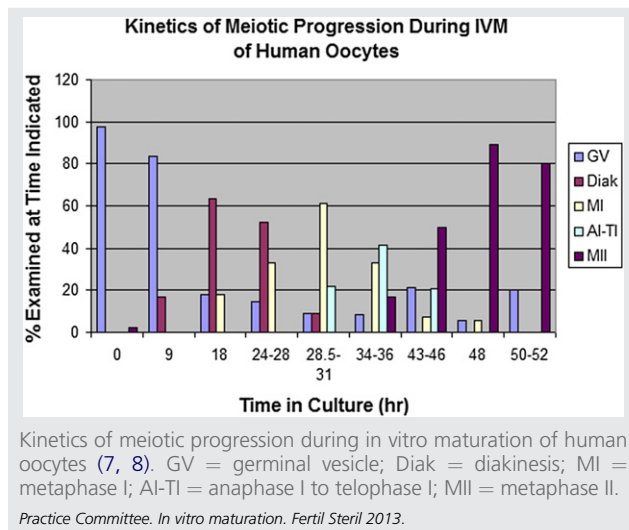
FIGURE 1

Kinetics of meiotic progression during in vitro maturation of human oocytes (7, 8). GV = germinal vesicle; Diak = diakinesis; MI = metaphase I; AI-TI = anaphase I to telophase I; MII = metaphase II.

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IVM can be used in patients who need to initiate gonado-toxic therapies without adequate time to undergo ovarian stimulation with retrieval of oocytes matured in vivo. Patients with hormonally sensitive tumors or other contraindications to sustained elevations of E2 also may consider using IVM in an effort to avoid the increased E2 concentrations achieved with ovarian stimulation.

The applicability of these indications may be limited by alternative strategies to minimize OHSS risk and, in the case of those with limited time, the delay incurred by coordinating treatment with the onset of the menstrual cycle, even in the absence of ovarian stimulation.

IVM IN CLINICAL PRACTICE
Patient Preparation

No randomized, controlled trials currently exist comparing IVM with IVF; however, there have been a number of clinical studies using in vitro matured human oocytes in PCO-like and PCOS patients (12–20) and in ovulatory women (15, 20–24). Several trials also have evaluated the utility of follicular priming versus the retrieval of oocytes from unstimulated antral follicles (12, 18–21, 25).

Three methods of follicular priming have been reported. One method is to prime oocytes with FSH for 3-6 days, followed by retrieval on cycle day 9-10; this is the strictest definition of IVM. Other approaches utilize hCG priming (and include single injections of 10,000 IU of hCG to "prime" intermediate-sized follicles 36 hours prior to oocyte retrieval) and the use of FSH stimulation in addition to hCG. Priming with hCG or FSH gives mixed results; however, priming with either agent appears to benefit PCOS patients in terms of implantation and pregnancy rates (14, 17, 19) over no priming at all (Table 1). The combination of gonadotropin and hCG priming compared to single-agent follicular priming has been studied with disparate results. While no difference was seen in one trial (18), a large randomized trial showed that the combination of FSH and hCG improved the percentage of oocytes that matured in vitro as well as implantation rates when compared to no priming or hCG alone. The clinical pregnancy rates were highest in the combination group (26%) compared to 11%, 5%, and 13% in the not primed, hCG-primed, or FSH-primed groups, respectively. The implantation rate of 16.4% was also significantly higher than in the no priming (9.2%) or the hCG-primed (4.0%) but not the FSH-primed group (10.6%) (25).

Interpretation of these studies is impaired by inconsistent reporting of the developmental stage of the oocytes at retrieval and the status of the cumulus at assessment and fertilization.

Oocyte Retrieval and Culture

There are no established criteria to identify the ideal timing or method for oocyte retrieval, with most studies using a lead-follicular diameter of up to 10 mm (17, 24, 26). Lead-follicle diameters greater than 13 mm have been associated with reduced numbers of oocytes collected and matured (27), possibly related to subsequent atresia of the non-dominant follicles from withdrawal of endogenous FSH support. Some protocols require an endometrial thickness of >5 mm while others do not include endometrial thickness as a criterion.

The aspiration technique also differs for immature oocytes compared to traditional IVF. Double lumen needles to flush follicles have been described but do not appear to be necessary to retrieve immature oocytes. The optimal aspiration pressure and needle design have yet to be determined with negative pressures ranging between 80 mm Hg and 300 mm Hg and needles ranging from 16 to 20 gauge described (24, 28). Extremely high aspiration pressure has been shown to negatively impact development (29).

There is no consensus on which formulation is best suited for the purpose of in vitro oocyte culture. Media are frequently supplemented with pyruvic acid along with essential and non-essential amino acids, such as tissue culture media (TCM).

Fertilization

Because of potential changes in the characteristics of the oocytes and zona pellucidae from culture of immature oocytes, intracytoplasmic sperm injection (ICSI) has been advocated as the preferred method for fertilization. While fertilization rates appear to be increased utilizing ICSI for IVM oocytes, developmental competence may be impaired as demonstrated in one comparative trial (20). Fertilization rates of matured oocytes in patients who did not receive gonadotropins were only 37.7% (229/608 matured oocytes) with conventional IVF compared to 69.3% (318/459 mature oocytes) when ICSI was used as the insemination technique. Despite lower fertilization results, the implantation rate was significantly higher in embryos derived from oocytes fertilized with conventional IVF compared to ICSI (24.2% vs. 14.8%; $P < .05$) as well as clinical pregnancy rates per embryo transfer procedure (34.5% vs. 20.0%; $P < .05$). Comparative trials comparing IVF to ICSI for fertilization of in vitro matured oocytes need to be conducted.

TABLE 1

In vitro maturation in PCOS and PCO-like patients.

Reference	N	Priming	Mean no. oocytes	Mean no. ET	PR/ET, %	IR, %	MC, %
13	203	None	15.5	5.0	21.9	5.5	37
14	13	hCG	7.8	2.8	38.5	16.6	40
	11	None	7.4	2.5	27.3	14.8	0
16	107	hCG	10.3	2.5	21.5	9.5	26
19	12	None	6.8	1.7	0	0	0
	24	FSH	6.5	1.8	33.0	21.6	63
17	45	hCG	11.4	2.5	22.5	10.9	40
18	35	FSH/hCG	21.9	3.8	31.4	9.7	13
	33	hCG	23.1	3.8	36.4	11.3	?

Note: ET = embryos transferred; PR/ET = pregnancies per embryo transfer procedure; IR = implantation rate (sacs/embryos transferred); MC = miscarriage rate.

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Clinical Outcome

Two large series have been reported (30). In the first, a total of 3,079 oocytes collected from PCOS patients following hCG priming, 78.8% of immature oocytes reached maturity and 69.2% successfully fertilized with ICSI. In the second, 73.2% of 6,860 oocytes matured in vitro following hCG priming with a fertilization rate of 79.0%. Although clinical pregnancy rates in these and other IVM series approach that of traditional IVF (23% to 34%), this is secondary to the increased number of embryos transferred (Table 1). As a consequence, implantation rates are a more reliable indicator of IVM efficiency and range between 5.5% and 21.6%, lower than expected for women of comparable age undergoing conventional IVF (Table 1). In the Society for Assisted Reproductive Technology (SART) report from cycles performed in 2010 in young women (<35 years of age), implantation rates of 36.9% and a mean number of embryos transferred of 2.0 yields a live birth rate per started cycle of 41.7% (31).

The developmental outcome of children conceived with IVM has been studied in small numbers and thus far are reported to be no different than children born through traditional IVF or ICSI (32, 33). However, the small number of children conceived through IVM limits the accuracy of malformation and anomaly rates, and developmental outcomes cannot be assessed accurately.

SUMMARY

- Although there are no RCT comparisons, available data indicate that IVM as described in this document results in lower-than-expected success rates.
- In many cases, nuclear maturation in vitro is evident; however, there is a failure to acquire developmental competence following in vitro culture secondary to cytoplasmic competence.
- The implantation and pregnancy rates of IVM are less than expected with ovarian stimulation and retrieval of in vivo matured oocytes.
- Because only a small number of children have been conceived with IVM, information on the safety of IVM with regard to malformation and developmental outcomes cannot be assessed accurately.

- Priming with hCG and/or FSH appears to improve implantation and pregnancy rates compared to no priming.

CONCLUSIONS

- Candidates for this technology include those at risk for OHSS, women with PCOS or PCO-like ovaries, women with estrogen-sensitive cancers, or those with limited time prior to initiating potentially gonadotoxic treatments for fertility preservation.
- ICSI does not appear to be necessary to achieve fertilization in oocytes matured in vitro.
- The initial results of in vitro maturation suggest the potential for clinical application. However, at this time, patients must be made aware that the implantation and pregnancy rates are significantly lower than with standard IVF, limiting more universal utilization.
- In vitro maturation should only be performed as an experimental procedure (34) in specialized centers for carefully selected patients evaluating both efficacy and safety. Informed consent must include information regarding pregnancy rates of IVM in comparison to conventional ART and alternative options, if any.

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