The in vitro maturation (IVM) of human oocytes for in vitro fertilization (IVF): is it time yet to switch to IVM-IVF?

Gary N. Piquette, Ph.D., H.C.L.D.

Northridge Center For Reproductive Medicine, Northridge, California

The recent study by Li et al. observed that human oocytes from patients with polycystic ovary syndrome (PCOS) matured in vitro exhibited a higher proportion of abnormal spindle structures and disturbed chromosomal configurations compared with in vivo–matured oocytes from a control group of PCOS patients. This article discusses the obstacles that must be overcome and factors that must be monitored when attempting to optimize conditions for the in vitro maturation of human oocytes, with particular attention to the strengths and weaknesses of the study by Li et al. (Fertil Steril® 2006;85:833–5. ©2006 by American Society for Reproductive Medicine.)

Since the birth of the first baby from IVF, there have been improvements in the pregnancy and birth rates with IVF. Improvements in birth rates with IVF have been directly attributed to advances in hormonal stimulation of patients with various controlled ovarian hyperstimulation (COH) protocols and improved culture media and culture systems for oocytes, sperm, and embryos. However, through all these improvements with stimulated cycles, there has been continued development with natural, unstimulated, or limited-stimulation cycles followed by in vitro maturation (IVM) of oocytes. Any protocol that would decrease the amount and duration of hormonal stimulation before oocyte retrieval would have an advantage over the more common COH/IVF protocols if resulting pregnancy rates were the same or improved.

In 1991, Cha et al. (1) reported a pregnancy from IVF with oocytes obtained from ovariectomy specimens and matured in culture. The idea that immature human oocytes extracted from the ovary could be coaxed into maturing from the germinal vesicle stage to the metaphase II (MII) stage in vitro and then fertilized and further result in a pregnancy led to further efforts to develop IVM techniques. The goal was then to acquire oocytes from the ovary before an LH surge or an hCG injection and to continue the development of the oocytes in vitro to produce oocytes ready for IVF. In 1994, Trounson et al. (2) reported the birth of a normal baby with IVM of immature oocytes from a polycystic ovary syndrome (PCOS) patient undergoing IVF who had not been triggered to ovulate.

Although there have been more than 300 births of babies with IVM procedures, including in patients with PCOS (3), IVM has not become mainstream in IVF, with ovulation induction cycles with oocyte retrieval of mature (MII) oocytes still the highly favored protocol. Some clinics are reporting no differences in pregnancy rates between IVM and in vivo–matured oocytes (4). However, in most clinics, the pregnancy and live birth rates with IVM do not match those reported for IVF cases using full hormonal protocols with triggered maturation in vivo. Therefore, only specific patients are currently considered for IVM, most notably PCOS patients who might be more sensitive to the elevated levels of gonadotropins and steroids experienced during an IVF protocol (2–4). Patients at risk for ovarian hyperstimulation syndrome (OHSS) might also benefit from IVM to avoid elevated levels of gonadotropins and estrogen that might trigger or worsen OHSS (5). To avoid complications from the hormones used in COH and considering the need for expediency, IVM might become the method of choice for patients diagnosed with cancer who want to undergo oocyte retrieval with the purpose of cryopreserving their oocytes. Delaying treatment for cancer in deference to preparing the ovaries for aspirating mature oocytes becomes an excruciating decision for the patient, and offering IVM could alleviate some of those concerns. Emerging technologies in oocyte banking and oocyte donation might also benefit from abbreviated COH protocols and IVM.

One hurdle that must be overcome before IVM becomes a mainstream procedure is the technical aspects of aspirating and handling immature oocytes. Compared with in vivo–matured oocytes, aspiration of immature oocytes from the

Received August 24, 2005; revised and accepted August 24, 2005.
Reprint requests: Gary N. Piquette, Ph.D., H.C.L.D., Northridge Center For Reproductive Medicine, 18546 Roscoe Boulevard, Suite 240, Northridge, California 91324 (FAX: 818-701-8100; E-mail: gpiquette@aol.com).

0015-0282/06/$32.00
doi:10.1016/j.fertnstert.2005.08.069

ovaries is more technically demanding, requiring adjustments in the aspiration needles, the pressures used, and the skills and patience required to navigate an ovary with small follicles. Furthermore, there is a possibility that patients undergoing IVF with IVM might have lower pregnancy rates that could be attributed to alterations in the uterus, specifically a diminished endometrial thickness that might impair implantation (6). Such technical and endocrine concerns can be addressed. More daunting is how the oocytes will be handled and prepared until they are ready to be fertilized.

In vitro maturation of oocytes is limited by the culture systems currently used, including the doses and duration of hormones and other factors that are added to the culture media that are needed to initiate and coordinate the events of oocyte maturation. Determining the optimal composition of the culture media, including the proper doses of hormones and an energy source, such as pyruvate, could be critical to the rate of oocyte maturation (7). While the oocytes are still in the ovary, the basic fundamental physiological system, the ovarian follicle, is still intact, along with the elaborate paracrine and endocrine interactions required for efficient oocyte maturation. Removing oocytes before exposure to hCG or an LH surge removes the elements of the granulosa and thecal cells and any influence they might have on oocyte maturation. To mimic the ovarian follicle system in vitro has been the challenge for making IVM successful. Culture systems, including the media composition, will need to be improved and optimized before IVM can become the method of choice for IVF. Until the culture systems for IVM are improved, it is expected that there might be differences between in vivo– and in vitro–matured oocytes.

The current study reported in this issue of *Fertility and Sterility* by Li et al. (8) investigates the spindles and chromosome arrangements that are critical in oocyte maturation in vitro and in vivo. Alterations in the oocyte internal structure, in particular spindles and chromosomes, have critical importance in the ability of an oocyte to be fertilized, develop into a normal embryo, and ultimately produce a healthy live birth. The movement of chromosomes within the oocyte is controlled by spindles, specialized components of the oocyte infrastructure that are composed of microtubules. This internal infrastructure is a type of scaffolding or superhighway, moving materials and structures within the oocyte. This constantly changing structure is influenced by its environment, including the hormonal milieu, osmolarity, temperature, pH, and salt concentrations (9–13). If the spindle is disrupted, then the movement of chromosomes during meiosis could also be affected and possibly lead to polyploidy and aneuploidy (14).

Li et al. (8) used confocal microscopy and fluorescent immunocytologic staining to analyze the appearance of spindles and chromosomes in IVM oocytes acquired from PCOS patients and compared their results with in vivo–matured oocytes from another group of PCOS patients. They reported that IVM oocytes were more likely to have abnormal chromosome configurations and disorganized meiotic spindle microtubules.

One of the strengths of this study by Li et al. (8) was that the two study groups compared were PCOS patients who were willing to donate some of their oocytes for this study, a situation that many patients might not agree to. However, the investigators do not mention what method was used to select the specific "donated" oocytes in each group—whether there was a random assignment from the total pool of oocytes collected from a patient or whether there was a selection of oocytes after each cumulus–oocyte complex was observed. It can only be assumed that appropriate unbiased, randomized methods of selection of oocytes from patients in each study group were followed.

There can also be technical difficulties with spindle arrangements and the possibility of introducing artifacts when handling oocytes at any time, even during processing for fixation. Profound alterations in meiotic spindles of human mature (MII) oocytes can be induced with even a brief 2- to 3-minute exposure to 0°C (10) or a cooling from 37°C to room temperature for 10 minutes (11). Such temperature-sensitive problems can be avoided by incubating and processing all the oocytes, including the fixation steps, continuously at 37°C, as Li et al. (8) have described. With the availability of polarization light microscopy, or PolScope™ (Cambridge Research and Instrumentation, Inc. [CRI], Woburn, MA) (13, 14), spindles and microtubules can be observed in living oocytes in real time with careful monitoring of temperature with a heated stage, without the disadvantages of processing and fixation that might introduce artifacts. Another difficulty with processing is the mechanical and enzymatic removal of cumulus cells from the oocyte with repeated pipetting through a small-bore pipette and hyaluronidase. It might be assumed that in vitro– and in vivo–matured oocytes were handled in similar ways when cumulus cells were stripped. However, it is possible that cumulus cells might be more resistant to removal in immature oocytes matured in vitro and thus require more handling than in vivo–matured oocytes, which usually have greatly expanded cumulus cells requiring little effort to remove. Cumulus cells have cellular processes studded with gap junctions that traverse the zona pellucida and enter the oocyte. Therefore, if removal of the cumulus cells is relatively difficult with extended exposure to the stresses of handling, it is possible that the oocyte internal structure could be compromised and consequently affect the integrity of microtubules and spindles within the oocyte. Furthermore, porcine oocytes collected from cumulus oocyte complexes rich in cumulus cells exhibit longer spindle lengths than those from complexes poor in cumulus cells, suggesting the influence of cumulus cells on oocyte spindle length (10).

Another drawback of this study is that because these are PCOS patients, it is difficult to extrapolate the results to patients with normal endocrine profiles. It might be possible that, because of the hormonal milieu they are exposed to before aspiration from the ovary, oocytes from PCOS pa-

tients that are matured in vitro might have a propensity for problems with chromosomes or microtubules compared with oocytes allowed to mature in vivo before aspiration. This study does not address what the effects of chronic hyperandrogenemia or insulin resistance with compensatory hyperinsulinemia associated with PCOS might have had on sensitizing the immature oocytes and influencing spindle arrangements after maturation in vitro. Until this study is repeated comparing oocytes from patients with normal hormone profiles, the differences noted in this study cannot be completely attributed to the effects of IVM.

In studies with mice, Moon et al. (15) reported that IVM oocytes had significantly lower rates of fertilization and blastocyst formation compared with in vivo–matured oocytes. The investigators noted that these rates might be associated with the significant differences in spindle arrangements they observed between the two types of oocytes. Porcine oocytes matured in vitro were also observed to have shortened spindles compared with in vivo–matured oocytes (10). However, in all these IVM studies with different species, one cannot presume that IVM culture conditions have been optimized in each case. Although nuclear maturation might be determined, with extrusion of polar bodies, there remains the problem of determining proper cytoplasmic maturation. Indeed, because these studies have observed that there can be differences in in vitro– and in vivo–matured oocytes, observing spindles and chromosomal arrangements could be an important tool to develop improved culture systems. It remains to be seen whether, if there are no differences between in vitro– and in vivo–matured oocytes with regard to chromosome arrangements and spindles, there will also follow an increased pregnancy rate and live birth rate for IVM procedures. Once oocytes reach the appropriate stage of nuclear and cytoplasmic maturation without there being any fear that the culture systems are imposing deleterious effects, then the oocytes can be prepared more confidently for proceeding through fertilization and embryo development during IVF procedures. Based on the current state of IVM technologies, it may be premature to use IVM exclusively in all IVF cases, but it might be beneficial for certain patients.

REFERENCES

1. 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.
2. Trounson A, Wood C, Kausche A. In vitro maturation and fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril 1994;62:353–62.
3. Chian RC, Lim JH, Tan SL. State of the art in in-vitro maturation. Curr Opin Obstet Gynecol 2004;16:211–9.
4. Cha KY, Chung HM, Lee DR, Kwon H, Chung MK, Park LS, et al. Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro fertilization-embryo transfer. Fertil Steril 2005;83:1461–5.
5. Schroder AK, Schopper B, Al-Hasani S, Diedrich K, Ludwig M. Unilateral follicular aspiration and in-vitro maturation before contralateral oocyte retrieval: a method to prevent ovarian hyperstimulation syndrome. Eur J Obstet Gynecol Reprod Biol 2003;110:186–9.
6. Requena A, Neuspiller F, Cobo AC, Aragones M, Garcia-Velasco JA, Remohi J, et al. Endocrinological and ultrasonographic variations after immature oocyte retrieval in a natural cycle. Hum Reprod 2001;16:1833–7.
7. Roberts R, Franks S, Hardy K. Culture environment modulates maturation and metabolism of human oocytes. Hum Reprod 2002;17:2950–6.
8. Li Y, Feng H-L, Cao Y-J, Zheng G-J, Yang Y, Mullen S, et al. Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured in vitro. Fertil Steril 2006;85:827–32.
9. Mullen SF, Agca Y, Broermann DC, Jenkins CL, Johnson CA, Critser JK. The effect of osmotic stress on the metaphase II spindle of human oocytes, and the relevance to cryopreservation. Hum Reprod 2004;19:1148–54.
10. Ueno S, Kurome M, Ueda H, Tomii R, Hiruma K, Nagashima H. Effects of maturation conditions on spindle morphology in porcine MII oocytes. J Reprod Dev 2005;51:405–10.
11. Zenzes MT, Bielecki R, Casper RF, Leibo SP. Effects of chilling to 0 degrees C on the morphology of meiotic spindles in human metaphase II oocytes. Fertil Steril 2001;75:769–77.
12. Pickering SJ, Braude PR, Johnson MH, Cant A, Currie J. Transient cooling to room temperature can cause irreversible disruption of the meiotic spindle in the human oocyte. Fertil Steril 1990;54:102–8.
13. Wang WH, Meng L, Hackett RJ, Oldenbourg R, Keefe DL. Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates. Fertil Steril 2002;77:1274–7.
14. Wang WH, Keefe DL. Predictions of chromosome alignment among in vitro matured human oocytes by spindle imaging with the Polscope. Fertil Steril 2002;78:1077–81.
15. Moon JH, Jee BC, Ku SY, Suh CS, Kim SH, Choi YM, et al. Spindle positions and their distributions in in vivo and in vitro matured mouse oocytes. Hum Reprod 2005;20:2207–10.