

Commercially available enhanced in vitro maturation medium does not improve maturation of germinal vesicle and metaphase I oocytes in standard in vitro fertilization cases

Improvements in in vitro maturation techniques have focused on culture optimization to increase oocyte maturation rates. Specialized culture media, now commercially available, did not perform significantly better than standard IVF culture media for maturation of immature oocytes in our normal IVF cases. (*Fertil Steril*® 2011;95:2645–7. ©2011 by American Society for Reproductive Medicine.)

Key Words: Human oocyte, immature oocyte, IVM, oocyte maturation, culture media

In vitro maturation (IVM) systems for treatment of infertility were originally designed for the maturation of immature oocytes to support the developing oocyte during its progression through meiosis. The clear advantage of IVM is that oocytes would be retrieved and matured without exposing the patient to exogenous gonadotropins. This would result in a reduction of gonadotropin-induced ovarian hyperstimulation syndrome, particularly in patients with polycystic ovarian syndrome, as well as reduced treatment costs. From a physiological standpoint, avoidance of gonadotropins has the potential to allow more natural endometrial development and lower treatment cycle costs. There are multiple reports documenting successful pregnancies and births from unstimulated, IVM oocytes (1–3).

More recently, however, IVM protocols have been more widely applied to include patients undergoing IVF cycles with controlled ovarian hyperstimulation. The rationale behind the use of IVM in stimulated cycles is that it could prove particularly beneficial for patients with poor response to gonadotropin treatment (as a rescue

technique), with diminished ovarian reserve, with premature ovarian failure, or for those undergoing oocyte cryopreservation cycles for medical or social indications. The primary goal in the application of IVM for these patients would be to maximize the number of mature oocytes available for potential fertilization and development. As with IVM in nonstimulated cycles, IVM oocytes from stimulated cycles also have been demonstrated to result in healthy live births (4–9). Despite these reports of successful treatment cycles, IVM efficiency, as measured by evidence of normal epigenetic reprogramming during meiosis, fertilization, pre- and postimplantation development, and live birth rates, remains uncertain. Furthermore, despite continued refinement of the IVM procedure, there are presently limited applications to the general infertile population.

Improvements in the IVM technique have largely focused on optimization of the culture media in an attempt to effectively support continued oocyte maturation in vitro. Commercialized IVM media are now available from numerous manufacturers (including Medicult, Sage/Cooper Surgical, Sage IVF Inc., and Cook) and with a variety of modifications to “standard” culture media with regard to concentration and content of amino acids, salts, carbohydrates, and antibiotics. Specialized IVM media are supplemented with components thought to support the specific needs of an oocyte throughout its maturation. These components can include FSH, LH, epidermal growth factor (EGF), growth hormone (GH), and hCG. Several studies have demonstrated the improvement of both nuclear and cytoplasmic maturity with growth factor supplementation. In one study, an increased maturation rate to the metaphase II (MII) stage was observed when EGF, a cytokine that is normally present within the ovary, was added to the culture media of denuded germinal vesicle (GV) stage oocytes (10). Another study observed significantly higher maturation to MII in addition to significantly higher numbers of day 2 and day 3 embryos using media supplemented with EGF family members than without (11). The addition of GH to culture media has been shown to stimulate cytoplasmic maturation and genetic competence in both denuded oocytes and those with intact cumulus complexes (9). Importantly,

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GV and MII oocytes and preimplantation embryos exhibit growth hormone receptor gene expression, suggesting that GH is an important component during folliculogenesis and subsequent embryo development (12).

It is clear that further studies will be needed to shed light on the necessity and value of specialized IVM media containing supplemental factors in achieving significant improvements in the subsequent developmental potential of oocyte conversion rates in both stimulated and nonstimulated cycles. Our study aimed to evaluate the value of such specialized media in supporting IVM of immature oocytes retrieved during a normal stimulated IVF cycle. Our purpose was to compare the effectiveness of two culture media, specialized IVM media and our standard culture media, with respect to their efficiencies in supporting the meiotic progression of immature oocytes at the GV or MI stage to full maturity at the MII stage.

A total of 28 patients were enrolled in the study. A total of 127 oocytes were analyzed, including 72 that were retrieved at the GV stage and 55 at the MI stage. All patients signed Institutional Review Board (IRB) consent to participate in the study by allowing assessment of tissue designated for discard. Oocytes were retrieved 36 hours post-hCG administration and were cultured in standard culture media, consisting of Quinn's Advantage Cleavage Media (Sage IVF, Inc.) supplemented with 5% human serum albumin for approximately 4 hours at 37°C in 5.5% CO₂. Oocytes were then treated with hyaluronidase (80 IU/mL) to remove cumulus cells to aid in the visualization of maturity. Once maturity was assessed, any immature oocytes were randomly assigned to group 1, standard culture media, or to group 2, Medicult IVM media (Medicult). The Medicult IVM media was prepared as directed with supplemental addition of both FSH and hCG (Gonal F and Ovidrel, EMD Serono). All oocytes were cultured for 24 hours at 37°C in 5.5% CO₂ and were reassessed for maturation conversion. A χ^2 analysis was performed, and $P < .05$ was considered statistically significant. Unfortunately, the clinical laboratory operational circumstances of required labeling of all clinical and research materials (Food and Drug Administration requirements) and the very limited quantity of IRB-approved experimental materials restricted our ability to conduct a blinded and more highly powered experiment.

The overall conversion rates observed after 24 hours of culture did not significantly differ between the standard media and Medicult IVM media. Table 1 reflects the maturation rates of each subgroup, namely, conversion from GV to MI stage, from GV to MII stage, and from MI to MII stage.

Immature oocytes retrieved during routine IVF from patients undergoing gonadotropin and hCG treatment may have clinical potential if they can be reliably and competently matured in vitro. Toward this goal, we compared the potential for our standard culture media and commercially available specialized IVM media to promote maturation of the immature oocytes that are normally unusable and discarded. Our study demonstrated that the specialized IVM media did not significantly improve the rate of maturation of any immature oocytes within a 24-hour period of culture.

In our study, the GV-to-MI transition subgroup was the only group to show a trend toward improved oocyte maturation when treated with specialized media ($P = .0849$). Regardless of its

TABLE 1

In vitro maturation rates of immature human oocytes.

IVM after 24 h	Standard culture media (%)	Medicult IVM media (%)	P value
GV to MI	4/38 (10.5)	10/34 (29.4)	.0849
GV to MII	19/38 (50.5)	14/34 (41.2)	.9895
MI to MII	25/31 (80.6)	16/24 (66.7)	.3853

Moschini. Commercial IVM media maturation rate. Fertil Steril 2011.

efficiency in maturing GV oocytes in vitro, however, its ability to ultimately support normal fertilization remains unclear (13). Our results showed a maturation efficiency comparable to past studies comparing the Medicult IVM media to other IVM media (TCM-199). It is of interest to note that this study used the Medicult media as originally intended, in unstimulated cycles without treatment with hCG (14). Based on our data, our standard media appears to perform comparably to media specifically customized for IVM in the conversion of immature oocytes to mature MII oocytes, without the additional cost and preparation time involved with the latter.

An important question of the IVM culture technique is whether the media is adequate to support normal epigenetic reprogramming and subsequent robust development of the embryo. As previously discussed, the in vitro development of immature oocytes exposed to gonadotropins could demonstrate a compromised developmental capacity. A comparison of nuclear quality between IVM and non-IVM derived embryos from stimulated cycles seems to lean more heavily toward IVM-derived embryos being intrinsically flawed, but there are also reports of normal epigenetic changes occurring. One group looked at histone acetylation in chromatin, an epigenetic modification regulating gene expression, and observed a down-regulation of two acetylation enzymes in IVM mouse oocytes and embryos up to the two-cell stage (15). However, this down-regulation did not persist after the two-cell stage. Another group looked at methylation patterns of the H19 differentially methylated region in IVM oocytes from stimulated cycles cultured in Medicult IVM media for a 24-hour period. While the GV and MI oocytes demonstrated a high percentage of hypermethylation, three-quarters of the oocytes reaching the MII stage showed a normal unmethylated pattern (16). The challenge of undergoing normal epigenesis may result in greater frequencies of chromosomal abnormalities. In one study, all embryos derived from IVM oocytes from stimulated cycles were found to be chromosomally abnormal, revealing mostly complex mosaic aberrations (13). Another study observed only a 60% aneuploidy rate in IVM-derived embryos (17). Lastly, one group did not find embryos cultured in GH-supplemented IVM media to be constitutionally different than standard IVF embryos after cytogenetic and DNA fragmentation analysis (18).

In our study, we demonstrate that the use of commercially available enhanced IVM media to rescue immature oocytes from standard cases of gonadotropin-stimulated IVF cycles did not improve oocyte maturation compared with the use of standard growth media. Although we did not attempt to fertilize these oocytes, examine them genetically, analyze them with transmission electron

microscopy, or transfer any embryos, it is clear that IVM culture of immature oocytes would require further study of nuclear and cytoplasmic competency and epigenetic safety before becoming more widely accepted into common clinical use. While the application of IVM in unstimulated cycles serves a very small niche of patients who cannot undergo ovarian stimulation, the greater potential of

this technique is to rescue the unusable immature oocytes currently retrieved in standard IVF or oocyte cryopreservation cycles. Ideally, optimal utilization of genetically normal oocytes, whether harvested in immature or mature states, will improve reproductive outcome. Further investigation must target the efficiency, efficacy, and safety of IVM oocytes.

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