Comparison of Blastocyst and Sage Media for In Vitro Maturation of Human Immature Oocytes

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Abstract

In vitro maturation (IVM) of human oocytes is an attractive alternative to conventional assisted reproductive technology (ART) treatment, as it involves no or minimal ovarian stimulation. Currently, commercialized media specifically designed for IVM are often used. These media are expensive, have limited shelf life, and must be ordered in advance. If standard culture media can be used in place of the specialized IVM media, it would simplify management and make IVM more feasible and more widely employed in ART centers around the world, especially in developing countries where resources are scarce. This study was, therefore, conducted to test the hypothesis that blastocyst medium was as good as commercial IVM medium to support maturation and developmental competence of human immature oocytes as previously shown in the mouse system. Immature oocytes were obtained by needle aspiration from 89 pregnant women during cesarean deliveries between April 2012 and February 2013. Sibling oocytes were allocated to Sage IVM media (512 oocytes) or blastocyst medium (520 oocytes) and assessed for maturation 36 hours later. Mature oocytes were inseminated by intracytoplasmic sperm injection and cultured up to 144 hours. There was no difference in maturation rate (65.0% vs 68.7%; $P = .218$) or fertilization rate (66.9% vs 66.4%; $P = .872$) of oocytes matured in vitro in both media. There was also no difference in the formation of good-quality blastocysts (46.6% vs 45.9%; $P = .889$) in the 2 groups. Further study should be done to ascertain implantation and pregnancy potential of these embryos.

Keywords

blastocyst medium, immature oocytes, in vitro maturation, in vitro maturation medium

Background

In vitro maturation (IVM) involves the collection of immature oocytes from nonstimulated or minimally primed follicles and then allowing them to mature in culture.¹ The IVM is less expensive, simpler, and safer than conventional assisted conception treatment because there is no or minimal ovarian stimulation and virtually no risk of ovarian hyperstimulation syndrome (OHSS).^{1,2}

The success of IVM depends on the choice of a suitable medium to allow IVM of the oocytes.³ MediCult IVM media (Origio A/S, Jyllinge, Denmark) and Sage IVM media (CooperSurgical, Trumbull, Connecticut) are often used, as they are commercially available and they have demonstrated reasonable fertilization, implantation, and pregnancy rates.⁴⁻⁶ However, these media are more expensive than standard culture media for in vitro fertilization (IVF). In addition, they need to be preordered well in advance and they have limited shelf life. Their use may, therefore, pose some problems to the average assisted conception centers, where IVM services are not performed on a routine basis. A recent study⁴

demonstrated that blastocyst medium was as suitable for IVM of immature mouse oocytes as Sage IVM media and tissue culture medium 199. The present study was, therefore, conducted to test the hypothesis whether blastocyst medium would be as good as conventional IVM medium for human immature oocytes as well. If routine IVF media could be used in place of the specialized IVM media, it would simplify logistic management and make IVM more feasible and economical to most fertility centers around the world.

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Methods

Participants

The Research Ethics Committee of Buddhachinaraj University Hospital approved this study. Pregnant women, who underwent cesarean delivery between April 2012 and February 2013, were invited to participate in the study. Patients were included if they met the following inclusion criteria: (1) aged between 19 and 40 years; (2) singleton pregnancy with a gestational age of 37 to 40 weeks; (3) elective repeated or emergency cesarean section not due to fetal distress; (4) had no chronic diseases or medical or obstetric complications that could be jeopardized by prolongation of anesthesia or surgery; and (5) consented to participate in the study. They were excluded if they (1) could not read or write or understand Thai language; (2) refused to participate in the study; and (3) had excessive blood loss or unstable vital signs during cesarean delivery.

Oocyte Collection, Maturation, Fertilization, and Embryo Development

Cesarean delivery was performed under general anesthesia or spinal block. After closure of uterine incision, all visible follicles were aspirated with a 22-gauge needle attached to a 5-mL syringe, filled with 1 mL of warmed flushing medium (FertiCult Flushing, MediCult, Beemem, Belgium). Aspirated fluid was examined using a stereomicroscope. Atretic oocytes and those with mechanical damage or without cumulus masses were discarded. Sibling oocytes from individual participants were allocated to Sage IVM media (group I) or blastocyst medium (group II). In the first group, oocytes were initially washed 2 times with Sage washing medium before transfer into Sage maturation medium. In the second group, oocytes were washed 2 times in flushing medium before cultured in blastocyst medium (Cook Medical, Bloomington, Indiana). They were cultured in groups of up to 3 oocytes in 25- μ L drops of maturation medium, supplemented with 75 IU/L of human menopausal gonadotropin (IVF-M, LG Life Sciences, Jeonbuk-do, Korea) under paraffin oil (MediCult) at 37°C in a humidified atmosphere of 6% CO₂ in air, without medium renewal.

The maturity of the oocytes was assessed under an inverted microscope at 36 hours in culture. Before maturity assessment, surrounding cumulus cells were removed by a 30-second exposure to 80 IU/mL hyaluronidase (Type VIII from bovine testis, Sigma H3757) in flushing medium and the use of a denuding pipette (Flexipet, Cook Medical). Mature oocytes were inseminated by intracytoplasmic sperm injection (ICSI), using spermatozoa from donors with normal semen analysis. Following ICSI, oocytes were cultured in groups of up to 3 in 20 μ L drops of Sage embryo maintenance medium in group I or cleavage medium (Cook Medical) in group II in a culture dish covered with paraffin oil.

Fertilization was checked 18 hours after ICSI. Fertilized oocytes were cultured in maintenance or cleavage medium for

Table 1. Nuclear Maturation and Developmental Competence of Immature Oocytes Cultured in Sage IVM Media and Blastocyst Medium.^a

	Sage Media	Blastocyst Medium
Immature oocytes	512 (100%)	520 (100%)
Mature oocytes	333 (65.0%)	357 (68.7%)
Fertilization	223 (66.9%)	237 (66.4%)
Cleavage	203 (91.0%)	213 (89.9%)
Blastocyst	104 (46.6%)	109 (45.9%)

Abbreviation: IVM, in vitro maturation.

^a Chi-square tests: no statistical difference between Sage and blastocyst media.

72 hours, without medium renewal. Embryos were then transferred into blastocyst medium (Cook Medical) and cultured for another 72 hours under the same conditions. Embryos were examined once daily. Only fully expanded blastocysts and hatching or hatched blastocysts, containing a distinct inner cell mass and trophectoderm layer, were considered to be good-quality blastocysts.

Statistical Analysis

Data were recorded using Microsoft Office Excel 2003 (Microsoft Corporation, Washington). Statistical analyses were performed using Stata Release 11 (StataCorp, College Station, Texas). Comparisons of frequency data between groups, such as fertilization and cleavage rates, were performed using chi-square tests. A *P* value <.05 was considered to indicate statistical significance.

Our sample size calculation showed that at least 400 oocytes in each arm would be required to demonstrate a 10% difference in maturation rate between oocytes in the 2 groups, given a type I error of 5% (2 tailed) and a type II error of 20%.

Results

A total of 220 pregnant women met eligibility criteria, and 89 of them gave their written informed consents to participate in the study. Patient characteristics were (mean $\pm$ standard deviation): age 28.8 $\pm$ 6.4 years (range 18-40 years), gravida 1.6 $\pm$ 0.6 (range 1-4), parity 0.5 $\pm$ 0.6 (range 0-3), gestational age 38.3 $\pm$ 0.9 weeks (range 37-40 weeks), and body mass index 28.7 $\pm$ 4.7 kg/m² (range 19.0-47.4 kg/m²).

A total of 1178 immature oocytes were recovered, but only 1032 oocytes met eligibility criteria and were included in the study (512 oocytes in group I and 520 oocytes in group II, respectively). There was no statistical difference in maturation rate at 36 hours, fertilization rate by ICSI, cleavage rate of fertilized oocytes, or the formation rate of good-quality blastocysts between the 2 groups (Table 1).

Discussion

In this study, we compared Cook blastocyst medium with Sage IVM media simply because they were already available in our

Table 2. Compositions of Sage IVM media and Blastocyst Medium.^a

Compositions	Sage IVM Media			Blastocyst Medium
	Washing Medium	Maturation Medium	Maintenance Medium	
Basic components ^b	✓	✓	✓	✓
Nonessential amino acids ^c	✓	✓	✓	✓
Essential amino acids ^d	✓	✓	✓	✓
Vitamins ^e	✓	✓		✓
Magnesium sulfate	✓	✓		
Magnesium chloride			✓	
Calcium chloride	✓	✓	✓	
Cysteamine			✓	
Sodium lactate			✓	
Taurine				✓
Plasma Protein Fraction	✓	✓	✓	
Human serum albumin				✓

Abbreviation: IVM, in vitro maturation.

^a Modified from Sage and Cook product brochures.

^b Sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, sodium pyruvate, sodium phosphate, gentamicin, glucose, phenol red, water.

^c Alanine, glutamine, asparagine, aspartic acid, glycine, proline, serine.

^d Arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine.

^e D-Calcium pantothenate, choline chloride, folic acid, inositol, pyridoxine, riboflavin, thiamine.

assisted reproductive technology (ART) program. Moreover, both media have very similar compositions (Table 2). Basically they contain (1) electrolyte solutions, bicarbonates, and energy substrates, such as glucose and pyruvate; (2) essential and nonessential amino acids; and (3) protein supplements, such as human serum albumin or plasma protein fraction. There are minor differences, such as the presence of taurine in blastocyst medium and cysteamine in Sage IVM media, which are absent in the other. These differences are, however, more apparent than real as some of the missing compounds can be produced in vivo from other precursors in the medium, and plasma protein supplement can supply additional factors, some of which could be involved in the regulation of oocyte maturation.^{7,8}

Oocyte maturation involves a complex set of nuclear and cytoplasmic changes that are not completely understood.^{3,9} Nuclear maturation occurs automatically in competent oocytes when they are removed from their follicular environment, as the maintenance of meiotic arrest is an undertaking that involves the entire follicle.⁹ Cytoplasmic maturation includes changes in the morphology and distribution of many cytoplasmic organelles and the reactivation of messenger RNA transcript to stockpile proteins and transcripts that are essential to sustain early embryonic development until genomic activation.^{3,9,10} In this study, we used fertilization rate and extended culture up to the blastocyst stage to indicate maturation and developmental competence of the oocytes, which should be better indicators than molecular or detailed morphological study.

Our oocyte maturation rate of 65% to 69% and fertilization rate of 66% to 67% were compatible with other studies using various IVM media, which reported a maturation rate of 44% to 67% after 28 to 36 hours of in vitro culture and a fertilization

rate of 40% to 77%.^{2,11-18} The rates of good-quality blastocyst formation in our study (45.9%-46.6%) were also in agreement with 2 previous studies in IVM oocytes that were obtained in the follicular phase (41.2%-47.4%).^{18,19} Our study showed that collection of oocytes during a cesarean section is safe and effective. It can be considered as an attractive alternative to conventional oocyte donation and also a promising option for those who become pregnant from a previous ART treatment and desire future treatment but do not have remaining cryopreserved oocytes/embryos. This approach could also be used to generate mature oocytes for use in stem cell research as well as for fertility.

This study included a large cohort of 1032 immature oocytes in a randomized sibling oocyte design, such that each patient served as their own controls. The weakness in our study was that we used the formation of good-quality blastocysts as the end point, without a detailed study of their chromosomal status or genomic imprinting pattern. However, animal studies and studies of children born after IVM are quite reassuring so far and show no evidence of an increased risk of congenital malformations, abnormal neonatal growth, or imprinting syndromes.^{20,21} Further study would be done to ascertain the implantation and pregnancy potential of the resulting embryos.

Conclusions

This was the first study to compare Sage IVM and blastocyst media in a randomized sibling oocytes design. We demonstrated that immature oocytes obtained from pregnant women could be successfully matured in vivo, fertilized, and cultured up to the blastocyst stage with comparable results in either commercial IVM or blastocyst media.

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Authors' Note

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Declaration of Conflicting Interests

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