

Comparison of Medicult and Sage Media for *In Vitro* Maturation of Immature Oocytes Obtained during Cesarean Deliveries

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Abstract

Background: The success of *in vitro* maturation (IVM) of oocytes depends on many factors. Culture medium is one crucial part of the technique, but evidence-based selection of the medium is difficult because very few trials are available on this subject. In this study we compared two widely available commercial media, namely Medicult (Origo, Måløv, Denmark) and Sage medium (Cooper Surgical, Trumbull, CT, USA) for IVM of human oocytes.

Methods: One thousand and fifteen immature oocytes were collected by needle aspiration from ninety-three women, who underwent cesarean deliveries during a five-month period at a University Hospital in Thailand. Sibling oocytes were allocated to either Medicult (509 oocytes) or Sage IVM medium (506 oocytes) and assessed for maturation after 36 hours in culture. Mature oocytes were inseminated by intracytoplasmic sperm injection and cultured up to 144 hours. χ^2 -tests were used to compare maturation, fertilization, cleavage and blastocyst formation rates between the two groups.

Results: There was no statistical difference ($p>0.05$) in maturation (65.0 vs. 64.2 %), fertilization (69.9 vs. 65.2 %), cleavage (61.7 vs. 61.2 %), or blastocyst formation (48.1 vs. 46.7 %) between oocytes in the two groups.

Conclusions: Despite the unfavorable hormonal milieu, immature oocytes obtained during pregnancies are developmentally competent. Given equal efficacy, the choice of Medicult or Sage IVM medium depends on availability, cost, and ease of use. This approach is an attractive alternative to conventional oocyte donation and may be used to generate mature oocytes for stem cell research.

Keywords: Immature oocytes; *In vitro* maturation; Medi Cult IVM media; Sage IVM media

Introduction

Ovarian stimulation is performed in assisted reproductive technology (ART) to increase the yield of oocytes and embryos. The stimulation involves a daily hormonal injection, which causes discomfort and inconvenience to the patients. The medication is expensive and the treatment requires frequent ultrasound scans and medical visits. Ovarian stimulation also exposes the patients to the risk of ovarian hyperstimulation syndrome (OHSS), with only modest increase in the success rate [1,2]. There are also concerns about the long-term side effects of ovarian stimulation and the increased risk of ovarian, endometrial and breast cancers [3,4]. *In vitro* maturation (IVM) of immature oocytes is an attractive alternative to overcome the disadvantages of conventional ART treatment [1,2,5]. Edwards reported early attempts of human IVM [6], but the first live birth was achieved nearly 30 years later [7]. Since then more than 5,000 children have been conceived from IVM of human oocytes [5]. However, pregnancy and live birth rates remain low after the transfer of embryos derived from IVM, compared with *in vivo*-matured oocytes [1,2].

Many factors influence the success of IVM, including patient selection, follicular priming, endometrial preparation, and culture conditions [1,8]. Various media have been used for IVM, such as human tubal fluid [8], tissue culture medium 199 [8-11], YS (Yeast extract-Soluble starch agar) medium [12], blastocyst medium [11], and specially formulated commercial media. As culture medium is one crucial part of the technique, the selection of an optimum medium for IVM is very important. To date, no randomized controlled trial has compared Medicult IVM media (Origo, Malov, Denmark) with Sage IVM media (Cooper Surgical, Trumbull, CT, USA). The purpose of this study was, therefore, to compare the performance of these two commonly used commercial media in terms of oocyte maturation, fertilization and subsequent embryo development up to the blastocyst stage.

Materials and Methods

Study population and participants

The Research Ethics Committee of Buddhachinaraj Hospital Medical School approved this study. Pregnant women were invited to participate in the study if they met the following inclusion criteria: 1) age 18-42 years; 2) singleton pregnancy with a gestational age of 37-40 weeks; 3) elective repeated or emergency cesarean section not due to fetal distress; 4) gave their written informed consent to participate in the study; 5) had no chronic diseases or medical or obstetric complications that could be jeopardized by prolonging either anesthesia or surgery. Patients were excluded if they: 1) could not read or write or understand Thai language; 2) had excessive blood loss or unstable vital signs during the operation.

Oocyte collection, maturation, fertilization and embryo development

Cesarean section was performed under general anesthesia or spinal block. After closure of uterine wound, all visible follicles were aspirated

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with a 22-gauge needle attached to a five ml syringe, filled with one ml of warmed flushing medium (Ferticult Flushing, MediCult, Beemem, Belgium). Aspirated fluid was examined using a dissecting microscope. Atretic oocytes and oocytes with mechanical damage or those without cumulus mass were discarded. Sibling oocytes, with multilayered or sparse cumulus, from individual women were randomly allocated to MediCult (Group I) or Sage IVM media (Group II). In the first group, oocytes were initially incubated in LAG medium for two to three hours prior to transfer into the final MediCult IVM medium (supplemented with 10% patient serum before use). In the second group, oocytes were washed three times with Sage washing medium before transfer into Sage maturation medium. They were cultured in groups of up to three oocytes in 25 µl 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 determined using an inverted microscope at 36 hours after culture. Oocyte handling was conducted on warm stages and plates at 37°C. Before maturity assessment, oocytes were denuded of their cumulus cells by a 30-second exposure to a dilution of 80 IU/ml hyaluronidase. Oocytes with first polar bodies were inseminated by intracytoplasmic sperm injection (ICSI). Spermatozoa for ICSI were obtained from healthy sperm donors, who had normal semen analysis, and were 20-40 years of age. Sperm was prepared by Sil-select Plus (Beemem, Belgium) gradient centrifugation. The sperm pellet was washed twice with two ml of flushing medium. Following ICSI, oocytes were cultured in groups of up to three in 20 µl drops of cleavage medium (Cook Medical, Bloomington, IN, USA) in Group I or Sage embryo maintenance medium in Group II in a culture dish covered with paraffin oil.

Fertilization was assessed 18 hours after ICSI for the appearance of two pronuclei and two polar bodies. After 72 hours of culture in cleavage or maintenance medium without medium renewal, the embryos were transferred into blastocyst medium (Cook), and cultured for another 72 hours under the same culture conditions. Embryos were examined once every 24 hours. We used the Gardner's system [13] to grade the quality of blastocyst. Only fully expanded blastocysts and hatching or hatched blastocysts, containing a distinct inner cell mass and trophoctoderm layer, were considered to be good-quality blastocysts.

Statistical analysis

Stata Release 11 (StataCorp, College Station, Tx, USA) was used for all statistical analyses. Comparisons of frequency data between groups, such as fertilization and cleavage rates, were performed using χ^2 -tests. A P-value <0.05 was considered to indicate statistical significance.

Results

Ninety-three pregnant women volunteered to participate in the study. Patient characteristics were as follows (mean ± SD): age 28.3 ± 6.4 years (range 18-42 years), gravida 1.5 ± 0.6 (range 1-4), parity 0.5 ± 0.6 (range 0-3), gestational age 38.2 ± 0.8 weeks (range 37-40 weeks), body mass index 27.7 ± 4.6 kg/m² (range 19.7-46.9 kg/m²). The average oocyte collection time was 9.5±3.2 minutes (range 4-15 minutes). A total of 1,015 immature oocytes (509 and 506 oocytes in Group I and II, respectively) were included in the study. On the average, 10.9 ± 5.2 oocytes (range 6-17 oocytes) were obtained per patient. There was no intra- or post-operative complication in any of the subjects.

There was no statistical difference in maturation rate, fertilization rate by ICSI, cleavage rate of fertilized oocytes or the formation rate of good-quality blastocysts between the two groups (Table 1).

Discussion

Human oocytes in the primordial follicles remain in the resting stage, until a process of initial recruitment initiates their growth. When the follicles reach a size of around five mm, the enclosed oocytes acquire developmental competence but they remain arrested at the first meiotic prophase by inhibitory molecules, such as cAMP, purines, and phosphodiesterase inhibitors [1,14]. The inhibitors pass from the granulosa/theca cells to the oocytes via gap-junctions. When immature oocytes are isolated from small competent follicles and cultured *in vitro*, they are capable of resuming and completing meiosis spontaneously [14]. The addition of follicle stimulating hormone (FSH) and luteinizing hormone (LH) to IVM medium may also enhance oocyte maturation by promoting the production of meiosis-activating substance [15]. Another important event is cytoplasmic maturation, which is still poorly understood. In contrast to nuclear maturation, the process of cytoplasmic maturation is not obvious when observed with the light microscope [1,14]. The process involves subcellular and biochemical alterations, such as the change in morphology and distribution of certain cytoplasmic organelles, and an increase in mRNA transcription that is essential to sustain subsequent embryonic development [14]. In this study, we collected germinal vesicle oocytes from primordial follicles, and used fertilization rate and embryo development up to the blastocyst stage as indicators of oocyte developmental competence, as they are more clinically relevant than the biochemical or detailed morphological study.

Both IVM media have very similar compositions (Table 2), except

	Medi Cult media	Sage media
Immature oocytes	509 (100 %)	506 (100 %)
Mature oocytes	311 (65.0 %)	325 (64.2 %)
Fertilization	208 (66.9 %)	212 (65.2 %)
Cleavage	192 (92.3 %)	199 (93.9 %)
Blastocyst	100 (48.1 %)	99 (46.7 %)

Table 1: Nuclear maturation and developmental competence of immature oocytes cultured in Medi Cult and Sage IVM media.

Compositions	Medicult IVM media		Sage IVM media		
	LAG medium	Maturation medium	Washing medium	Maturation medium	Maintenance medium
Basic components ^a	✓	✓	✓	✓	✓
Nonessential amino acids ^b		✓	✓	✓	✓
Essential amino acids ^c		✓	✓	✓	✓
Vitamins ^d		✓	✓	✓	
Magnesium sulfate	✓	✓	✓	✓	
Magnesium chloride					✓
Calcium chloride	✓	✓	✓	✓	✓
Cysteamine					✓
Sodium lactate					✓
PPF ^e	✓		✓	✓	✓
Patient's own serum		✓			
Nucleotide base		✓			

^aSodium chloride, Potassium chloride, Calcium chloride, Sodium bicarbonate, sodium pyruvate, sodium phosphate, gentamicin, glucose, phenol red, water;

^bAlanyl-glutamine, asparagine, aspartic acid, glycine, proline, serine;

^cArginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine;

^dD-Calcium pantothenate, choline chloride, folic acid, inositol, pyridoxine, riboflavin, thiamine;

^ePPF = Plasma Protein Fraction

Table 2: Composition of MediCult and Sage IVM media (from MediCult and Sage product brochure).

for the presence of glutathione, alanine, ribose, deoxyribose, and adenosine triphosphate (ATP), cholesterol, and vitamins A, D₂, E, C, niacin and niacinamide in Medicult IVM medium and asparagine and nicotinamide in Sage IVM medium that are absent in the other. These minor differences are more apparent than real because some of the missing compounds can be produced *in vivo* from other precursors in the medium. In addition, both media were supplemented with either patient's serum or plasma protein fractions. These macromolecules are known to harbor a variety of additional compounds, some of which could be involved in the regulation of oocyte maturation [16,17]. The use of patient's own serum in the case of Medicult IVM medium was a drawback, because the composition of serum could vary from patient to patient and there was a potential risk of infectious contamination, such as human immunodeficiency virus (HIV) or hepatitis virus. It also added another step to medium preparation and made the medium less convenient to use.

In this study, we compared the whole process of two commercial IVM systems, and not just the IVM media themselves. As such, immature oocytes were exposed sequentially to LAG medium or Sage washing medium, then to Medicult or Sage IVM medium and finally to routine culture medium or Sage maintenance medium, as recommended by the manufacturers. We believed the results of such a comparison would be more relevant, as this is the usual way that commercial IVM systems are intended to be used in a real clinical setting.

Our oocyte maturation rate of 64-65% and fertilization rate by ICSI of 65-67% were compatible with other studies using various IVM media, which reported a maturation rate of 44-67% after 28-36 hours of *in vitro* culture, and a fertilization rate of 40-77% [8-10,18-25]. Only three studies cultured embryos that were derived from *in-vitro* matured oocytes up to the blastocyst stage. They reported the rates of good-quality blastocyst formation of 48.6% [20], 44.8% [23], and 41.2-47.4% [25], which were compatible with this study (46.7-48.1%).

Our PubMed search found few studies that compared the efficacy of different IVM media. One study reported tissue culture medium (TCM) 199 to be better than human tubal fluid medium for IVM of oocytes obtained from women with polycystic ovary syndrome (PCOS) [8]. Another retrospective study reported no difference between TCM-199 and Medicult IVM medium in terms of oocyte maturation, fertilization, early embryo development and pregnancy rates in PCOS women [9]. One study in the mouse found Sage IVM and blastocyst culture medium to be superior to TCM-199 for oocyte maturation, fertilization, and subsequent embryo development [11]. There were two studies using denuded immature oocytes from stimulated cycles for IVM. One study reported that the new IVM medium, which had a composition similar to Sage IVM media, gave a better maturation and blastulation rate than TCM-199 [26]. The other study compared cleavage medium with Medicult IVM medium, and found no difference in maturation rates [27]. Our study was the first to compare Medicult with Sage IVM medium in a randomized sibling oocytes design. We included a large cohort of >1,000 immature oocytes, and had a post-hoc statistical power of >90% to demonstrate a difference of ≥15% in nuclear maturation and fertilization rate and >85% to detect a difference of ≥20% in blastulation rate, given a type I error of 5% (two-tailed).

The current research was an experimental study, and it remained a challenge if the model can be translated into a real clinical setting. At present, oocyte donation involves ovarian stimulation and an oocyte retrieval procedure that the donor will not otherwise need. Ovarian stimulation exposes the donor to short and unknown long-term risks, such as ovarian hyperstimulation syndrome and ovarian cancer [28,29]. Collection of immature oocytes during a cesarean section is, therefore,

an attractive alternative to conventional oocyte donation. Our research showed that this procedure was quite safe and effective, and it added only 10 minutes or so to the operative time. Such a procedure is also a promising option for those who are pregnant from an IVF treatment, and desire future treatment but do not have any remaining cryopreserved oocytes/embryos. Moreover, the approach can be used to generate mature oocytes for stem cell research.

The weakness of this 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 follow-up studies of children born after IVM are quite reassuring, and they show no evidence that IVM is associated with an increased risk for congenital malformations, abnormal neonatal growth or imprinting syndromes [2,30]. Nevertheless available data are still limited and more well-designed human studies are urgently needed. Without embryo transfer in our study, we had no information on implantation, clinical pregnancy and miscarriage rate, which can be considered to be the ultimate indicators of oocyte developmental competence.

Conclusion

Despite the unfavorable hormonal milieu, immature oocytes obtained during pregnancies are developmentally competent. Further clinical studies are needed to clarify the implantation potential and the risk of miscarriage of *in vitro* matured oocytes retrieved during the luteal phase. Given equal efficacy, the choice of Medicult or Sage IVM medium depends on availability, cost, and ease of use.

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References

1. Mahajan N, Singh S (2012) *In vitro* maturation- current scenario. In: Talwar P (Eds) Manual of assisted reproductive technologies and clinical embryology. Jaypee Brothers Medical Publishers, New Delhi.
2. Fadini R, Mignini Renzini M, Dal Canto M, Epis A, Crippa M, et al. (2013) Oocyte *in vitro* maturation in normo-ovulatory women. *Fertil Steril* 99: 1162-1169.
3. Brinton LA, Moghissi KS, Scoccia B, Westhoff CL, Lamb EJ (2005) Ovulation induction and cancer risk. *Fertil Steril* 83: 261-274.
4. van Leeuwen FE, Klip H, Mooij TM, van de Swaluw AM, Lambalk CB, et al. (2011) Risk of borderline and invasive ovarian tumours after ovarian stimulation for *in vitro* fertilization in a large Dutch cohort. *Hum Reprod* 26: 3456-3465.
5. Chian RC, Uzelac PS, Nargund G (2013) *In vitro* maturation of human immature oocytes for fertility preservation. *Fertil Steril* 99: 1173-1181.
6. Edwards RG (1965) Maturation *in vitro* of human ovarian oocytes. *Lancet* 2: 926-929.
7. Cha KY, Koo JJ, Ko JJ, Choi DH, Han SY, et al. (1991) 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* 55: 109-113.
8. de Araujo CHM, Nogueira D, de Araujo MCPM, de Paula Martins W, Ferriani RA, et al. (2009) Supplement tissue culture medium 199 is a better medium for *in vitro* maturation of oocytes from women with polycystic ovary syndrome women than human tubal fluid. *Fertil Steril* 91: 509-513.
9. Filali M, Hesters L, Fanchin R, Tachdjian G, Frydman R, et al. (2008) Retrospective comparison of two media for *in vitro* maturation of oocytes. *Reprod Biomed Online* 16: 250-256.
10. Söderström-Anttila V, Mäkinen S, Tuuri T, Suikkari AM (2005) Favourable pregnancy results with insemination of *in vitro* matured oocytes from unstimulated patients. *Hum Reprod* 20: 1534-1540.

11. Kim M, Hong SJ, Lee JH, Min CK, Hwang KJ, et al. (2011) Comparison of in vitro maturation media of immature oocytes: the effectiveness of blastocyst culture media. *Fertil Steril* 95: 554-557.
12. Son WY, Yoon SH, Lee SW, Ko Y, Yoon HG, et al. (2002) Blastocyst development and pregnancies after IVF of mature oocytes retrieved from unstimulated patients with PCOS after in-vivo HCG priming. *Hum Reprod* 17: 134-136.
13. Balaban B, Yakin K, Urman B (2006) Randomized comparison of two different blastocyst grading systems. *Fertil Steril* 85: 559-563.
14. Hennet ML, Combelles CM (2012) The antral follicle: a microenvironment for oocyte differentiation. *Int J Dev Biol* 56: 819-831.
15. Byskov AG, Yding Andersen C, Hossaini A, Guoliang X (1997) Cumulus cells of oocyte-cumulus complexes secrete a meiosis-activating substance when stimulated with FSH. *Mol Reprod Dev* 46: 296-305.
16. Gardner DK, Lane M, Spitzer A, Batt PA (1994) Enhanced rates of cleavage and development for sheep zygotes cultured to the blastocyst stage in vitro in the absence of serum and somatic cells: amino acids, vitamins, and culturing embryos in groups stimulate development. *Biol Reprod* 50: 390-400.
17. Meintjes M (2012) Media composition: macromolecules and embryo growth. *Methods Mol Biol* 912: 107-127.
18. Trounson A, Wood C, Kausche A (1994) In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil Steril* 62: 353-362.
19. Barnes FL, Kausche A, Tiglias J, Wood C, Wilton L, et al. (1996) Production of embryos from in vitro-matured primary human oocytes. *Fertil Steril* 65: 1151-1156.
20. Cobo AC, Requena A, Neuspiller F, Aragon s M, Mercader A, et al. (1999) Maturation in vitro of human oocytes from unstimulated cycles: selection of the optimal day for ovum retrieval based on follicular size. *Hum Reprod* 14: 1864-1868.
21. Smith SD, Mikkelsen A, Lindenberg S (2000) Development of human oocytes matured in vitro for 28 or 36 hours. *Fertil Steril* 73: 541-544.
22. Mikkelsen AL, Andersson AM, Skakkebaek NE, Lindenberg S (2001) Basal concentrations of oestradiol may predict the outcome of in-vitro maturation in regularly menstruating women. *Hum Reprod* 16: 862-867.
23. Son WY, Lee SY, Lim JH (2005) Fertilization, cleavage and blastocyst development according to the maturation timing of oocytes in in vitro maturation cycles. *Hum Reprod* 20: 3204-3207.
24. Gremeau AS, Andreadis N, Fatum M, Craig J, Turner K, et al. (2012) In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. *Fertil Steril* 98: 355-360.
25. Walls M, Junk S, Ryan JP, Hart R (2012) IVF versus ICSI for the fertilization of in-vitro matured human oocytes. *Reprod Biomed Online* 25: 603-607.
26. Chian RC, Tan SL (2002) Maturation and developmental competence of cumulus-free immature human oocytes derived from stimulated and intracytoplasmic sperm injection cycles. *Reprod Biomed Online* 5: 125-132.
27. Moschini RM, Chuang L, Poleshchuk F, Slifkin RE, Copperman AB, et al. (2011) Commercially available enhanced in vitro maturation medium does not improve maturation of germinal vesicle and metaphase I oocytes in standard in vitro fertilization cases. *Fertil Steril* 95: 2645-2647.
28. Sauer MV, Kavic SM (2006) Oocyte and embryo donation 2006: reviewing two decades of innovation and controversy. *Reprod Biomed Online* 12: 153-162.
29. van Leeuwen FE, Klip H, Mooij TM, van de Swaluw AM, Lambalk CB, et al. (2011) Risk of borderline and invasive ovarian tumours after ovarian stimulation for in vitro fertilization in a large Dutch cohort. *Hum Reprod* 26: 3456-3465.
30. Anckaert E, De Rycke M, Smits J (2013) Culture of oocytes and risk of imprinting defects. *Hum Reprod Update* 19: 52-66.

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