Sequential versus Monophasic Media Impact Trial (SuMMIT): a paired randomized controlled trial comparing a sequential media system to a monophasic medium

Marie D. Werner, M.D.,[a,b] Kathleen H. Hong, M.D.,[a,b] Jason M. Franasiak, M.D.,[b] Eric J. Forman, M.D., H.C.L.D.,[a,b] Christine V. Reda, B.S.N., R.N.,[a] Thomas A. Molinaro, M.D., M.S.C.E.,[a,b] Kathleen M. Upham, B.S.,[a] and Richard T. Scott Jr., M.D., H.C.L.D.[a,b]

[a] Reproductive Medicine Associates of New Jersey and [b] Robert Wood Johnson Medical School of Rutgers University, Basking Ridge, New Jersey

Objective: To determine whether sequential or monophasic media is the more optimal formulation for blastocyst development and sustained implantation rates (SIR) in IVF.
Design: Paired randomized controlled trials.
Setting: Academic.
Patient(s): Infertile couples (N = 192) with female partner ≤42 years old and normal ovarian reserve.
Intervention(s): Fertilized zygotes from each patient were randomly divided into two groups: [1] cultured in sequential media and [2] cultured in monophasic medium. Sequential media consisted of Quinn's Advantage Cleavage Medium (SAGE) followed by Blast Assist (Origio). The monophasic medium used was Continuous Single Culture (Irvine Scientific). Paired ETs were accomplished by transferring the best euploid blastocyst from each media group. DNA fingerprinting was used to link outcomes.
Main Outcome Measure(s): The primary outcome measure was the proportion of blastocysts suitable for clinical use. Secondary outcome measures included timing of blastulation, aneuploidy rates, and SIR. Sustained implantation rate is defined as the number fetal heart beats at 8–9 weeks of gestation, divided by the number of embryos transferred.
Result(s): A total of 192 patients had their 2PN embryos (N = 2,257) randomized to each culture system. Sequential media had higher blastulation rate than monophasic medium (55.2% vs. 46.9%). No differences were found in the day of blastulation or aneuploidy rate. Of the 168 patients who had euploid blastocysts suitable for transfer, 126 completed a paired ET. Among the double ETs, there was no difference in implantation between groups.
Conclusion(s): This is the first randomized controlled trial to examine paired euploid transfers of sibling zygotes cultured in sequential versus monophasic media. This study demonstrates that the usable blastocyst rate is greatest after culture in the sequential media tested in comparison with the monophasic formulation selected for study. However, no difference exists in timing of blastulation, aneuploidy, or SIR. Whether these observations are generalizable to other media systems remains to be determined.
Clinical Trial Registration Number: NCT01917240. (Fertil Steril® 2016;105:1215–21. ©2016 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).)
Key Words: Embryo culture, media for IVF, embryo development, in vitro fertilization, culture media

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/wernerm-sequential-monophastic-medium/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received August 28, 2015; revised and accepted January 5, 2016; published online January 27, 2016.
M.D.W. has nothing to disclose. K.H.H. has nothing to disclose. J.M.F. has nothing to disclose. E.J.F. has nothing to disclose. C.V.R. has nothing to disclose. T.A.M. has nothing to disclose. K.M.U. has nothing to disclose. R.T.S. has nothing to disclose.
Reprint requests: Marie D. Werner, M.D., 140 Allen Road, Basking Ridge, New Jersey 07920 (E-mail: mwerner@rmanj.com).

Fertility and Sterility® Vol. 105, No. 5, May 2016 0015-0282

http://dx.doi.org/10.1016/j.fertnstert.2016.01.005

Two widely used but distinct approaches of culture media have emerged to support embryonic growth to the blastocyst stage of development: sequential and monophasic media. Sequential media, otherwise known as the "back to nature," approach is a two-step formulation designed to accommodate changes in embryo metabolism before and after the compaction stage of development (1, 2). In contrast, monophasic formulations are one step and capable of supporting embryonic growth through all stages of development. Functionally, monophasic formulations "let the embryo choose" which nutrients are required whereas sequential formulations may mimic in vivo conditions more closely (3, 4). Both culture systems have demonstrated excellent clinical outcomes. However, at present, there is no consensus among clinical programs as to which approach is optimal as evidenced that both systems remain in widespread clinical use.

The first attempts at blastocyst culture used monophasic formulations, as referenced, but these were replaced by sequential culture systems, which purported better implantation rates (5–7). Sequential culture systems reflected an increased understanding of the physiologic requirements of the growing embryo in vivo (8–11). Step 1 primarily consists of nonessential amino acids, ethylenediaminetetraacetic acid (EDTA), pyruvate, and a reduced concentration of glucose to support before compaction development. In a temporally correct manner, step 2 removes EDTA, adds essential amino acids, decreases the concentration of pyruvate and increases the concentration of glucose to support after compaction development. Most of the pioneering work in support for sequential media was performed using animal models that demonstrated improved fetal development and increased implantation rates (12–14).

A continued strong interest remained in optimizing monophasic formulations, as they are simpler to use and less expensive in practice. Monophasic medium incorporates all the necessary ingredients for culture to blastocyst stage including nonessential and essential amino acids, EDTA, glucose, pyruvate, and lactate in a constant concentration. Consequently, monophasic medium requires less manipulation of the embryo and fewer resources to support development, especially if medium is not renewed on day 3 of development. The improvement in monophasic culture systems was rigorously developed in animal model using simplex optimization (4, 15).

Several sequential and monophasic media formulations are US Food and Drug Administration approved and commercially available. Ready availability of these high quality culture media has likely contributed to increased utilization of blastocyst culture in clinical settings. Some studies suggest equivalent outcomes in embryological parameters when tested among sibling zygotes, but are underpowered to assess differences in implantation and have not yet assessed aneuploidy risk (16–18). At present, there has not been an adequately powered randomized rigorously controlled trial to evaluate blastocyst culture in contemporary IVF practice. The goal of the present analysis is to perform a paired, prospective randomized controlled trial to assess usable blastulation rates, aneuploidy risk, and sustained implantation rates among sibling zygotes cultured in monophasic compared with sequential media. For the purposes of this analysis the sequential media tested consisted of Quinn's Advantage Cleavage Medium (SAGE) followed by Blast Assist (Origio). The monophasic media used was Continuous Single Culture (Irvine Scientific).

MATERIALS AND METHODS

Patient Population

Infertile couples attempting conception through IVF at a single center from August 2013 to March 2015 were evaluated to determine study eligibility. Given the paired design where each patient served as her own control, it was critical to recruit patients who met the American Society for Reproductive Medicine guidelines for having two (or more) embryos transferred (19). All study participants had also elected to use comprehensive chromosomal screening to assess for embryonic aneuploidy.

Inclusion criteria reflected the paired nature of the study design and targeted patients with normal ovarian reserve, therefore each patient would serve as her own control. Patients $\leq$42 years old at the time of IVF cycle were eligible for inclusion if the FSH level was $<$12 IU/L, AMH $\geq$1.2 ng/mL, and basal antral follicle count $\geq$12. To exclude confounding variables, patients with the following characteristics were not offered enrollment [1] chronic endometrial insufficiency, [2] use of oocyte donation or gestational carriers, [3] medical contraindication to double embryo transfer or IVF, [4] severe male factor infertility requiring surgical sperm extraction, or [5] single gene disorders.

The protocol was Institutional Review Board approved and registered with clinicaltrials.gov (NCT01917240). All patients that met inclusion criteria and expressed the desire to participate in the study were consented before ovarian stimulation.

IVF Cycle Management

There were no restrictions on stimulation protocols as the study intervention began after fertilization was confirmed. Due to the paired nature of the study design every patient had their zygotes randomized to sequential and monophasic media. This design allowed for a comparison of zygotes exposed to identical endocrine milieus during follicular stimulation, thereby eliminating confounding variables associated with follicular stimulation.

Standard regimens for controlled ovarian hyperstimulation (COH) were employed using purified urinary FSH or recombinant FSH and LH activity in the form of low-dose hCG or hMG along with GnRH agonist (long down-regulation or microdose flare) or GnRH antagonist to prevent a premature LH surge. Monitoring of IVF cycles were per practice routine. Oocyte maturation was induced with recombinant hCG, purified urinary hCG, or with GnRH agonist. This was performed when at least two follicles reached 17–18 mm or when the follicular cohort was deemed to be mature by the patient's primary physician. Transvaginal oocyte aspiration was performed approximately 36 hours later. Cumulus