Blastocyst utilization rates after continuous culture in two commercial single-step media: a prospective randomized study with sibling oocytes

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Abstract

Purpose The aim of this study is to determine whether blastocyst utilization rates are different after continuous culture in two different commercial single-step media.

Methods This is a paired randomized controlled trial with sibling oocytes conducted in infertility patients, aged ≤40 years with ≥10 oocytes retrieved assigned to blastocyst culture and transfer. Retrieved oocytes were randomly allocated to continuous culture in either Sage one-step medium (Origio) or Continuous Single Culture (CSC) medium (Irvine Scientific) without medium renewal up to day 5 post oocyte retrieval. Main outcome measure was the proportion of embryos suitable for clinical use (utilization rate).

Results A total of 502 oocytes from 33 women were randomly allocated to continuous culture in either Sage one-step medium ($n = 250$) or CSC medium ($n = 252$). Fertilization was performed by either in vitro fertilization or intracytoplasmic sperm injection, and embryo transfers were performed on day 5. Two patients had all blastocysts frozen due to the occurrence of severe ovarian hyperstimulation syndrome. Fertilization and cleavage rates, as well as embryo quality on day 3, were similar

in the two media. Blastocyst utilization rates (%; 95% CI) [55.4% (46.4–64.1) vs 54.7% (44.9–64.6), $p = 0.717$], blastocyst formation rates [53.6% (44.6–62.5) vs 51.9 (42.2–61.6), $p = 0.755$], and proportion of good quality blastocysts [36.8% (28.1–45.4) vs 36.1% (27.2–45.0), $p = 0.850$] were similar in Sage one-step and CSC media, respectively.

Conclusions Continuous culture of embryos in Sage one-step and CSC media is associated with similar blastocyst development and utilization rates. Both single-step media appear to provide adequate support during in vitro preimplantation embryo development. Whether these observations are also valid for other continuous single medium protocols remains to be determined.

Clinical trial registration number: NCT02302638.

Keywords Embryo culture · Single step · Continuous culture · Blastocyst formation · Utilization rate

Introduction

In recent years, there has been renewed interest in the use of single culture media, which are designed to support the entire embryo preimplantation development based on a single formulation. Single media are based on the principle of allowing developing embryos to choose the nutrients they need, while at the same time minimizing stress from exposure to different culture environments [1, 2].

Embryo culture in single-step media is associated with several benefits, one of the most important being the compatibility with the increasingly popular time-lapse imaging systems [3–8]. As a result, an increasing number of single media have become commercially available and have been swiftly introduced in embryology laboratories worldwide.

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A recent study comparing the composition and blastocyst formation rates among four single-step culture media using a mouse model showed significant compositional differences which seem to affect mouse embryo development [9].

A comparison between single media in human in vitro fertilization (IVF) has been previously performed in only a single clinical trial, which failed to show any significant differences in blastocyst development and utilization between different single-step formulations [10].

The aim of this randomized controlled trial was to determine whether blastocyst utilization rates are different after continuous culture in two commercial single-step media, namely Sage one-step medium and Continuous Single Culture (CSC) medium.

Materials and methods

Study design

This was a single-center prospective randomized study with sibling oocytes performed from October 2014 to April 2015. Patients were ≤ 40 years of age, had at least 10 cumulus-oocyte complexes (COCs) retrieved, and underwent blastocyst culture. Cases with oocyte cryopreservation, surgical sperm recovery, triggering final oocyte maturation using GnRH agonist, or preimplantation genetic diagnosis were excluded. Patients could enter the study only once. Doctors, patients, and the assessing embryologist were blinded to the type of medium used.

The study was approved by the Unit's Institutional Review Board (IRB approval number 01/11-9-14). Informed consent was obtained from all individual participants included in the study. The study was registered at clinicaltrials.gov (NCT02302638) before the onset of patient recruitment.

Ovarian stimulation

Patients were treated using a flexible GnRH antagonist protocol, as previously described [11]. Patients were administered 150 IU/day recombinant FSH (Gonal-F, Merck Serono, Geneva, Switzerland) as starting dose from day 2 of the cycle. The dose was adjusted after day 5 of stimulation, depending on the ovarian response, as assessed by estradiol levels and ultrasound. Administration of rFSH continued daily thereafter until and including the day of human chorionic gonadotropin (hCG) administration. GnRH antagonist cetrorelix 0.25 mg (Cetrotide, Merck Serono, Geneva, Switzerland) was initiated when at least one of the following criteria was fulfilled: (i) the presence of at least one follicle measuring >14 mm, (ii) E_2 serum levels >600 pg/ml, and (iii) LH serum levels >10 IU/l and continued daily thereafter until and including the day of hCG administration.

When at least three follicles of diameter ≥ 17 mm were present, final oocyte maturation was triggered by i.m. injection of 10,000 IU hCG (Pregnyl; MSD, Oss, The Netherlands) or 5000 IU hCG in high risk for ovarian hyperstimulation syndrome (OHSS) patients (≥ 19 follicles on the day of triggering [12]) to reduce the possibility of OHSS development, as previously described [13, 14]. Transvaginal ultrasound-guided oocyte retrieval was performed 36 h later. The luteal phase was supplemented with daily vaginal administration of 600 mg natural micronized progesterone in three separate doses (Utrogestan; Besins, Brussels, Belgium) starting 1 day after oocyte retrieval and continued until 7 weeks of gestation if pregnancy was achieved.

Oocyte randomization

Randomization of COCs was performed at the time of oocyte retrieval. A 1:1 randomization was achieved using a computer-generated randomization list. Allocation concealment was achieved using sequentially numbered, opaque, sealed envelopes, as has been previously described [15–17]. During oocyte retrieval, COCs from each patient were placed in an alternated manner, as they were retrieved, in different dishes, labeled either group A or group B. The study embryologist then opened a sealed envelope designating which medium group A and group B corresponded to.

Oocyte recovery, fertilization, and embryo culture

COCs were recovered in a MOPS-buffered medium (GMOPS, Vitrolife, Goteborg, Sweden) and then placed in Universal IVF medium (Origio, Malov, Denmark) until insemination or denudation. Oocytes were fertilized with conventional IVF or intracytoplasmic sperm injection (ICSI) depending on semen quality and previous patient history. In the case of conventional IVF, COC was inseminated in groups of five with 150,000/ml spermatozoa in 1 ml CSC medium (CSC group) (Santa Ana, CA, USA) or Universal IVF medium (Sage one-step group) (Origio, Malov, Denmark). In the case of ICSI, cumulus cells were removed using 80 IU hyaluronidase solution (SynVibro Hyadase, Origio, Malov, Denmark), and stripped oocytes were placed in either CSC medium (CSC group) or Universal IVF medium (Sage one-step group) until injected. Immediately after ICSI, oocytes were placed in either CSC medium or Sage one-step medium, as assigned. Fertilization was assessed 16–18 h after insemination, and fertilized oocytes (2PN) were cultured in either 1 ml CSC medium or 1 ml Sage one-step medium under oil in one-well IVF dishes (Falcon, Corning, NY, USA). Embryos were cultured in groups continuously without media changes up to day 5.

Embryos from both groups were cultured in parallel in an atmosphere of 6.5% CO_2 /5% O_2 /88.5% N_2 on the same shelf of the same incubator (Heracell 240, Thermo Fisher Scientific,

Waltham, USA) for the entire duration of culture, aiming for a pH range 7.3 ± 0.1 , as recommended by the culture media manufacturers.

There was continuous pH monitoring of CSC and Sage one-step media during embryo culture using pH online recording system (MTG, Bruckberg, Germany) in order to ensure optimal performance of all media used.

Embryo transfer was performed on day 5 in hyaluronate-containing UTM medium (Origio, Malov, Denmark). Supernumerary embryos were cryopreserved either on day 5 or day 6.

Both media used in the current study were ready to use and were already supplemented with 5 mg/ml human serum albumin by the manufacturers.

Embryo assessment and transfer

Cleavage-stage embryos were assessed on day 3 (68 ± 1 h post insemination) using the Istanbul criteria [18], based on morphology (number, size, and shape of blastomeres, degree of fragmentation, and multinucleation), and were categorized in three grades [grade 1 (highest) to grade 3 (lowest)]. Embryos with four cells on day 2 and embryos with six to eight cells on day 3 and $<10\%$ fragmentation were regarded as top quality embryos (grade 1). Blastocyst development was assessed on day 5 (116 ± 2 h post insemination), taking into account the degree of expansion and the appearance of inner cell mass and trophectoderm, according to the Gardner scale [19–21]. Good quality blastocysts should have a score of at least 3BB.

No attempt was made to transfer embryos from only one medium, and selection for transfer was based on blastocyst quality regardless of the medium used.

Embryo assessment was performed by a blinded embryologist to avoid bias.

Outcome measures

Main outcome measure was the proportion of embryos suitable for clinical use (utilization rate, i.e., number of embryos transferred and cryopreserved over number of 2PN).

Secondary outcomes included blastocyst formation rate on day 5 (proportion of 2PN reaching at least early blastocyst), proportion of good quality blastocysts (at least 3BB) on day 5, fertilization rate, cleavage rate, number of good quality embryos on day 3, number of embryos selected for transfer, and number of supernumerary good quality blastocysts cryopreserved. Positive hCG, clinical pregnancy (presence of fetal sac with positive heartbeat at 7 weeks of gestation), ongoing pregnancy (presence of fetal sac with positive heartbeat at 12 weeks of gestation), live birth rate (after 24 weeks of gestation), and implantation rate (number of sacs with positive heartbeat over number of embryos transferred) were also assessed in cycles where transfer was performed using embryos originating

exclusively from one medium (either CSC or Sage one-step) or both (mixed transfers).

Statistical analysis

It was calculated that 32 patients would be required to show a mean paired difference of 10% in embryo utilization rates assuming $\beta = 0.8$ and $\alpha = 0.05$, using a two-sided Wilcoxon test.

Paired *t* test or a Wilcoxon signed-rank test were used for paired comparison of outcome measures between the two culture media depending on the normality of their distribution.

Statistical analysis was performed with the use of IBM SPSS Statistics (Version 24, IBM Corporation). Statistical significance was accepted when $p < 0.05$.

Results

A total of 502 COCs were retrieved from 33 women and were randomly allocated to continuous culture in either Sage one-step medium ($n = 250$) or CSC medium ($n = 252$).

Patient and cycle characteristics are shown in Table 1.

Conventional IVF and ICSI were used in 12 and 21 patients, respectively. Data were combined as no significant effects were observed due to the fertilization technique.

The proportion of embryos suitable for clinical use [mean (95% CI)] was similar in Sage one-step and CSC medium [55.4% (46.4–64.1) vs 54.7% (44.9–64.6), $p = 0.717$] (Table 2).

Table 1 Patient and cycle characteristics

Parameter	Mean $\pm$ SD
Number of patients	33
Age (years)	35.7 ± 4.5
BMI (kg/m^2)	23.5 ± 4.1
Number of previous attempts	0.6 ± 1.1
Years of infertility	3.7 ± 2.0
Antral follicle count	11.1 ± 3.3
Basal FSH (IU/l)	6.9 ± 2.6
Basal LH (IU/l)	5.6 ± 1.7
Basal progesterone (ng/ml)	0.7 ± 0.3
Number of follicles >11 mm on hCG day	15.1 ± 6.9
Oestradiol on hCG day (pg/ml)	2399.9 ± 945.3
Progesterone on hCG day (ng/ml)	1.1 ± 0.5
Total FSH dose (IU)	1720.7 ± 540.4
Number of COCs retrieved	15.2 ± 3.9
Number of 2PN	9.9 ± 3.9
Days of stimulation	10.5 ± 1.9
Number of embryos transferred	2.2 ± 0.5

Table 2 Characteristics of embryos cultured in Sage one-step and CSC media

	Sage one-step Mean (SD) 95% CI	Irvine CSC	<i>p</i>
Mature oocytes (% of oocytes retrieved)	86.0 (12.4) 81.6–90.4	85.6 (14.8) 80.4–90.9	0.733 ^b
Fertilized oocytes, 2PN (% of oocytes retrieved)	68.6 (21.2) 61.1–76.1	72.4 (17.6) 60.4–73.9	0.359 ^a
Cleavage (% of 2PN)	95.4 (9.0) 92.2–98.5	98.9 (6.5) 96.5–100	0.092 ^b
Day 3			
≥6 cells (% of 2PN)	84.6 (17.0) 78.6–90.6	84.6 (21.7) 76.9–92.3	0.915 ^b
≤10% fragmentation (% of 2PN)	77.5 (28.0) 67.6–87.4	75.2 (25.9) 66.0–84.4	0.467 ^b
Good quality embryos (% of 2PN)	70.2 (28.5) 60.1–80.3	69.5 (26.6) 60.1–79.0	0.700 ^b
Day 5			
Blastocysts (% of 2PN)	53.6 (25.2) 44.6–62.5	51.9 (27.3) 42.2–61.6	0.755 ^a
At least full blastocysts (% of 2PN)	45.0 (25.0) 36.1–53.8	44.0 (25.0) 35.1–52.8	0.896 ^a
Good quality blastocysts (% of 2PN)	36.8 (24.4) 28.1–45.4	36.1 (25.1) 27.2–45.0	0.850 ^a
ICM grade A (% of full blasts)	64.7 (35.0) 51.4–78.0	56.7 (38.4) 42.1–71.3	0.732 ^b
TE grade A (% of full blasts)	67.2 (30.2) 55.7–78.6	44.1 (37.1) 30.7–57.5	0.035 ^b
Embryo transfer and utilization			
Embryos transferred (% of 2PN)	25.0 (18.2) 18.5–31.5	17.5 (14.8) 12.2–22.7	0.104 ^b
Blastocysts frozen (% of 2PN)	30.4 (26.4) 21.1–39.8	37.3 (30.9) 26.3–48.2	0.178 ^b
Embryo utilization (% of 2PN)	55.4 (24.5) 46.4–64.1	54.7 (27.9) 44.9–64.6	0.717 ^b

^a Paired samples *t* test^b Related samples Wilcoxon signed-rank test

Oocyte maturation rates [86.0 (81.6–90.4) vs 85.6 (80.4–90.9), $p = 0.733$], fertilization rates [68.6% (61.1–76.1) vs 72.4% (60.4–73.9), $p = 0.359$], and cleavage rates [95.4% (92.2–98.5) vs 98.9% (96.5–100), $p = 0.092$] were similar in Sage one-step and CSC, respectively. On day 3, proportion of embryos with good morphology [70.2% (60.1–80.3) vs 69.5% (60.1–79.0), $p = 0.700$], proportion of embryos with ≥6 cells [84.6% (78.6–90.6) vs 84.6% (76.9–92.3), $p = 0.915$], and proportion of embryos with ≤10% fragmentation [77.5% (67.6–87.4) vs 75.2% (66.0–84.4), $p = 0.467$] were comparable in Sage one-step and CSC, respectively.

On day 5, blastocyst formation rates [53.6% (44.6–62.5) vs 51.9 (42.2–61.6), $p = 0.755$], proportion of at least full blastocysts [45.0% (36.1–53.8) vs 44.0% (35.1–52.8), $p = 0.896$], proportion of good quality blastocysts (≥3BB) [36.8% (28.5–45.1) vs 36.1% (27.5–44.7), $p = 0.850$], and proportion of blastocysts with grade A inner cell mass [64.7% (51.4–78.0) vs 56.7% (42.1–71.3), $p = 0.732$] were similar in Sage one-step and CSC media, respectively. The proportion of blastocysts with grade A trophoderm [67.2% (55.7–78.6) vs 44.1% (30.7–57.5), $p = 0.035$] appeared to be higher in Sage one-step medium compared to CSC medium. The proportion of embryos selected for transfer [25.0% (18.5–31.5) vs 17.5% (12.2–22.7), $p = 0.104$] and cryopreservation [30.4% (21.1–39.8) vs 37.3% (26.3–48.2), $p = 0.178$] was similar in Sage one-step and CSC media (Table 2).

The pH achieved during the study period was 7.24 ± 0.02 for Sage one-step and 7.31 ± 0.02 for CSC.

Thirty-one patients had an embryo transfer on day 5, while two patients had all blastocysts cryopreserved on day 5 due to OHSS development.

There were six live births (75.0%; 95% CI 40.9–92.9) resulting from Sage-only transfers, three live births (75.0%; 95% CI 30.1–95.4) from CSC-only transfers, and nine live births (47.4%; 95% CI 27.3–68.3) from mixed transfers.

Discussion

The present study suggests that continuous culture of human embryos in two different single media formulations is associated with similar blastocyst utilization rates.

The implementation and optimization of extended culture conditions of human embryos to the blastocyst stage has attracted attention for nearly two decades [21, 22]. Blastocyst culture may be performed using either a single medium or sequential media, which represent two fundamentally different approaches to human embryo culture, both in principle and composition.

Sequential culture media were designed to meet the changing metabolic and nutritional requirements of the developing preimplantation embryo, reflecting the fluid composition of the female reproductive tract [19, 23, 24], and made human

blastocyst culture a possibility in routine clinical practice. On the other hand, single media were designed to support the entire embryo preimplantation development based on a single formulation [1–3]. More recently, single-step embryo culture has received renewed attention, with new generation single media becoming commercially available and challenging the necessity of their sequential counterparts.

Numerous studies have compared the efficacy of single and sequential media, and evidence to date suggest that single media appear to perform better than sequential ones in terms of blastocyst formation rates [4, 5, 7, 17, 25–30], although pregnancy rates appear similar using the two types of media [30].

However, direct comparisons of different single media are currently lacking in the literature. One previous study comparing two commercial single media reported no differences in blastocyst formation and utilization rates [10], corroborating the findings of the present study.

Two basic aspects should be considered when comparing different single medium protocols: (a) the formulation of the media and (b) the renewal or not of medium on day 3.

There is evidence from animal [31] and human studies [1, 32] to suggest that embryo quality and blastocyst development are similar when single media are used in either a continuous or interrupted fashion.

Continuous uninterrupted culture without media renewal may confer several advantages, including sustained presence in the medium of embryo-secreted autocrine/paracrine factors, reduced stress to the embryo from pipetting and fluctuation of culture conditions, lower exposure to contaminants, lower probability of unintentional handling errors, reduced staff labor, and lower cost of consumables [3, 4, 33]. Moreover, compatibility with time-lapse systems is undoubtedly a significant benefit of continuous single medium protocols [5–8].

However, at the same time, the success of continuous embryo culture largely relies on closely monitored optimal laboratory conditions and a series of precautions against atmospheric fluctuations and accumulation of volatile organic compounds and ammonium, which may negatively affect the culture environment [42–45].

There seem to be more similarities than differences in the compositions of Sage one-step and CSC media in terms of amino acid, organic acid, and ion content [9]. CSC is primarily based on simplex optimized KSOM [2], while Sage one-step seems similar to CSC, although concentrations of inorganic ions are different and share many characteristics with its predecessor Sage sequential series [34]. In addition, both media are presupplemented with human serum albumin; they both contain EDTA, and they both include glutamine and alanyl in the form of a stable alanyl-glutamine dipeptide to minimize ammonia production [9, 34].

A marked difference in the composition of the two media was that hyaluronan was present in Sage one-step but was

absent in CSC. Hyaluronan in combination with human serum albumin (HSA) has been found to increase embryo development and to improve embryo cryosurvival [35]. In addition, evidence suggests improved clinical pregnancy and live birth rates with the use of hyaluronic acid as an adherence compound in the embryo transfer medium [36]. A putative beneficial effect of hyaluronan in Sage one-step on blastocyst development and pregnancy rates could not be demonstrated in the present study.

Another difference between the two media is the level of glucose. The media composition analysis by Morbeck et al. [9] showed lower glucose levels in Sage one-step (0.19 mM) compared to CSC (0.47 mM), while puruvate, lactate, and lactate/pyruvate ratios were similar in the two media. The presence of glucose has been shown to be a requirement in the post compaction phase of *in vitro* embryo development, but its effect on early precompaction embryos is still surrounded by controversy [21, 37]. Notably, the proportion of blastocysts with a top quality trophectoderm (grade A: layer with many cells forming a cohesive epithelium) were significantly higher after culture in Sage one-step compared to CSC. A strong trophectoderm layer is essential for successful hatching and implantation, and trophectoderm morphology has been previously suggested to predict pregnancy outcome and live birth [38–41]. Whether better trophectoderm morphology, observed in the present study in Sage one-step, may be attributed to the presence of hyaluronan or lower levels of glucose remains to be elucidated. Although the patient population of the present study is too small to make meaningful comparisons of reproductive outcomes, an eventual impact of better trophectoderm on cumulative pregnancy rates between the two media should be investigated in future studies.

In conclusion, both commercial single media appear to equally promote blastocyst development and to yield similar utilization rates when used continuously without renewal, with the exception of higher incidence of grade A trophectoderm associated with culture in Sage one-step compared to CSC medium. Whether these observations are also valid for other continuous single medium protocols remains to be determined. Future research is necessary to compare reproductive outcomes in a RCT with patients as the randomization unit.

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Author contributions IAS conceived the study, performed acquisition, analysis and interpretation of data, writing and revision of the manuscript, and embryology work. EMK, GTL, and CAV participated in the analysis and interpretation of data and writing and revision of the manuscript. GKP and BCT participated in the interpretation of data and revision of the manuscript. TGL had the general supervision of the study, participated in study design, analysis and interpretation of data, writing and revision of the manuscript, and performed clinical work. All authors read and approved the final manuscript.

Compliance with ethical standards

Study funding Sage one-step medium was provided by Origio, and CSC medium was provided by Biocare Europe.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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