ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Composition of single-step media used for human embryo culture

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Objective: To determine compositions of commercial single-step culture media and test with a murine model whether differences in composition are biologically relevant.
Design: Experimental laboratory study.
Setting: University-based laboratory.
Animal(s): Inbred female mice were superovulated and mated with outbred male mice.
Intervention(s): Amino acid, organic acid, and ions content were determined for single-step culture media: CSC, Global, G-TL, and 1-Step. To determine whether differences in composition of these media are biologically relevant, mouse one-cell embryos were cultured for 96 hours in each culture media at 5% and 20% oxygen in a time-lapse incubator.
Main Outcome Measure(s): Compositions of four culture media were analyzed for concentrations of 30 amino acids, organic acids, and ions. Blastocysts at 96 hours of culture and cell cycle timings were calculated, and experiments were repeated in triplicate.
Result(s): Of the more than 30 analytes, concentrations of glucose, lactate, pyruvate, amino acids, phosphate, calcium, and magnesium varied in concentrations. Mouse embryos were differentially affected by oxygen in G-TL and 1-Step.
Conclusion(s): Four single-step culture media have compositions that vary notably in pyruvate, lactate, and amino acids. Blastocyst development was affected by culture media and its interaction with oxygen concentration. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Culture media, embryo culture, mouse embryo assay, quality control

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Assisted reproductive technology (ART) success, defined as delivery of a healthy child, depends importantly on quality of the embryo culture environment. The foundation of this environment is the culture medium, yet detailed composition of this medium is not provided by manufacturers, despite requests from the embryology community (1, 2).

Routine time-lapse imaging for embryo culture introduced a paradigm shift for embryo culture, because more information is obtained with less handling compared with conventional culture (3). This shift favors single-step media over sequential media and in turn has led to the introduction of new single-step media from companies that previously only offered sequential media (4). A new culture medium can be introduced to the market without extensive clinical testing and without disclosure of detailed composition. Furthermore, although media composition varies considerably (5), the scientific rationale for different formulations is rarely provided.

Identification of the best and safest conditions for embryo culture to support optimal embryo development remains elusive. Assisted reproductive technology laboratories are faced with choices without having detailed compositional analysis. Details of the long-term effects of culture media on assisted reproductive technology are limited by a lack of data (1, 6, 7).

To address this gap, we analyzed the composition of single-step culture media from four suppliers and determined whether differences in media composition were biologically relevant by culturing single-cell murine embryos to the blastocyst stage. Cultures were performed with reduced (5%) and ambient (20%) oxygen, because we had previously demonstrated medium × oxygen interactions that altered mouse embryo development (5).

MATERIALS AND METHODS

Culture Media Analysis

Culture media sources. Culture media were purchased in April and May 2015 from four suppliers and included Global (Global-Total, LifeGlobal, IVFOnline), CSC (Irvine Scientific), G-TL (Vitrolife), and 1-Step (Origio). Global is provided with 10 mg/mL human

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serum albumin (HSA), whereas the other three media contained 5 mg/mL HSA.

Amino acid and organic acid analysis. Amino acids were quantified in duplicate (5). Organic acids (lactate, pyruvate, and citrate) were prepared as oximated silyl-derivatives for stable isotope dilution for capillary gas chromatography–mass spectrometry analysis. Briefly, 100 μL of each sample was diluted 1:1 into isotopic internal standard mix (containing D^4-citrate, D^4-lactate, $^{13}C_2$-pyruvate), followed by oximation with pentafluorobenzyl hydroxylamine. Organic oximes were extracted with ethyl acetate and derivatized with N,O,-bis-(trimethylsilyl) trifluoroacetamide and 1% trimethylchlorosilane to form silyl-derivatives. Oximes (1 μL) were injected into an Agilent 6890/5973 gas chromatograph–mass spectrometer using a splitless/split combination (50:1 split mode). Quantification of analytes was performed by comparing measured peak areas of quantifying ions against unique quantifying ions of known concentrations of isotope-labeled internal standards. Coefficients of variation for citrate, lactic acid, and pyruvate at concentrations measured were 6.8%, 1.9%, and 2.1%, respectively.

Inorganic ion analysis. Calcium (colorimetric o-cresolphthalein complexone), chloride (ion-selective electrode [ISE]), potassium (ISE module), magnesium (colorimetric), sodium (indirect ISE), and phosphorus (photometric) were quantified in duplicate using Roche Cobas reagents and chemistry analyzers (Cobas 6000 c501 or Cobas 8000 ISE, c701, c502 modules) as previously described (5).

Other analytes. Glucose (hexokinase) was quantified in duplicate using Roche Cobas chemistry analyzers (Cobas 6000 c501 or Cobas 8000 ISE, c701, c502 modules), and lactate was assayed by lactate oxidase/peroxidase (dry slide chemistry) on a Vitros 350 chemistry analyzer (Ortho Clinical Diagnostics) as previously described (5). pH and osmolality were determined as previously described (8).

Mouse Embryo Assay

The Mayo Clinic Institutional Animal Care and Use Committee approved procedures involving animals. Mice were obtained from Charles River Laboratories. Six- to nine-week-old FVB mice were superovulated with 5 IU of intraperitoneal pregnant mare serum (National Hormone and Peptide Program), followed 48 hours later with 5 IU of intraperitoneal hCG (APP Pharmaceuticals). Females were caged individually with male CF1 mice overnight, and mating was confirmed by presence of a vaginal plug. Cumulus surrounded zygotes were isolated from oviducts obtained 18 hours after hCG administration.

After denuding zygotes with hyaluronidase (250 u/mL; H3506, Sigma Chemical), embryos were transferred individually into 25 μL of media in an EmbryoSlide (Unisense Fertilitech), and each slide was inserted into an EmbryoScope (Unisense Fertilitech). A 4 × 2 factorial study was performed with four media at 5% and 20% oxygen. All experiments were performed in triplicate at 37°C and 6.2% CO_2, with 10 to 11 embryos for each protein/condition combination. Expanded blastocyst at 96 hours of culture was the primary endpoint. Data for precise cell division timings using time-lapse imaging were obtained; all annotations were performed by one technician.

Data and Statistical Analysis

Developmental and time-lapse data were analyzed using a one-way analysis of variance (ANOVA) with Tukey's test for pairwise comparisons of blastocyst development and cell division timings. Analyses were performed in two steps. First, a 4 × 2 factorial ANOVA was used to compare blastocyst development and timings for four media at low and high oxygen. Second, because the primary treatment effect observed was a media × oxygen interaction, a Student *t* test ANOVA was used to compare blastocyst development and timings between low and high oxygen within each medium. Statistical analyses were performed using JMP statistical software (SAS Institute).

RESULTS

Media Composition

Two of the culture media (G-TL and 1-Step) were only available presupplemented with protein (HSA), therefore all experiments used presupplemented media. Protein supplementation constitutes addition of undefined components, but our analysis represents only components reported by manufacturers or known components of albumin preparations (e.g., octanoate). Manufacturer-reported components that were not analyzed include ethylenediaminetetraacetic acid, gentamicin, dipeptide forms of glutamine, calcium pantothenate, pyridoxine, riboflavin, thiamine, sodium bicarbonate, and sodium hyaluronate. Age of culture media may impact composition (9), therefore all analyses were performed within 60 days of media manufacture date.

Glucose and organic acids. Pyruvate, lactate, and glucose are the primary energy substrates for preimplantation embryos. All four media contained these carbohydrates, but concentrations differed (Table 1). Global and 1-Step had similar concentrations of all three substrates. G-TL had a fivefold higher concentration of glucose and twofold higher concentrations of pyruvate and lactate than Global. The ratio of pyruvate to lactate was similar for all four media.

TABLE 1

Concentrations of glucose and organic acids, and the lactate to pyruvate (L/P) ratio in embryo culture media.

	Medium			
Variable	**Global**	**CSC**	**G-TL**	**1-Step**
Glucose (mM)	0.18	0.47	0.97	0.19
Citrate (mM)	0	0.02	0.01	0
Octanoate (mM)	0.681	0.324	0.344	0.355
Lactate (mM)	4.9	5.71	10.01	4.35
Pyruvate (mM)	0.24	0.28	0.55	0.22
L/P ratio	20	21	18	20

Morbeck. Composition of continuous culture media. Fertil Steril 2017.