Composition of commercial media used for human embryo culture

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Objective: To determine the composition of commercially available culture media and test whether differences in composition are biologically relevant in a murine model.
Design: Experimental laboratory study.
Setting: University-based laboratory.
Animal(s): Cryopreserved hybrid mouse one-cell embryos were used in experiments.
Intervention(s): Amino acid, organic acid, ions, and metal content were determined for two different lots of media from Cook, In Vitro Care, Origio, Sage, Vitrolife, Irvine CSC, and Global. To determine whether differences in the composition of these media are biologically relevant, mouse one-cell embryos were thawed and cultured for 120 hours in each culture media at 5% and 20% oxygen in the presence or absence of protein in an EmbryoScope time-lapse incubator.
Main Outcome Measure(s): The compositions of seven culture media were analyzed for concentrations of 39 individual amino acids, organic acids, ions, and elements. Blastocyst rates and cell cycle timings were calculated at 96 hours of culture, and the experiments were repeated in triplicate.
Result(s): Of the 39 analytes, concentrations of glucose, lactate, pyruvate, amino acids, phosphate, calcium, and magnesium were present in variable concentrations, likely reflecting differences in the interpretation of animal studies. Essential trace elements, such as copper and zinc, were not detected. Mouse embryos failed to develop in one culture medium and were differentially affected by oxygen in two other media.
Conclusion(s): Culture media composition varies widely, with differences in pyruvate, lactate, and amino acids especially notable. Blastocyst development was culture media dependent and showed an interaction with oxygen concentration and presence of protein. (Fertil Steril® 2014;102:759–66. ©2014 by American Society for Reproductive Medicine.)
Key Words: Culture media, quality control, mouse embryo assay, embryo culture

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Humans conceived in vitro have an increased incidence of low birth weight (1), preterm birth, and perinatal complications (2). Low birth weight is associated with increased risk for type 2 diabetes (3), hypertension, and cardiovascular disease (4). The underlying etiology of low birth weight in children conceived in vitro remains unclear, obscured by multiple factors, including infertility, increased incidence of multiple births, and genetic background (5). Thus, the popularity of assisted reproductive technology (ART) and the potential for health complications compels further investigations into which aspects of the in vitro culture environment are most influential.

In vivo, the composition of the early embryo's environment (oviductal and uterine fluids) is largely determined by the composition of the maternal diet (6). In contrast, the environment of an IVF embryo is the culture medium. Epidemiologic studies demonstrating the effects of maternal nutrition on birth weight and risks for metabolic and cardiovascular disease (7) support the hypothesis that the composition of culture media may affect offspring health (8, 9). Although reports on the association between culture media and birth weight in humans are conflicting (10, 11), studies in mammalian animal models demonstrate strong relationships between media composition and multiple aspects of embryonic, fetal, and offspring health (12).

Types and concentrations of nutrients provided to preimplantation

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embryos vary among brands of culture media, but the only information provided by manufacturers is a list of ingredients. Unless detailed descriptions of the composition of these media are disclosed, it is impossible to determine which components might be responsible for differences in preimplantation development or birth weight, or to generate hypotheses for future study.

To address this gap in available information, we performed a systematic analysis of the composition of culture media from seven suppliers. We then determined whether observed differences in media composition were biologically relevant for blastocyst development using mouse embryos, the standard model used for development of media used for human embryo culture. Two components of culture that introduce additional sources of variation are oxygen and protein. Because oxygen is a metabolic nutrient, and the amount of oxygen used varies in clinical practice, we compared mouse embryo development at both reduced (5%) and ambient (20%) oxygen to determine whether there were any media × oxygen interactions. Studies were also conducted with and without protein supplementation, because protein supplements, which are highly heterogeneous (13), perform many nonspecific functions and carry many undefined components that may mask differences in culture media.

MATERIALS AND METHODS

Culture Media Analysis

Culture media sources. Culture media (two lots per medium) were purchased from seven suppliers and analyzed. Two single-step ("Global," LifeGlobal, IVFOnline; CSC ["Irvine"], Irvine Scientific) and five sequential (Sydney IVF Cleavage and Blastocyst Media ["Cook"], Cook Medical; IVC1 and IVC3 ["IVC"], In Vitro Care; ISM1 and BlastAssist ["Origio"], Origio; Sage Quinn's Advantage Cleavage and Quinn's Advantage Blastocyst Medium ["Sage"], Cooper Surgical; and G1v5 and G2v5 ["Vitrolife"], Vitrolife) media were analyzed. All media were protein-free, except Cook and Origio, which are only available with human serum albumin (HSA).

Amino acid and organic acid analysis. Amino acids (AAs) and organic acids were quantified in media using established methods for liquid chromatography–tandem mass spectrometry and gas chromatography–mass spectrometry, respectively (14, 15).

Elemental analysis. Calcium (colorimetric o-cresolphthalein complexone), chloride (ion selective electrode [ISE]), potassium (ISE module), magnesium (colorimetric), sodium (indirect ISE), and phosphorus (photometric) were quantified using Roche Cobas chemistry analyzers (Cobas 6000 c501 or Cobas 8000 ISE, c701, c502 modules) and Roche Cobas reagents. Samples were serially diluted, and analyte recovery after dilution was verified. In samples in which the analyte concentration was at the low end of the analytic measuring range (magnesium, phosphorus), spiked recovery of analyte was performed to verify accurate measurement in the sample matrix. Samples for trace metal analyses (aluminum, chromium, cobalt, copper, iron, manganese, selenium, and zinc) were collected in polyethylene containers demonstrated to be free of metal contamination. Quantification was performed by inductively coupled plasma mass spectrometry on samples diluted in 1% ultra-pure nitric acid. Calibration was performed in the same matrix using reference materials acquired from Venture Analytics.

Other analytes. Glucose (hexokinase) was quantified using Roche Cobas chemistry analyzers (Cobas 6000 c501 or Cobas 8000 ISE, c701, c502 modules) and Roche Cobas reagents. Insulin was measured with a Roche Cobas e immunoassay analyzer. Lactate was assayed by lactate oxidase/peroxidase (dry slide chemistry) on a Vitros 350 chemistry analyzer (Ortho Clinical Diagnostics). Samples were serially diluted, and analyte recovery after dilution was verified. In samples in which the analyte concentration was at the low end of the analytic measuring range (glucose, insulin), spiked recovery of analyte was performed to verify accurate measurement in the sample matrix.

Mouse Embryo Assay

Cryopreserved one-cell in vivo–fertilized mouse embryos from F1 hybrid mice (bcl/B6 × B6/bcl) were obtained from Embryotech Laboratories. After thawing (survival rate >98%), embryos were transferred to 25 μL of media in individual wells in an EmbryoSlide (Unisense Fertilitech), and each slide was inserted into an EmbryoScope (Unisense Fertilitech). Two sets of media were not available without protein, and it is known that protein can obscure the nature of culture media; therefore, SICM/SIBM and ISM1/BA were included in the study but not included in the statistical analysis of developmental data. Thus a 5 × 2 × 2 factorial study was performed with five culture media (Global, Irvine, IVC, Sage, and Vitrolife) with or without protein (5 mg/mL HSA; IVFOnline), and cultured at 20% or 5% oxygen. All experiments were performed in triplicate at 37°C and 6.2% CO_2 (pH 7.2–7.3 for each media), with 10 to 11 embryos for each media/condition combination. Expanded blastocyst at 96 hours of culture was the primary endpoint. Data for precise cell division timings using time-lapse imaging were also obtained and are presented in the online supplement.

Data and Statistical Analysis

Developmental and time-lapse data were analyzed using a one-way analysis of variance (ANOVA) with Tukey's test for pair-wise comparisons of blastocyst development. Analyses were performed in three steps. First, a 2 × 7 factorial ANOVA was used to compare blastocyst development and timings for all seven media containing protein at low and high oxygen. Second, a 2 × 2 × 5 factorial ANOVA was used to compare blastocyst development for the five media available without protein, tested with and without protein at low and high oxygen. Statistical analyses were performed using JMP statistical software (SAS Institute).

RESULTS

Media Composition

Because Cook and Origio were not available without protein, data from these media are presented despite the potential presence of contaminants from the protein. In all of the