ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Noninferiority, randomized, controlled trial comparing embryo development using media developed for sequential or undisturbed culture in a time-lapse setup

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Objective: To study whether a culture medium that allows undisturbed culture supports human embryo development to the blastocyst stage equivalently to a well-established sequential media.
Design: Randomized, double-blinded sibling trial.
Setting: Independent in vitro fertilization (IVF) clinics.
Patient(s): One hundred twenty-eight patients, with 1,356 zygotes randomized into two study arms.
Intervention(s): Embryos randomly allocated into two study arms to compare embryo development on a time-lapse system using a single-step medium or sequential media.
Main Outcome Measure(s): Percentage of good-quality blastocysts on day 5.
Result(s): Percentage of day 5 good-quality blastocysts was 21.1% (standard deviation [SD] ±21.6%) and 22.2% (SD ±22.1%) in the single-step time-lapse medium (G-TL) and the sequential media (G-1/G-2) groups, respectively. The mean difference (−1.2; 95% CI, −6.0; 3.6) between the two media systems for the primary end point was less than the noninferiority margin of −8%. There was a statistically significantly lower number of good-quality embryos on day 3 in the G-TL group [50.7% (SD ±30.6%) vs. 60.8% (SD ±30.7%)]. Four out of the 11 measured morphokinetic parameters were statistically significantly different for the two media used. The mean levels of ammonium concentration in the media at the end of the culture period was statistically significantly lower in the G-TL group as compared with the G-2 group.
Conclusion(s): We have shown that a single-step culture medium supports blastocyst development equivalently to established sequential media. The ammonium concentrations were lower in the single-step media, and the measured morphokinetic parameters were modified somewhat.
Clinical Trial Registration Number: NCT01939626.
Key Words: Blastocyst, sequential media, single-step medium, time-lapse, undisturbed culture

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During the last three decades we have seen a major increase in the understanding of embryo physiology and improvements in the in vitro culture environment. The subsequent improvements have resulted in more embryos available for transfer and thus a need for more effective cryopreservation protocols for the supernumerary embryos. Effective cryopreservation protocols allow all stages (oocyte through blastocyst) to be cryopreserved and used in a subsequent frozen embryo transfer, with outcomes equivalent to those of fresh transfers (1–4). With these advances and the move toward elective single-embryo transfer, attention has turned toward increasing the ability to select the most viable embryos for transfer (5–8). The ultimate goal is therefore to perform elective single-embryo transfer, resulting in a singleton healthy live birth.

Currently, morphologic features are the most prominent and, the majority of the time, the only method used to select which embryo(s) to transfer. In 2011, the Alpha Scientists in Reproductive Medicine group and European Society of Human Reproduction and Embryology (ESHRE) published a consensus statement that outlined the time at which embryos should be scored during development (9). Such periodic observations indicate whether the embryo is developing at an appropriate rate. Embryos can then be further assessed with grading schemes to give a final indicator of potential viability and enable an informed, if somewhat subjective, selection of the embryo(s) to transfer.

It is acknowledged that periodical observations can only provide minimal insight into the highly dynamic process of embryo development, along with a limited ability to detect certain abnormalities during mitotic divisions. Thus, time-lapse imaging has been suggested as a solution to observe the dynamic morphology by identifying abnormal cleavage patterns and more closely observing the morphokinetics of development. This could improve embryo selection or more likely deselection (10, 11). Furthermore, time-lapse technology has the potential to increase the consistency of quality embryo selection by minimizing the level of subjectivity in embryo scoring within clinics and more widely the field of in vitro fertilization (IVF) (11–13).

Time-lapse systems allow for automated image acquisition of embryos with a relatively high temporal resolution (5–20 minutes) without the need to remove the embryos from the incubator. This in itself is likely to improve embryo development and viability, as any kind of manipulation subjects the embryo and/or its culture environment to fluctuations in temperature, gas concentrations (causing pH shifts), light, and humidity. With time-lapse there is no need to remove the embryos from the incubator for observation on day 3 when culturing to the blastocyst stage. Thus, one might question the practical compatibility of time-lapse with sequential media.

Sequential media were developed to mimic the nutrient changes from the oviduct to the uterus and the change in metabolic requirements of the preimplantation embryo (14, 15). Currently, to use sequential media, one must remove the dish from the incubator and physically move the embryos from the first- to second-phase medium, which is maintained in a separate culture dish. Moving embryos into a fresh culture drop is even recommended in some single-step medium protocols. Therefore, the embryos could be subjected to the same fluctuations as outlined here. With each manipulation of the embryos, there is also an increased risk in physical damage or even loss. Thus, there could be major advantages to using a medium with time-lapse systems that does not require a change or refresh, but rather can be used from day 1 through days 5 to 6 and the time of embryo transfer or cryopreservation (16).

Our study prospectively compared the embryo development of randomized sibling embryos cultured in a single-step medium designed for time-lapse (G-TL) that can be used continuously and undisturbed from day 1 through to days 5–6 with the development of embryos in sequential media (G-1/G-2) with a mandated day 3 changeover.

MATERIALS AND METHODS

Overall Study Design

This study was a two-arm, prospective, randomized, controlled clinical trial performed at four independent IVF clinics: two in Sweden (Fertilitetscentrum, Gothenburg; and Reproductive Medicine Centre, Skåne University Hospital, Malmö) and two in the United States (Pacific Fertility Center, San Francisco; and Frisco Institute for Reproductive Medicine in Texas). The study was performed from September 2013 to February 2014. Institutional review board approval was obtained for each site in the United States through Schulman Associates (Cincinnati, OH), protocol number TL-SCM-2013; and ethics approval was obtained for the Swedish clinics though the local ethics committee in Gothenburg, Sweden (988-12). The trial was registered at ClinicalTrials.gov (NCT01939626). We prospectively randomized sibling embryos at the two pronuclei (2PN) stage to compare embryo development on a time-lapse device (Primo Vision; Vitrolife Sweden AB) using a continuous, undisturbed, single-step, time-lapse medium (G-TL) or sequential media (G-1/G-2).

The primary end point was the percentage of good-quality blastocysts (GQB) on day 5 per randomized patient. The secondary end points were [1] the percentage of good-quality embryos on day 3 of development, [2] the blastocyst utilization rate (number of blastocysts transferred and/or cryopreserved on day 5 and 6) per 2PN, and [3] total blastocyst rate (total number of blastocysts per 2PN).

Recruitment

Couples with female, male, or unexplained infertility intending to undergo IVF or intracytoplasmic sperm injection (ICSI) who had no medical contraindications for the treatment were informed of the study based on the inclusion and exclusion criteria. The women were required to be $\leq$40 years old. On the day of fertilization check (day 1) the couple needed $\geq$6 normally fertilized oocytes as exhibited by 2PN. As the primary end point was GQB on day 5, all patients were required to allow their embryos to be cultured to the blastocyst stage (days 5–6). Patients were not considered for the study if they had previously participated or the sperm was derived from testicular or epididymal biopsy. Written informed