Improved Clinical Outcomes with Continuous Single Culture™ - A True Single Step Non-Interrupted Culture Medium Protocol That Does Not Require Medium Renewal During Six Days of Human Preimplantation Embryo Culture

Matthew D. VerMilyea¹, James R. Graham¹, Michael J. Tucker¹,²
¹Shady Grove Fertility Reproductive Science Center, Rockville, MD
²Georgia Reproductive Specialists, Atlanta, GA

Introduction: For over a decade, the culture of preimplantation mammalian embryos has been approached by one of two culture protocols separated by opposing philosophies. The sequential, two-step protocol is thought to mimic the physiological interaction between specific developmental stages of the embryo and its vicinity within the oviduct and uterus. This protocol is based on the ‘back to nature’ principle that consists of medium renewal approximately midway in the extended culture period; either day-3 or -4 of embryo culture. The opposing system is based on the ‘let the embryo choose’ principal that is analogous to a single step culture medium. By providing the embryo with the appropriate concentration of components as defined through biological standardizations, the embryo is ultimately allowed to determine when and which nutrients and compounds are needed for suitable development. Although the ‘back to nature’ dogma of a two-step protocol is thought to be optimal for zygote to blastocyst development, we now show that blastocyst development and implantation potential are strikingly similar, if not better, in an uninterrupted, non-renewed single step culture medium protocol.

Materials and Methods: Irvine Scientific’s Continuous Single Culture™ (CSCM) was compared with Quinn’s Advantage™ Cleavage and Blastocyst (QA, Sage) sequential media system with respect to human embryo development, blastocyst utilization rates and pregnancy outcomes. On day-1, following fertilization assessments, sibling embryo cohorts from 60 patients were randomly allocated equally into the control (QA) or test (CSCM) culture media and cultured in reduced oxygen (6% CO2, 6% O2 and 88% N2) conditions. A combined total of 923 embryos were cultured in groups of up to five in 0.5ml volumes of their assigned medium. Embryos cultured in the QA two-step media (n=448) were transferred from cleavage to blastocyst medium early on day-4 of culture. Embryos cultured in the single step CSCM medium (n=475) supplemented with 10% Serum Substitute Supplement (Irvine Scientific) remained uninterrupted in the same medium for the duration of their culture up to six days. Embryos were assessed daily as per normal protocol using the Shady Grove grading system nomenclature. The best quality embryo(s), regardless of treatment, were chosen for transfer on day-3, -5 or -6. Transfer quality embryos that were not selected for embryo transfer were vitrified according to protocol. Two appropriately rising $\beta$hCG levels were indicative of a positive biochemical pregnancy, followed by clinical pregnancies showing FCA activity as determined by ultrasound. In addition to extended embryo culture, 61 oocytes from eight patients were conventionally inseminated in CSCM medium to determine if the medium composition supported fertilization. Normal fertilization was determined by the presence of two pronuclei.

Results: The number of transfer and vitrification-quality blastocysts available on day-5/6 in the assigned embryo cohorts determined the Blastocyst Utilization Rate (BUR). Both the control and test media had identical BURs of 36%. A total of 31 elective Single Embryo Transfers (eSET) were conducted from embryos cultured in Continuous Single Culture™ medium compared to 22 in the control QA sequential two-step media protocol. Implantation rates (IR) per elective Single Embryo Transfer (eSET) were 51.6% and 50% respectively. The total implantation rate per transfer from each cohort was 44.9% in the single step medium group compared to 37.9% in the sequential media group. Interestingly, BUR as determined per day of culture showed a 7.3% increase in embryos cultured in the CSCM test medium compared to the QA control media on day-5 (26.7% (n=127) and 19.4% (n=87) respectively). On day-6, however, the BUR was reversed being 12.5% (n=56) in the control QA media and 6.1% (n=29) in the CSCM test medium. No miscarriages in either media were reported. In addition, the fertilization rate of mature oocytes by conventional insemination was higher in the single step test medium (74%; n=47) compared to the sequential test media (68%; n=50).
Comparison of Implantation Rates According to Embryo Culture Medium

**Conclusion:** This study demonstrates that a true single step, non-interrupted culture medium protocol not only delivers the necessary components for successful human oocyte fertilization, but also supports the development of extended preimplantation embryos through day-6 of in-vitro culture. Embryos cultured in Continuous Single Culture™ uninterrupted exhibited a 7% increase in implantation potential and an increase in the production of more suitable embryos available for transfer or vitrification on day-5 compared to sibling embryos cultured in Quinn’s Advantage™ sequential two-step media. While we did not detect any significant differences within the media groups we noticed what appears to be a positive trend associated with implantation rates and embryos cultured in Continuous Single Culture™. This may prove to be significant with a larger sample size. However, in addition to an increase in implantation potential and superior embryo development, there are other significant benefits with use of a continuous single medium protocol. These benefits include: a reduction in embryo stress by eliminating the exposure to varying culture constituents and osmotic concentrations, reduced temperature and pH fluctuations, reduced potential for embryo loss and/or introduction of contaminants by pipetting errors, reduction in cost of materials and less labor intensive for culture dish preparation overall.

**References**


