Continuous Single Culture™ Comparison of Clinical Outcomes using a Single-Step Uninterrupted Culture Medium Protocol Requiring No Medium Renewal vs. a Traditional Three-Step Sequential Medium System

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Introduction: Previous reports have suggested that a sequential media system is required for optimal development of human embryos to the blastocyst stage. These sequential media culture systems require the use of one culture medium for fertilization, a fresh medium during the cleavage stages of development (day-3/4), followed by a third different medium for final culture to the blastocyst stage. The three culture media used differ significantly in composition and/or concentration of components. Although a three-step culture protocol has traditionally been the standard for in-vitro culture of embryos, more recently advances in the development of a single-step culture medium have occurred. We present here a comparison of pregnancy rates, from transfer of fresh and frozen embryos, which were grown either in a sequential medium protocol, previously used in our IVF clinic, or more recently in an uninterrupted single-medium protocol that is our current protocol. We also outline the cost and timesaving benefits of a single-step medium. Furthermore, we challenge the concept that a sequential medium protocol supports more optimal blastocyst development as compared to a single-step, non-renewal culture medium.

Materials and Methods: Irvine Scientific’s Continuous Single Culture™ (CSCM) was compared with Quinn’s Advantage™ Fertilization, Cleavage and Blastocyst (QA, Sage) sequential medium system with respect to human embryo development, blastocyst utilization rates and pregnancy outcomes. Embryos from 892 oocyte retrievals were cultured in CSCM compared to 2423 in QA in reduced oxygen (6% CO₂, 6% O₂ & 88% N₂) conditions during 2011. Embryos cultured in the QA three-step medium protocol were transferred from cleavage to blastocyst medium early on day-4 of culture. Embryos cultured in the single-step CSCM medium remained uninterrupted in the same medium until day-6 of culture. Embryos were cultured in groups of up to five in 0.5ml medium. Embryos were assessed daily, and the best quality embryo(s) were chosen for either a cleavage stage embryo or blastocyst transfer on day-3, or day-5 respectively. Transfer quality embryos that were not selected for embryo transfer (ET) were vitrified on day-5 or day-6. Vitrification and warming of blastocyst were done as per standard protocol for subsequent Frozen Embryo Transfer (FET). In an attempt to report the most recent results from this study, positive clinical pregnancies were established by quantitative βhCG level ≥ 50mIU/ml approximately 12 days after transfer. Ongoing pregnancies are defined by confirmation of positive fetal cardiac activity (FCA) with ultrasound at approximately 28 days pregnancy. Data were analyzed by Chi-square analysis or Fisher’s exact test with P≤ 0.05 considered as significant.

Results: Overall clinical pregnancy (CP) rate per ET when considering all autologous (non-donor oocyte) patients (all ages) was not different from the 885 ETs from CSCM when compared to the 2257 ETs of embryos cultured in QA (48% vs. 48%, P=0.835). The CP rate for donor oocyte ET cycles was 62% (194 ET) compared to 57% (506 ET) respectively (P=0.163). Overall, significantly more embryos cultured in CSCM were transferred at the blastocyst stage, compared to those cultured in QA (64% vs. 59%, P=0.019). The number of blastocyst transfers from donor cycles was equal (89% vs. 89%, P=0.892). Interestingly, autologous patients between the ages of 38-40yrs had significantly more blastocyst transfers as a result of Continuous Single Culture™, when compared to QA (56% vs. 44%, P=0.005). The ongoing pregnancy rate per frozen embryo transfer (FET) was significantly improved (P=0.001) from embryos cultured in CSCM (63%), when compared to those cultured in QA (43%).

Conclusion: In this retrospective comparative study, the CP rate per transfer from embryos cultured in the single-step culture medium (Continuous Single Culture™) was as good as that obtained from embryos cultured in the traditional sequential medium system (Quinn’s Advantage™ Fertilization, Cleavage and Blastocyst Media). Our results agree with previous studies that show no advantage of sequential medium systems over a single medium for culture of the human embryo. However, our data demonstrates an overall significant increase in blastocyst transfers from a true single-step culture media protocol, refuting the belief that sequential medium culture systems support better overall embryo development to blastocyst. The establishment of significantly more pregnancies, following the warming and transfer of embryos, confirmed the potential viability of vitrified blastocysts cultured in CSCM. In addition, we have calculated a 9% reduction in cost per patient, and a timesaving of 15% when using the Continuous Single Culture™ medium routinely in our laboratory.
Comparison of Clinical Pregnancy Rates According to Embryo Culture Medium

Comparison of Day5/6 Blastocysts Transfers

Comparison of Frozen Embryo Transfers

References