## Challenging Traditional Embryo Culture Techniques with a Simplified, Continuous Single Medium Protocol

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## Introduction

Mammalian preimplantation embryos develop in vitro with some degree of plasticity, as evidenced by the varied culture media and techniques in use today. There are a number of commercial media formulations for stage-specific use; media for fertilization, sequential media for growth of fertilized ova, and complex single medium formulations designed to present all components to the embryos during all stages of post-fertilization in vitro development. The historical progression of culture media, discussions of culture media components, and a brief history of media redesigned from somatic cell culture media for use in clinical IVF is reviewed elsewhere (1-6).

There are three static formats for embryo culture, as discussed in detail by Biggers and Summers, 2009 (6): 1) two different media e.g. sequential, where culture is interrupted on day three with movement of embryos to a new dish with the next sequential medium, 2) one medium, where culture is interrupted by movement of embryos to a new dish with the same, single medium on day three, and 3) one medium, continuous uninterrupted single medium culture, where embryos are not moved to new dishes nor is medium renewed. And recently, a fourth format is challenging

the static culture methods, where there is continuous medium renewal, without movement of embryos, via microfluidic device culture (7-10), although as promising as this technique is, the microfluidic culture method is not easy to apply to routine clinical IVF.

In a previous quality assurance study, Reed et al., 2009 (11) evaluated development of sibling embryos within patients, comparing a sequential media protocol to a continuous uninterrupted single medium culture protocol. The study results demonstrated that sibling embryo development to the blastocyst stage was significantly better using the continuous uninterrupted single medium protocol vs. the sequential media protocol already in use, and that under the laboratory conditions described, it was concluded that there was no advantage to the use of the sequential media protocol. Based on the results of that study, the protocol for continuous uninterrupted single medium culture was adopted for routine IVF cases at this clinic, beginning calendar year 2009.

The purpose of this study was to verify the clinical utility of the continuous uninterrupted single medium protocol, by performing a retrospective evaluation of cycle characteristics for the 162 IVF patients receiving treatment in calendar year 2009.