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# Human blastocyst development: A randomized comparison of sibling zygotes cultured in Vitrolife G-TL™ to Life Global® single-step media

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## Abstract

The ability to grow good quality embryos in vitro is crucial to optimizing Assisted Reproduction Technologies (ART) success. The advent of time-lapse imaging (TLI) has allowed embryo development determinations to be performed without removing the embryos from the incubator. In turn, one-step culture media formulations that grow human zygotes continuously to the blastocyst stage have become more popular.

**Objective:** Compare a new Vitrolife G-TL™ culture medium to our control Global® (Life Global) formulation in 21 cycles randomizing 287 sibling zygotes.

**Methodology:** Routine IVF stimulation and ICSI was performed with patient's zygotes being randomly subdivided between Global® and G-TL™ media treatments. Culture performance was evaluated by TLI of individually cultured embryos. Treatment differences were evaluated by comparing Day 3 quality grades, blastocyst development, euploidy and pregnancy results. Single euploid embryo transfers (SEET) were performed based on randomized treatment selection. Chi-square and t-Test analysis were used to determine significance.

**Results:** Although no single TLI comparison was significant, some differences in embryo development favored Global® media. The number of 6-10 cell embryos and blastocysts ≥3BB were greater ($p<0.05$) for Global® than G-TL™ media (89%, 60.6% vs 70%, 51.7%, respectively). Meanwhile, there was no difference in blastocyst euploidy rates (43% to 56%), nor did the 17 single euploid embryo transfer cycles show a difference in blastocyst implantation, ongoing clinical pregnancy or live birth rates between Global® (9 of 10, 90%) or G-TL™ (4 of 7, 57.1%) groups.

**Conclusion:** G-TL™ proved to be an effective continuous culture media product. Any differences observed in this study (i.e., slower development), may have been a by-product of our experimental condition. That is, controlling for what is optimal pH conditions for Global® may have been suboptimal to G-TL™. Continuous culture in single-step media must be optimized within each independent laboratory, if reliable blastocyst development and high implantation success is to be attained.

## Introduction

In the late-1990's, the concept of sequential media formulations to meet the specialized needs of different early embryonic stages (i.e., cleavage and blastocyst development) gained scientific and clinical acceptance [1]. The original and highest quality sequential media was G-series media (G1 and G2), produced by Vitrolife (Englewood, CO), which continued to make modification enhancements to their formulations up until 2009 (version 1 up to version 5). By 2002, a new and improved complete, single-use medium (KSOM$^{AA}$) was introduced for human embryo use [2] and was later produced and marketed by Life Global® (Global®; Guilford, CT). Both media have been employed in the IVF industry and are equally regarded today for their superior quality control practices. Our laboratory has attained high pregnancy success using Global® medium, in conjunction with paraffin oil (Ovoil™, Vitrolife) since 2010, proving the efficacy of this continuous complete medium [3]. Reed (2009) showed that a change in culture dishes containing fresh media is unnecessary on Day 3 with the single-use media containing the stable Alanyl-Glutamine dipeptide [4]. Conversely, it had been shown by Lane and Gardner that a detrimental build-up of metabolic waste products (e.g. $NH_3$) can occur within 72 hours, if media containing Glutamine alone was not replaced [5].

The introduction of time-lapse imaging (TLI) equipment has made it possible to continuously monitor the development of an embryo inside the incubator, thus reducing the need to handle the embryo outside the incubator [6]. Time-lapse monitoring of embryo development is ideally suited for single, continuous culture medium, in contrast to other commonly used sequential media which require a media change on Day 3. Because of a growing shift toward improved single-use media for early embryo development, Vitrolife validated a continuous culture, single-step medium (G-TL™) in 2014. Although, compared to G-series sequential media fewer good quality Day 3 developed (50.7% vs. 60.8%, respectively), there was no differences in total blastocyst production, implantation rate or live birth rates [7]. The ability of monoculture media to effectively neutralize the physiological advantages of sequential media has been explained [5]. In this investigation a randomized, controlled sibling zygote trial was

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applied to comparatively evaluate the development of human embryos in Vitrolife G-TL™ and Global® media.

## Materials and methods

Twenty-one infertility patients consented to participate in an IRB approved (Aspire IRB, SCIRS-2014) randomized clinical trial in 2015. Patients followed a standard regime of gonadotropin injections to achieve controlled ovarian hyperstimulation. After 10-14 days of hormonal stimulation, 7500-10000 IU of hCG was administered to induce final maturation of the cumulus oocyte complex (COC). Oocytes were harvested from the patients' ovaries by transvaginal ultrasound-guided follicular aspiration (36 hours post-hCG), using a temperature/$CO_2$ gas controlled Isolette/IVF Workstation. Once the COC's were collected and trimmed of excess cumulus cells/bloody residue, they were transported via portable incubator directly to the ART laboratory within 5 min.

In the laboratory, the COC dish was placed into a holding incubator (2-4 hours) until the time the oocytes were prepared for intracytoplasmic sperm injection (ICSI). Following ICSI, all oocytes were placed into fresh Global® media dishes containing 10% synthetic serum substitute in 25 µl droplets in Nunc 58mm dishes under Ovoil™. Upon overnight incubation, 287 normal fertilized (2PN) sibling zygotes were randomly subdivided into two different culture media treatment groups (Global®, n=142; G-TL™, n=145). All zygotes were individually isolated in Vitrolife TLI wells in a group 60 µl volume under Ovoil™. The GTL medium was complete, being pre-supplemented with 5% recombinant human serum albumin. All in vitro embryo development was performed in mini MCO-5 tri-gas incubators (Sanyo/Panasonic), with $CO_2$ levels varying from 5.3-6.5 to achieve pH levels of 7.32 for cleavage stages (Day 1-3) and 7.35 for blastocyst development (Day3-6). Each media type was controlled for pH by adjusting $CO_2$ levels, thus requiring the use of separate incubators. $CO_2$ levels were calibrate accordingly, based of standard titration curve quality control analysis at 5.0, 5.5, 6.0 and 6.5% $CO_2$.

Incubators were each equipped with a pair of Primo Vision EVO time-lapse embryo monitoring units. The dishes used for each TLI device were designed to culture and analyze up to 9 individual embryos/dish. A snapshot of each embryo was taken every 10 minutes over the course of 6 days. The images of each embryo were saved and analyzed in a double-blinded manner, independent of morphologic grading/scoring or PGS outcomes. The TLI analysis looked at first cleavage division to 2-cell (T0-T2), to 5-cell (T2-T5), to (T2-T8), to Morula ($T_M$), and 8 cell-blastocyst (T8-$T_{BL}$).

All embryos underwent standard quality grading of Day 3 to assess cell number, symmetry and fragmentation and assigned an overall grade of A, B or C (good to poor, respectively). All blastocysts were graded on Day 5 and 6 according to modified Gardner criteria (accounting for premature herniation of blastocyst due to Day3 laser zona drilling [8,9]; were the inner cell mass (ICM) and trophectoderm (TE) were assigned A, B and C dual grades. Blastocyst formation was documented and those possessing ≥3BB grade underwent TE biopsy procedures and array-CGH aneuploidy determination as previously detailed [3,9]. All biopsied blastocysts were subsequently vitrified using a microSecure vitrification (µS-VTF) procedure [10]. Upon euploid status (i.e., genetically normal, 23 paired chromosomes) determination, aneuploid embryos were discard per patient consent.

Subsequently, one euploid embryo was selected for transfer back to each patient in a standard vitrified embryo transfer (VFET) cycle. Transfer treatment group selection was randomized and then the best quality euploid blastocyst(s) was selected for warming 2-3 hr prior to transvaginal-ultrasound guided ET. VFET procedures were performed on the 6th day of luteal support and all implantations (with cardiac activity) and ongoing pregnancies confirmed by ultrasound between the 6th to 12th week of pregnancy. This study only compared first-time ET attempts. Direct comparisons between treatment groups (rates, %) were made using a Chi-square analysis and /or comparison of means using T-test or Fisher Exact Test, where appropriate. Whereas, the relationship between embryo development and TLI measurements used analysis of variance and correlation regression analyses.

## Results

The mean patient age is this study was 33.6±2.6 (±SD). No differences in TLI interval analyses were detected between culture medium treatments. Differences in embryo development, however, favored ($p<0.05$) Global® medium. More 6-10 cell embryos on Day 3 and blastocysts ≥3BB by Day 6 were produced ($p<0.05$) in Global® than G-TL™ media (Table 1). Although more biopsy quality (QG≥3BB) and top quality (QG-AA) blastocysts were produced by Global®, the euploidy rates were not different between treatments, varying from 43% to 56%. Not surprisingly, when a single euploid embryo was transferred no difference in blastocyst implantation, ongoing clinical pregnancy or live birth rate was observed between Global® (9 of 10, 90%) or G-TL™ (4 of 7, 57.1%) treatments.

## Discussion

This study randomly compared sibling zygotes evenly split between two culture media treatment groups. There was no difference in Day 3 embryo quality, but Global® medium did produce more advanced cleaved embryos and eventually more biopsied blastocysts. Differences in blastocyst quality have been previously shown to not be correlated to the euploid status of human embryos [9]. Overall, the total blastocyst production rate reported herein for G-TL™ and Global® are similar to those previous published [3,7]. Variations in the chemical ingredients between G-TL™ and Global® have been evaluated [11] and may account for the observed developmental outcomes. However, the apparent difference in growth potential may also be an artifact of the experimental design. In an attempt to keep treatment group numbers even, we chose to randomize culture groups for TLI after the fertilization check. Therefore, zygotes produced in our control Global® medium and transferred to G-TL™ medium could have experienced a biochemical stress. Such a stress could have influenced embryo physiology, which may have adversely impacted some zygotes [5]. Furthermore, in an attempt to best control media lot differences between culture treatments we standardized pH levels by altering %$CO_2$ tri-gas incubation conditions. It is possible that the optimized culture conditions used for Global® medium may not necessarily be optimal for G-TL™.

**Table 1.** Developmental differences between sibling zygotes cultured in two different single-step media treatment groups

| | Global® | G-TL™ | p Value |
|---|---|---|---|
| # Zygotes (2PN) | 142 | 145 | |
| Day3: 6 to 10cells | 127 (89.4%) | 106 (73.1%) | p<0.05 |
| % QG - A | 74.6 | 70.3 | n/s |
| % QG - B | 20.4 | 17.2 | n/s |
| Day 5 Blastocysts | 49 (34.5%) | 37 (25.5%) | p<0.05 |
| Day 6 Blastocysts | 39 (27.5%) | 39 (26.9%) | n/s |
| Total Blastocysts | 88 (62.0%) | 76 (52.4%) | p<0.10 |
| % QG - AA | 58.0 | 46.1 | p<0.05 |
| % QG – AB | 14.8 | 14.5 | n/s |
| % QG – BA | 6.8 | 10.5 | n/s |
| % QG - BB | 10.2 | 17.1 | n/s |
| Blastocysts ≥3BB | 86 (60.6%) | 75 (51.7%) | p<0.05 |

Although the TLI data in this pilot study did not confirm significant trends in embryo development, the Primo Vision EVO units did prove to be a resourceful device. Its portable size and mobility allowed for easy placement into our miniature box-style incubators. Such devices may be useful in clinical practice [6], especially if they can be adopted at an affordable price point. The potential impact of TLI analysis is lessened in programs, like ours, that emphasize blastocyst culture and biopsy/PGS. The high overall implantation rate observed in this study (76.4%) is consistent with our past clinical experiences applying single euploid embryo transfer [3,8]. Overall, we see no reason to change our current standard of care using Global® medium or to adopt TLI routinely based on this study. Furthermore, we do believe that G-TL™ proved to be effective but requires independent optimization for clinical use by each laboratory.

## Disclosure

The study was sponsored by Vitrolife and Genesis Genetics. The authors have no conflicts of interest to disclose.

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