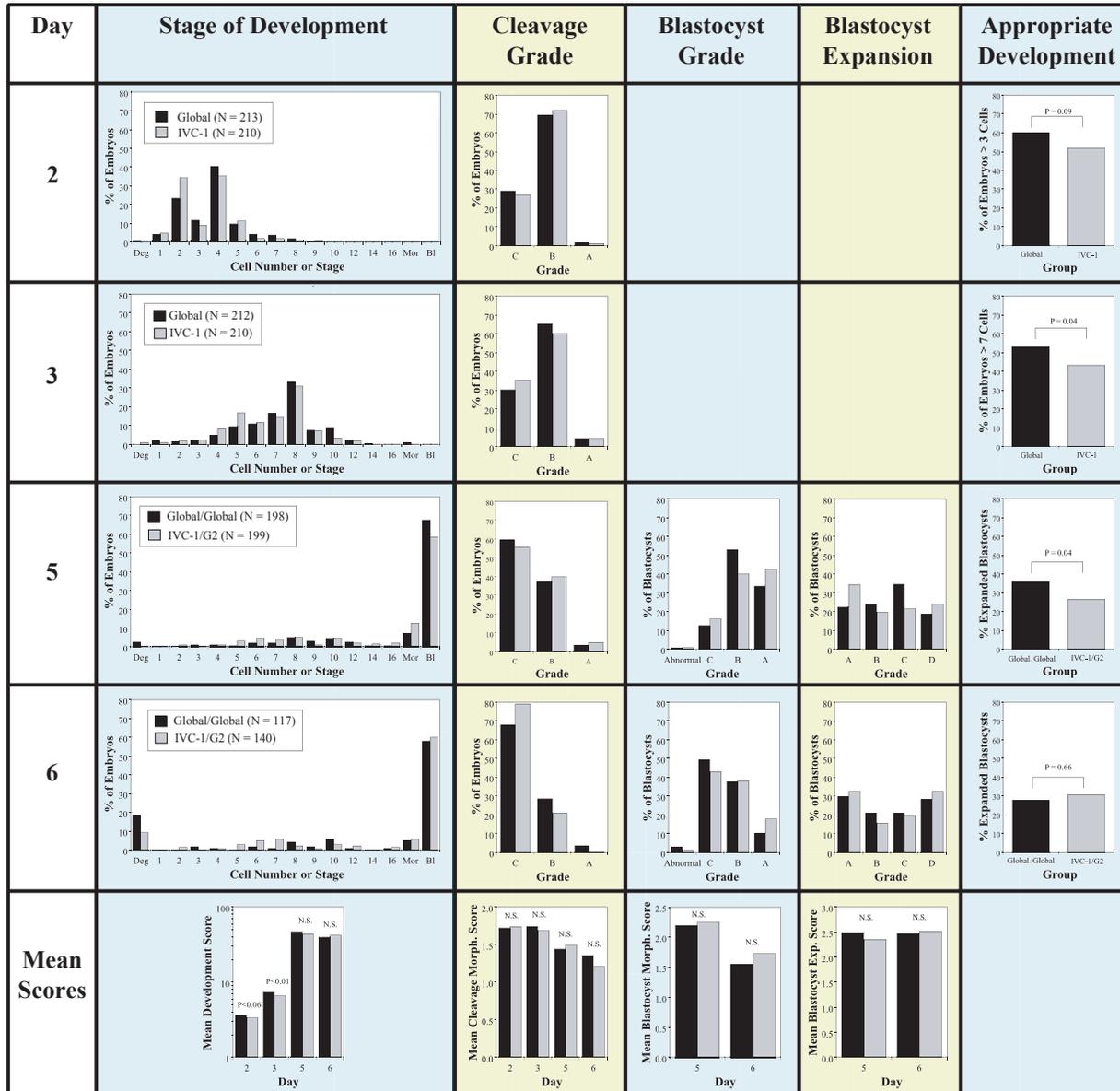


# COMPARISON OF A SINGLE MEDIUM WITH SEQUENTIAL MEDIA FOR DEVELOPMENT OF HUMAN EMBRYOS TO THE BLASTOCYST STAGE

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## Introduction

It has been suggested that culture of human embryos to the blastocyst stage requires the sequential use of one medium for culture to Day 3, and a second medium for culture thereafter. However, it has been shown that human embryos can be successfully cultured in the same medium throughout. At the Nashville Fertility Center, embryos are routinely cultured in IVC-One (InVitroCare) from Day 1 to Day 3, and then in G2 (Gardner & Lane, Hum Reprod Update, 3, 367-382, 1997). from Day 3 to Day 5 or Day 6. The objective of this experiment was to directly compare the development of embryos in this sequential media system (IVC-1/G2) with development in a single medium (Global, LifeGlobal) from Day 1 to Day 3, and from Day 3 to Day 5 or Day 6.

## Materials and Methods

Oocytes were retrieved from 37 women (mean age = 32.1; range 23-39) undergoing IVF treatment between December, 2002 and March, 2003. Following fertilization in Quinn's Advantage medium (Cooper Surgical), the zygotes from each retrieval were divided as equally as possible into two groups.

The embryos of the Global group (N = 213) were cultured individually in 10 µl droplets of Global medium under oil until Day 3 and then transferred into fresh droplets of Global medium and cultured until Day 5 or Day 6. The embryos of the IVC-1/G2 group (N = 210) were similarly cultured in IVC-One medium until Day 3, and then in G2 medium until Day 5 or Day 6.

Embryo development and morphology were evaluated on Days 2, 3, 5 and 6, according to the criteria shown in Table 1. Appropriate development was defined as being >3 cells on Day 2, > 7 cells on Day 3, and expanded blastocyst on Day 5 or Day 6. After evaluation on each day, embryos were removed from the treatment groups if they were degenerate or retarded, or to be transferred or frozen (Table 2).

For the statistical analyses, the scores for embryonic development, morphology, and blastocyst expansion on each day were compared between treatment groups using Kruskal-Wallis tests. The proportion of embryos with appropriate development on each day was compared between treatment groups by Chi-Square analysis.

## Results

The results are displayed in the table of graphs. The top four rows show the results for each day. The columns show the measures of development, morphology, blastocyst expansion and appropriate development. The bottom row shows the mean scores for the measures for all days. The results for the Global group are shown in black (■), and the results for the IVC-1/G2 group in gray (□).

Developmental score was greater in the Global group than in the IVC-1/G2 group on Day 2 and on Day 3 but was not different between the Global and IVC-1/G2 groups on Day 5 or Day 6.

The morphological quality of cleavage stage embryos and blastocysts was not different between treatment groups on any of Days 2, 3, 5 or 6.

Blastocyst expansion was not different between treatment groups on Day 5 or 6.

The proportion of embryos having appropriate development was greater in the Global group than in the IVC-1/G2 group on Days 3 and 5, but not on Day 2 or Day 6.

**Table 1. Grades and Scores for Embryo Development and Morphology**

Measure	Grade	Description	Numeric Score	
Embryo Development		Degenerated	0	
		1-16 Cell	1-16	
		Morula	32	
		Blastocyst	64	
Cleavage Stage Morphology	A	Symmetrical blastomeres, no fragmentation	3	
	B	Unsymmetrical blastomeres, or <10% fragment.	2	
	C	10-25% fragmentation	1	
Blastocyst Morphology	A	<b>Inner Cell Mass</b> Tightly packed, many cells	<b>Trophectoderm</b> Many cells forming a cohesive epithelium	3
	B	Loosely grouped, several cells	Few cells forming a loose epithelium	2
	C	Very few cells	Very few large cells	1
	Abn	Abnormal	Abnormal	0
Blastocyst Expansion	A	Early blastocyst with a blastocoele just appearing as one or more vacuoles	1	
	B	A distinct single cavity equal to one-quarter to one-half of the volume of the embryo	2	
	C	A full blastocyst with a blastocoele completely filling the embryo. The diameter is unchanged	3	
	D	Fully expanded with a thinning zona, and a distinct ICM and trophectoderm layer	4	

**Table 2. Number and Disposition of Embryos on Each Day**

	Medium		Total
	Global/Global	IVC-1/G2	
<b>Day 2</b>	213	210	423
Removed: Degenerate	1	0	1
<b>Day 3</b>	212	210	422
Removed: Degenerate	0	2	2
1-cell	4	2	6
Transferred	10	7	17
<b>Day 5</b>	198	199	397
Removed: Degenerate	5	0	5
Transferred	42	34	76
Frozen	34	26	60
<b>Day 6</b>	117	140	257
Frozen	26	37	63
Discarded	91	103	194

## Discussion and Conclusions

Development, morphological quality, and blastocyst expansion of human embryos cultured in a single medium (Global) were as good, or better, than those for embryos cultured in a sequential media system (IVC-One/G2).

These observations agree with the report of Biggers and Racowsky (Reprod. Biomed. Online 5, 133-40, 2002) who showed that blastocyst formation rates were not different between human embryos cultured in KSOM(AA) from Day 1 to Day 5, compared with embryos cultured in a sequential media system.

The use of a single medium eliminates the possibility of osmotic or other stress associated with moving the embryos from one medium to another. In addition, only one medium need be maintained and/or tested in the laboratory.

Poster Number  
**0659**