The Development and Clinical Studies of global® Medium for the Culture of Human Embryos and the global® Family of ART Media

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LifeGlobal Group, LLC
Vision, Science, Research, Standards, and Quality

The global® Family of products are developed through innovative scientific research, have been proven independently in centers worldwide and are manufactured and tested to the highest Quality Control standards and its on-going quality is assured by independent testing of every batch.

global® medium is used in Fertility centers worldwide, to help increase the number and quality of human embryos for transfer embryos on either Day 3 culture or at the blastocyst stage, yielding more embryos available for transfers and increased pregnancy rates.

Fertility clinics who have adopted the use of global® have reported improved embryonic development, blastocyst development and an increase in the number of embryos available for fresh transfer or for cryopreservation.

global® has proven to be stable in its use; it maintains its pH and osmolality. It is formulated to produce the lowest levels of ammonium build-up over time. Superior stability, quality, and performance.

LifeGlobal®, ‘The Art Media Company’ was established to meet the industry needs for high quality, specially designed media products. All products are designed with the nutritional needs of the human embryo in mind, with the strictest quality controls, tested ingredients and are FDA compliant. Each batch is independently tested. Our research team of John Biggers, Jacques Cohen, Klaus Wiemer, and Don Rieger have years of research and clinical experience. Together, they have developed these products to offer you the best quality and performance. LifeGlobal® has a well established worldwide distribution network to assure you quality customer service, and fresh delivery of the products.

The LifeGlobal® scientific team now includes John Biggers, Jacques Cohen, Klaus Wiemer, Don Rieger, and many outside expert consultants in the industry. Together, they are working on exciting projects; using breakthrough technologies to develop and improve products for embryo culture, stem cell culture, gamete and embryo freezing, and many other aspects of human ART.

What makes our team outstanding is not only their commitment and dedication to scientific research, but also their openness to communication in the industry by encouraging multiple center testing and research collaboration, and their willingness to help with clinical applications and testing. Our team is your team, and open to work with you and answer any question you might have on the scientific background or clinical use of global® or any other of the family of LifeGlobal® ART media.

Please feel free to contact our team at: experts@LifeGlobal.com
I. Historical Background

A. Ringer's Saline Solution and its Derivatives

The history of defined tissue culture media begins with Ringer (1882) who developed a simple salt solution based on the constituents of blood serum, for the study of the beating frog heart, in vitro. It was composed of sodium chloride (NaCl), potassium chloride (KCl), calcium chloride (CaCl₂) and a low concentration of sodium bicarbonate (NaHCO₃, 2.7 mM). This was modified by Locke and Rosenheim (1907), notably by the addition of 11.1 mM glucose, for the study of rabbit heart. Tyrode (1910) added sodium phosphate (NaH₂PO₄) and magnesium chloride (MgCl₂) and increased the concentration of NaHCO₃ to 11.9 mM for use in the study of rabbit intestine. Krebs and Henseleit (1932) used no glucose, but increased the concentration of NaHCO₃ to 25 mM, for the study of nitrogen metabolism by rat tissues. The 25 mM concentration of NaHCO₃ in this Krebs-Ringers bicarbonate solution (KRB) is what is conventionally used in conjunction with 5% CO₂ in many tissue culture media to produce a physiological pH. (see The Biological Bulletin, Compendium of Physiological Solutions: http://www.mbl.edu/BiologicalBulletin/COMPENDIUM/Comp-TabCont.html)

B. Embryo Culture Media based on KRB

Whitten (1956) showed that 8-cell mouse embryos would develop to the blastocyst stage when cultured in KRB supplemented with 5.55 mM glucose, and McLaren and Biggers (1958) showed that such blastocysts would produce live young when transferred to recipient mothers. Whitten (1957) added lactate to his 1956 formulation and was able to culture out-bred 2-cell mouse embryos, but not zygotes, to the blastocyst stage. This observation led to the concept of the “two-cell block” to mouse embryo culture. In a series of papers, Brinster showed that phosphoenolpyruvate, pyruvate, lactate and oxaloacetate, but not glucose, could support the development of the 2-cell mouse embryo to the 8-cell stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage. This observation led to the concept of the “two-cell block” to mouse embryo culture. In a series of papers, Brinster showed that phosphoenolpyruvate, pyruvate, lactate and oxaloacetate, but not glucose, could support the development of the 2-cell mouse embryo to the 8-cell stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage, while glucose could support the development of the 8-cell embryo to the blastocyst stage.

Based on these findings, Brinster modified Whitten’s medium by reducing the calcium concentration and adding 0.27 mM pyruvate to produce BMOC2, which would support the development of the mouse 2-cell embryo to the blastocyst stage, at high rates. Whittingham (1971) modified Brinster’s medium by decreasing the concentration of lactate and increasing the concentration of pyruvate to produce M16 medium.

Brinster’s medium and M16 were significant advances for mouse embryo culture and were widely used. However, except for inbred strains of mice, they could not overcome the 2-cell block. Blocks to development in vitro were similarly found for hamster (2-cell), cattle (8-16 cell), pig (4-8 cell) and human (4-8 cell) embryos, all approximately coincident with the major onset of expression of the embryonic genome (see Rieger 1992). The 2-cell block to in-vitro development of the mouse embryo was finally overcome by Chatot et al. (1989) with CZB medium, a modified version of BMOC2 with no glucose, 1.0 mM glutamine, and 0.1 mM EDTA. This led to the misconception that glucose is inhibitory to early embryo development.

C. Embryo Culture Media based on the Composition of Oviduct and Uterine Fluids

As noted above, much of the development of embryo culture media was based on simple salt solutions. An alternative approach was the formulation of media based on the measured concentrations of the components of oviduct and uterine fluids. These include SOF (synthetic oviduct fluid), based on ovine oviduct fluid (Tervit et al., 1972); B2, based on bovine oviduct and uterine fluids (Ménézo 1976); HTF (human tubal fluid), based on human oviduct fluid (Quinn et al., 1985); and MTF (mouse tubal fluid), based on mouse oviduct fluid (Gardner and Leese 1990).

Based on measured differences in the composition of oviduct and uterine fluids, and on measured changes in the metabolic activity of embryos during early development, Gardner and Lane (2002) have suggested that “in order to optimize mammalian embryo development in culture, sequential media are required, each designed to meet the changing requirements of the developing embryo.” Although this seems logical, this “back to nature” approach relies on several questionable assumptions.
First, as noted by Summers and Biggers (2003), the measurements of the components of oviduct and uterine fluids are highly variable, and almost certainly subject to physiological inductance. Second, such measurements only reflect the overall composition of the tract fluids and not the micro-environment around the embryo. Third, as shown in Figure 1, the physical and chemical environment of the embryo in vivo is completely different from its environment in vitro. Except in pathological conditions, there is no pool of fluid in the reproductive tract. The embryo is surrounded by a thin layer of fluid and is in close apposition to the maternal tissues, allowing rapid exchange of nutrients, gases, wastes, and effectors between the embryo and the mother. In contrast, in vitro, the embryo is bathed in a relatively large pool of fluid in which the nutrients are continually decreasing and the waste products are continually increasing during culture. Clearly, the stresses on the embryo in vitro are very different from those in vivo, and culture media must be designed to optimize embryo development under in-vitro conditions.

D. Embryo Culture Media Designed by Simplex Optimization

In a radical departure from the traditional methods for designing embryo culture media, Lawitts and Biggers (1991; 1992) applied the principles of simplex optimization to determine the optimal concentration of each component. They began the process with a generating medium, based on M16 and CZB, containing NaCl, KCl, potassium phosphate (KH2PO4), magnesium sulphate (MgSO4), lactate, pyruvate, glucose, bovine serum albumin (BSA), ethylenediaminetetraacetic acid (EDTA), and glutamine. Ten other test media were derived from the generating medium, each containing a high concentration of one of the components. These eleven media formed the START simplex. Four cycles of the simplex optimization process showed that high concentrations of NaCl, pyruvate, KH2PO4, and glucose were detrimental to mouse embryo development (Lawitts and Biggers, 1991). A further 16 cycles of optimization resulted in the formulation of Simplex Optimization Medium (SOM) which was marked by a low NaCl concentration, and was able to overcome the mouse 2-cell block (Lawitts and Biggers, 1992). In a subsequent study, it was found that blastocyst development was improved by increasing the concentration of KCl from 0.25 mM to 2.5 mM and this modified version was called KSOM (Erbach et al., 1994). Of particular interest, glucose was found to have no inhibitory effect on mouse embryo development in KSOM, even at a relatively high concentration (Summers et al., 1995). Embryo development was further improved by the addition of amino acids to KSOM (KSOM-AA) (Ho et al., 1995; Biggers et al., 2000; Summers et al., 2000). Biggers et al. (2004) and Summers et al. (2005) subsequently showed that the development of mouse embryos is better when the medium includes the glycyl-glutamine dipeptide, compared to either glutamine or alanyl-glutamine.

In addition to many studies on mouse embryos, KSOM, with or without amino acids, has been shown to support the development of cattle (Liu and Foote 1995), rabbit (Liu et al. 1996), rhesus monkey (Weston and Wolf 1996), pig (Machaty et al., 1998), rat (Zhou et al., 2003), and human (Biggers and Racowsky 2002, Figure 2) embryos.

![Fig. 1. A comparison of the interaction of an embryo with its environment, in vivo and in vitro.](https://example.com/fig1.png)
For more detailed and extensive reviews of the history of the development of embryo culture media, see Biggers (1998), Hammer (1998), and Summers and Biggers (2003).

II. Clinical Studies of \( \text{global}^\circledR \) Medium in Human ART

Based on the reports of the successful culture of mouse embryos in KSOM-AA, Klaus Wiemer and his colleagues showed that a modified version could support high rates of development of Day 3 human embryos to the blastocyst stage (Wiemer et al., 2002; Anderson et al., 2002). The medium was subsequently further modified for human use, and marketed as \( \text{global}^\circledR \).

As shown in the following graphs, \( \text{global}^\circledR \) medium has been successfully used for the culture of human embryos from Day 1 to Day 3 (Figures 3-12), as a “blastocyst medium” for culture from Day 3 onward (Figures 13-16), for two-step (interrupted) single-medium culture from Day 1 to 3 and then from Day 3 to Day 5-6 (Figures 17-32), and for single-step (uninterrupted) culture from Day 1 to Day 5-6 (Figures 33-41).

A. Culture of Human Embryos in \( \text{global}^\circledR \) from Day 1 to Day 3

![Graph showing cumulative embryo score comparison between Global and HTF](image1)

Fig. 3. Embryos were cultured in \( \text{global}^\circledR \) or HTF from Day 1 to 3. The composite embryo score was significantly greater for embryos cultured in \( \text{global}^\circledR \) than for those cultured in HTF on Day 2 and Day 3. The pregnancy rates were not significantly different. (Neal et al., 2004)

![Graph showing percentage of transfers comparison between Global and Cook](image2)

Fig. 4. Embryos were cultured from Day 1 to 3 in \( \text{global}^\circledR \) or Cook medium. The proportion of patients that had embryos cryopreserved, and the pregnancy rate were significantly greater for embryos cultured in \( \text{global}^\circledR \). (Mellon et al., 2005)
Fig. 5. Embryos were cultured from Day 1 to 3 in global® or G1. There were no significant differences in the proportions of good quality embryos, pregnancy, or implantation rates between culture media. (Racowsky, 2005)

Fig. 6. Embryos were cultured from Day 1 to 3 in global® or G1. There were no significant differences in the proportions of good quality embryos on Day 3 or in pregnancy rates between culture media. (Cairó et al., 2006)

Fig. 7. Embryos were cultured in global® or HTF from Day 1 to 3. The proportion chosen for cryopreservation, and the pregnancy rate tended to be greater for embryos cultured in global®. The implantation rate was significantly greater for embryos cultured in global®. (Riqueros et al., 2007)

Fig. 8. Embryos were cultured from Day 1 to 3 in global® or IVF. Cleavage rate and the proportions of good quality embryos on Day 2 and Day 3 were significantly greater for embryos cultured in global®. (Verza et al., 2007)

Fig. 9. In-vitro development and pregnancy rate for single transfers of embryos cultured from Day 1 to 3 in global®. (Zech et al., 2007)

Fig. 10. Embryo quality and pregnancy outcomes for human embryos cultured from Day 1 to 3 in global® or G1. There were no significant differences in embryo morphology or pregnancy rates. (Karakocsokmensuer et al., 2005)
B. Culture of Human Embryos in \( \gamma \)-global\(^\circ\) from Day 3 to Day 5

**Fig. 11.** Embryos were cultured from Day 1 to 3 in \( \gamma \)-global\(^\circ\), Sage medium, or G5 Plus. The proportions of good quality embryos, embryos chosen for freezing, and clinical pregnancy rates were not significantly different among the culture media treatments. (Herreros et al., 2009)

**Fig. 12.** Oocytes were fertilized using conventional ICSI or by IMSI, and the embryos cultured in \( \gamma \)-global\(^\circ\) until transfer at Day 3. (González-Ortega et al., 2010)

**Fig. 13.** Embryos were cultured in HTF from Day 1 to 3, followed by culture in \( \gamma \)-global\(^\circ\) from Day 3 until transfer on Day 5. (Wiemer et al., 2002)

**Fig. 14.** Embryos were cultured in P-1 from Day 1 to 3, then in \( \gamma \)-global\(^\circ\) or IBM until frozen on Day 5 or 6, and transferred in a later cycle. The clinical pregnancy and implantation rates were significantly greater for embryos cultured in \( \gamma \)-global\(^\circ\). (Unpublished data from Dr. T.B. Pool, Fertility Center of San Antonio, San Antonio, TX, USA)

**Fig. 15.** Embryos were cultured in HTF from Day 1 to 3, and then in \( \gamma \)-global\(^\circ\) or MultiBlast until Day 5. There was no difference in the proportion of embryos that reached the blastocyst stage or in the cell number. A significantly larger proportion of embryos hatched in \( \gamma \)-global\(^\circ\). (Desai et al., 2007)

**Fig. 16.** Embryos were cultured in HTF from Day 1 to Day 3 and then vitrified. After thawing, the embryos were cultured in \( \gamma \)-global\(^\circ\) for 48 hours until transferred at the morula or blastocyst stage. (Desai et al., 2010)
C. Two-step (Interrupted) Culture of Human Embryos in global® from Day 1 to Day 5 or 6

Fig. 17. Embryos were cultured in global® from Day 1 to 3 and Day 3 to Day 5-6, or in G1 from Day 1 to 3 and then in G2 from Day 3 to Day 5 or 6. The proportion of embryos reaching the blastocyst stage by Day 6 was significantly greater in global®. There were no significant differences in pregnancy or implantation rates between the media treatments. (Greenblatt et al., 2005)

Fig. 18. Embryos were cultured in global® from Day 1 to 3 and Day 3 to 5, or in G1 from Day 1 to 3 and then in G2 from Day 3 to 5. The proportion of embryos reaching the blastocyst stage by Day 5 was significantly greater in global®. The pregnancy rates were not different between the culture media treatments. (Angus et al., 2006)

Fig. 19. Embryos were cultured in global® from Day 1 to Day 3 and from Day 3 to 5, or in G1 from Day 1 to 3 and then in G2 from Day 3 to 5. The proportion of embryos reaching the blastocyst stage by Day 5, and the pregnancy and implantation rates were significantly greater for embryos cultured in global®. (Zech et al., 2006)

Fig. 20. Embryos were cultured in global® from Day 1 to 3 and Day 3 to 5, or in BAS1 from Day 1 to 3 and then in BAS2 from Day 3 to 5. The proportion of embryos having 6 or more cells on Day 3 was significantly greater in global®. The blastocyst rate on Day 5 and the pregnancy rate were not different between media treatments. (Matsubara et al., 2006)

Fig. 21. Embryos were cultured in global® from Day 1 to 3 and Day 3 to 5, or in Cleavage medium from Day 1 to 3 and then in Multiblast from Day 3 to 5. Development on Day 3 and Day 5 was not different between the media treatments. (Kumagai et al., 2006)

Fig. 22. Embryos were cultured in global® from Day 1 to 3 and Day 3 to 5, or in G1 from Day 1 to 3 and then in G2 from Day 3 to 5. The proportion of embryos reaching the blastocyst stage by Day 5 was significantly greater in global®. Pregnancy and implantation rates were not different between media treatments. (Vansteenbrugge et al., 2007)
Fig. 23. Embryos were cultured in \textit{global} from Day 1 to 3 and Day 3 to Day 5 or 6, or in IVF on Day 1 to 2, a 50:50 mix of IVF and CCM on Day 2 to 3, and then in CCM from Day 3 to Day 5-6. The implantation rate was significantly greater for embryos cultured in \textit{global}. The pregnancy rates were not different between media treatments. (Herrero \textit{et al.}, 2007)

Fig. 24. Embryos were cultured in \textit{global} or G series media from Day 1 to Day 5. The proportion of embryos judged as transferable blastocysts on Day 5 was significantly greater for embryos cultured in \textit{global}. The pregnancy and implantation rates were not different between the culture groups. (Pomerory \textit{et al.}, 2009)

Fig. 25. Embryos were cultured in \textit{global} from Day 1 to 3 and Day 3 to 5, or in ECM from Day 1 to 3 and then in Multiblast from Day 3 to 5. The proportion that developed to the blastocyst stage by Day 5, and the implantation rate were significantly greater for embryos cultured in \textit{global}. The clinical pregnancy rate was not different between the culture media treatments. (Sepulveda \textit{et al.}, 2009)

Fig. 26. Embryos were fertilized in \textit{global} and then cultured in \textit{global} from Day 1 to 3 and Day 3 to Day 5 or 6, or fertilized in Quinn’s Advantage Fertilization medium and then cultured in Cleavage from Day 1 to 3 and then in Blastocyst from Day 3 to Day 5 or 6. The proportion of embryos at the blastocyst stage by Day 5-6 was significantly greater in \textit{global}. The clinical pregnancy and implantation rates were not different between the culture groups. (Carrillo and Yalcinkaya, 2010)

Fig. 27. Embryos were cultured in \textit{global} from Day 1 to 3 and Day 3 to 5, or in G1 from Day 1 to 3 and then in G2 from Day 3 to 5. Development to blastocyst by Day 5, and the implantation rate were significantly greater for embryos cultured in \textit{global}. Pregnancy rates were not significantly different between the culture media treatments. (Wirleitner \textit{et al.}, 2010)

Fig. 28. Embryos were cultured in \textit{global} from Day 1 to 3 and Day 3 to 6, or in HTF from Day 1 to 3 and then in \textit{global} from Day 3 to 6. Development to blastocyst was significantly greater in \textit{global} than in HTF/\textit{global}. Pregnancy and implantation rates were not significantly different between the culture media treatments. (Desai \textit{et al.}, 2011)
Fig. 29. Sibling oocytes were fertilized and the resulting embryos cultured in global® from Day 1 to 3 and Day 3 to 6, or fertilized and cultured in Quinn’s Advantage Cleavage medium from Day 1 to 3 and then in Blastocyst medium from Day 3 to 5. Development to ≥ 6 cells on Day 3, development to blastocyst on Day 5, and embryos chosen for transfer or cryopreservation were significantly greater for embryos cultured in global®. (Khoury et al., 2012)

Fig. 30. Sibling zygotes were cultured in global® from Day 1 to 4 and Day 4 to 6, or in Sage media from Day 1 to 4 and Day 4 to 6. Compaction on Day 4, development to blastocyst on Day 5-6, and the proportion chosen for transfer or cryopreservation were significantly greater for embryos cultured in global®. The implantation rates were not significantly different between the culture media treatments. (Liebermann et al., 2012a,b)

Fig. 31. Sibling zygotes were cultured in global® from Day 1 to 3 and Day 3 to 5, or in Origio ISM from Day 1 to 3 and BlastAssist media Day 3 to 5. Development to blastocyst, proportion of good quality blastocysts, and utilization rate transfer or cryopreserved) were all significantly greater for embryos cultured in global®. (Sfontouris et al., 2014)

Fig. 32. Patients were randomized to have their embryos cultured in global® or Sage sequential media. Good quality blastocysts were vitrified and subsequently warmed for transfer. Development to blastocyst, clinical pregnancy rate, and implantation rate were all significantly greater for embryos cultured in global®. (Apyrshko et al., 2015)

D. Single-step (Uninterrupted) Culture of Human Embryos in global® from Day 1 to Day 5 or 6

Biggers and Summers (2008) have suggested that single-step (uninterrupted) culture may be preferable to both sequential culture, and two-step (interrupted) culture in a single medium, for culture of human embryos from the zygote to the blastocyst stage. Practical advantages of single-step culture include reduced quality control procedures, labour, and cost. Physiological advantages of single-step culture include the possibility of accumulation of endogenous growth factors, and a decrease in stress to the embryo. However, single-step culture carries the risk of accumulation of embryo-toxic volatile organic compounds (VOC) from the culture dishes, lab gases, and the environmental air into the culture oil and then the culture medium. (See Cohen et al., 1997; Schimmel et al., 1997; Hall et al., 1998; Merton et al., 2007). Consequently, a careful comparison of single-step vs. two-step culture should be made before introducing single-step culture as general practice in any ART laboratory.
Fig. 33. Embryos were cultured without interruption in global® or SSM from Day 1 to Day 6. The proportions of good quality embryos on Day 3 and blastocysts on Day 5 were not significantly different between culture treatments. The proportion of good quality blastocysts on Day 5 was significantly greater in the global® group. (Sumimoto et al., 2008)

Fig. 34. a) Embryos were cultured in the same droplet of global® from Day 1 to 5, or in G1 from Day 1 to 3 and in G2 from Day 3 to 5. Development to blastocyst on Day 5, and the proportion of embryos that were selected for transfer were significantly greater for embryos cultured in global®. (Recalculated from Reed et al., 2009) b) Pregnancy outcomes for embryos cultured in the same droplet of global® from Day 1 to 5, in a subsequent study. (Reed et al., 2010)

Fig. 35. Sibling zygotes were cultured from Day 1 to 3 in global® and then in fresh drop of global® from Day 3 to Day 6 (interrupted), or in the same drop of global® from Day 1 to Day 6 (uninterrupted). Development to blastocyst and the proportion chosen for transfer or cryopreservation (GQ) were significantly better for embryos in 1-step culture. (Singh et al., 2012)

Fig. 36. Results of a total of 7744 cycles. Embryos were cultured in the same droplet of global® from Day 1 to 6, or in sequential media from Day 1-3 and Day 3-6. The clinical pregnancy rate was significantly better for embryos cultured in global® compared with sequential media for patients < 35 and 38-40 years of age. (Keskintepe, 2012, & Pers. comm.)

Fig. 37. Embryos from donor oocytes were cultured from Day 1 to 5 in global® total® in an Embryoscope with a change to fresh medium on Day 3 (interrupted) or not (uninterrupted). There was no significant effect of culture treatment on any of the morphokinetic parameters, development to blastocyst, proportion of good quality blasts, or implantation rate. (Costa-Borges et al., 2013).
Fig. 38. Sibling embryos from donor oocytes were cultured from Day 1 to 5 in global® with a change to fresh medium on Day 3 (interrupted) or not (uninterrupted). There was no significant effect of culture treatment on development to blastocyst, proportion of early or late blast, or blastocysts suitable for vitrification. (Bormann et al., 2013)

Fig. 39. Embryos from donor oocytes were cultured from Day 1 to 5 in global® with a change to fresh medium on Day 3 (interrupted) or not (uninterrupted). There was no significant effect of culture treatment on good quality embryos on Day 3, development to blastocyst, or proportion of good quality blastocysts. (Portella et al., 2013)

Fig. 40. Sibling zygotes were cultured from Day 1 to 5 in global® in an Embryoscope with a change to fresh medium on Day 3 (interrupted) or not (uninterrupted). There was no effect of culture treatment on development to blastocyst, proportion of good quality blastocysts, or implantation rate. (Çetinkaya & Kahraman, 2013)

Fig. 41. Embryos were cultured from Day 1 to 5 in global® in an Embryoscope with a change to fresh medium on Day 3 (interrupted) or not (uninterrupted). Development to blastocyst and embryo utilization were significantly greater with uninterrupted culture, while there was no effect of culture treatment on the implantation rate. (Rambha et al., 2014)

E. Conclusions

- The results presented above clearly demonstrate that global® can support the development of human embryos at all stages from the zygote to the blastocyst.
- The use of global® as a single medium from Day 1 onward reduces the possibility of stress associated with changing the medium composition at Day 3.
- Equally important, the use of a single medium for all phases of embryo culture reduces the requirement for maintenance and quality control of media, and reduces the chances for error in the clinical human ART laboratory.
- Single-step (uninterrupted) culture in global® may offer some additional advantages over two-step culture in global®. However, a careful comparison of single-step vs. two-step culture should be made before introducing single-step culture as general practice in any ART laboratory.
III. The \textit{global®} Family of ART Media

A. Background

As documented in the previous section, clinical studies have shown that the use of a single medium, \textit{global®}, for culture of the human embryo from the zygote to the blastocyst stage, results in better in-vitro development than a variety of sequential media systems (Greenblatt et al., 2005; Angus et al., 2006; Zech et al., 2006; Matsubara et al., 2006; Vansteenbrugge et al., 2007; Pomeroy et al., 2009; Sepulveda et al., 2009; Reed et al., 2009; Carrillo and Yalcinkaya, 2010; Wirleitner et al., 2010). Moreover, pregnancy and implantation rates are as good or better for embryos cultured in \textit{global®} compared to sequential media (Zech et al., 2006; Herrero et al., 2007; Sepulveda et al., 2009; Wirleitner et al., 2010). These observations strongly support the suggestion of Biggers and Racowsky (2002) that “The switching of embryos from one medium to another may cause additional osmotic or other shock to the embryo …”

In view of the clinical results using \textit{global®} medium for embryo culture, a family of media based on \textit{global®} have been developed for human ART procedures. The overarching philosophy is to maintain, as closely as possible, the same chemical environment throughout all stages of oocyte and embryo handling and culture, in order to minimize the stress of changing from one medium to another.

In addition to \textit{global®}, for embryo culture from the zygote to the blastocyst stage, the family includes \textit{global®} Collect®, for oocyte collection and handling, \textit{global®} for Fertilization, for oocyte culture and conventional in-vitro fertilization; \textit{global®} w/ HEPES, for ICSI and embryo handling; and \textit{global®} LG PGD Biopsy Medium, for the removal of cells for pre-implantation genetic diagnosis. The components are shown in Table 1.

Table 1: The components of the \textit{global®} family of ART media

<table>
<thead>
<tr>
<th>Component Type</th>
<th>\textit{global®}</th>
<th>\textit{global®} for Fertilization</th>
<th>\textit{global®} w/ HEPES</th>
<th>\textit{global®} Collect®</th>
<th>LG PGD Biopsy Medium</th>
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*Must be supplemented with protein before use, typically at 5-10 mg protein/ml.

B. \textit{global®} for Fertilization

In conventional in-vitro fertilization, the medium must support the metabolic requirements of three cell types: the oocyte, its surrounding cumulus cells, and the spermatozoa. The oocyte itself metabolizes little or no glucose (Biggers et al., 1967; Rieger and Loskutoff 1994). Rather, the cumulus cells metabolize glucose to pyruvate, lactate, and other intermediates which are passed to the oocyte for oxidative metabolism (Biggers et al., 1967; Leese and Barton, 1984; Downs et al., 1996). Human sperm also metabolize glucose (Rees et al., 1990), and capacitation, the quality of motility, and the ability to penetrate the oocyte depend on glucose (Rogers and Perreault, 1990).
In general, conventional IVF is carried out in HTF or similar media, which contain pyruvate, lactate and a relatively high concentration of glucose (Quinn et al., 1985). However, the ionic composition of HTF is quite different from \( \text{global} \) and it contains no amino acids. In some circumstances, \( \text{global} \) works well as a fertilization medium (Carrillo and Yalcinkaya, 2010). However, \( \text{global} \) contains a relatively low concentration of glucose, which may be insufficient to satisfy the metabolic requirements of fertilization if there are large numbers of cumulus cells and/or spermatozoa present. Consequently, \( \text{global} \) for Fertilization was formulated with a glucose concentration equal to that of HTF, but with all the other components present in the same concentrations as in \( \text{global} \).

C. \( \text{global} \) w/ HEPES

All human embryo culture media include approximately 25 mM sodium bicarbonate (\( \text{NaHCO}_3 \)), which, when placed in an incubator containing 5-7% \( \text{CO}_2 \), results in a physiological pH of approximately 7.30. However, when removed from the incubator, the pH of bicarbonate-buffered media rises very rapidly. ART procedures such as ICSI and assisted hatching are performed outside of the incubator, and consequently the medium for such procedures must use an acid-base buffer that does not require \( \text{CO}_2 \) usually HEPES (Good et al., 1966).

Quinn et al. (1984) have suggested that the use of a HEPES-buffered medium for manipulating embryos in the absence of an atmosphere containing 5% \( \text{CO}_2 \) is an important factor in the success of human IVF. However, the use of relatively simple media like HEPES-buffered HTF may be stressful to the embryo, because of differences in ionic composition and the lack of amino acids. For that reason, \( \text{global} \) w/ HEPES contains all of the salts, energy substrates, amino acids, and other components, in the same concentrations, as in \( \text{global} \). It is important to note that \( \text{global} \) w/ HEPES does contain a low concentration of bicarbonate, because bicarbonate is important for embryo development in some way unrelated to acid-base buffering (Kane 1975).

D. \( \text{global} \) Collect®

As noted above, the cumulus cells surrounding the oocyte metabolize glucose to pyruvate, lactate, and other intermediates which are passed to the oocyte for oxidative metabolism. Consequently, \( \text{global} \) Collect® was formulated with a glucose concentration equal to that of HTF in order to satisfy the metabolic requirements of the cumulus-oocyte-complex during retrieval, washing, and handling. As for \( \text{global} \) w/ HEPES, and for the same reason, \( \text{global} \) Collect® contains a low concentration of bicarbonate.

E. HSA-supplemented \( \text{global} \) media

Media used for the handling, manipulation, and culture of human oocytes and embryos are supplemented with protein, typically at 5-10 mg protein/ml. Originally, bovine serum albumin, human cord serum, and autologous maternal serum were used for this purpose, but these have been entirely replaced with highly purified therapeutic-grade human serum albumin (HSA) or HSA together with \( \alpha- \) & \( \beta \)-globulins. The most obvious practical advantage of protein supplementation is as a surfactant to prevent the gametes and embryos from sticking to pipettes, tubes and culture dishes. In addition, it has been suggested that HSA may function to control pH, osmotic pressure and membrane stability, as a carrier of growth factors, as a scavenger of heavy metals and other toxins, and as a source of amino acids (see Blake et al., 2002).

Originally, ART media were supplemented with protein by the ART laboratory, using concentrated HSA or other protein solutions. Increasingly, ART laboratories prefer ready-to-use media that are supplemented with HSA by the media suppliers. This offers several advantages including convenience and the elimination of the possibility of error. In addition, when media are supplemented in the lab, the concentration of most of the components of the media is reduced by dilution with the protein solution. Interestingly, the concentration of NaCl is actually increased when the media are supplemented with protein in the ART lab because the concentration of NaCl is higher in the protein stock solutions than in most ART media. Conversely, when protein supplementation is done by the media supplier, the other components can be maintained at the same concentrations as in the unsupplemented media.
LifeGlobal provides two series of ready-to-use media supplemented with HSA. \( \text{global}^\circ \text{total}^\circ \) w /HSA, \( \text{global}^\circ \text{total}^\circ \) for Fertilization w/ HSA, and \( \text{global}^\circ \text{total}^\circ \) w/ HEPES w/ HSA all contain 10 mg/ml HSA. \( \text{global}^\circ \text{total}^\circ \) LP, \( \text{global}^\circ \text{total}^\circ \) LP for Fertilization, and \( \text{global}^\circ \text{total}^\circ \) LP w/ HEPES, all contain 5 mg/ml HSA. Aside from the protein, all of the components and their concentrations are exactly the same as in the respective unsupplemented media (\( \text{global}^\circ \), \( \text{global}^\circ \) for Fertilization, and \( \text{global}^\circ \) w/ HEPES).

F. \( \text{LG PGD Biopsy Medium} \)

Preimplantation diagnosis of cleavage-stage human embryos for chromosomal or gene defects requires that a cell be removed, usually at the 6-8 cell stage, on Day 3 of development. However, at about that time, as a prelude to compaction, adhesion between cells increases; partly mediated by epithelial cadherins (calcium-dependent adhesion molecules, see Alikani 2005). Because of the adhesion between the cells, separation of a cell from its neighboring cells may cause stress or damage to the embryo. Consequently, it is advisable to temporarily disrupt the E-cadherin connections and reduce cell-cell contact. This is typically done by placing the embryo into a calcium- and magnesium-free medium (Grifo et al., 1990).

Although the embryo is exposed to the biopsy medium for a very short time, usually not more than 5 minutes, the composition of the biopsy medium can affect the subsequent development and viability of the embryo following return to culture. Hill and Li (2004) found that the clinical pregnancy rate for biopsied embryos was significantly better if the biopsy medium was supplemented with alanyl-glutamine, EDTA, pyruvate and sodium lactate, compared with unsupplemented biopsy medium. They concluded that “We believe this step – that of using embryo biopsy media that contain essential amino acids and nutrients found in the primary culturing milieu – to be critical in maximizing clinical outcomes.” Based on their observations, and in collaboration with Dr. Santiago Munné, (Reprogenetics, Livingston, NJ, USA), \( \text{LG PGD Biopsy Medium} \) was formulated without calcium chloride and magnesium sulfate, but with all the energy substrates, amino acids and other components, at the same concentrations, as in \( \text{global}^\circ \). Based on the experience of Munné and his colleagues, sucrose was added to cause the cells to shrink slightly and thus make it easier to remove the biopsy. \( \text{LG PGD Biopsy Medium} \) is HEPES-buffered, and contains human serum albumin to make it ready-to-use. As for \( \text{global}^\circ \) w/HEPES, and for the same reason, \( \text{LG PGD Biopsy Medium} \) contains a low concentration of bicarbonate.

G. \( \text{global}^\circ \) Blastocyst Fast Freeze® and \( \text{global}^\circ \) DMSO Blastocyst Vitrification Kits

Blastocyst vitrification involves equilibration with very high concentrations (> 5 M) of potentially toxic cryoprotectants, rapid cooling (up to 20,000 C°/min), rapid warming, and rehydration beginning with high concentrations of sucrose. These osmotic excursions and temperature changes are highly stressful to the embryos and therefore it is advantageous to minimize other stresses, as much as possible. To this end, two vitrification/fast freezing kits have been developed using \( \text{global}^\circ \) w/HEPES as the base medium, as shown in Table 2.

Table 2: The components of \( \text{global}^\circ \) Blastocyst Fast Freeze® and \( \text{global}^\circ \) DMSO Blastocyst Vitrification Kits

<table>
<thead>
<tr>
<th>Components</th>
<th>( \text{global}^\circ ) Blastocyst Fast Freeze® and Thawing Kits</th>
<th>( \text{global}^\circ ) DMSO Blastocyst Vitrification and Warming Kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Medium</td>
<td>( \text{global}^\circ ) w/ HEPES</td>
<td>( \text{global}^\circ ) w/ HEPES</td>
</tr>
<tr>
<td>Cryoprotectants</td>
<td>Glycerol Ethylene Glycol</td>
<td>DMSO Ethylene Glycol</td>
</tr>
<tr>
<td>Rehydration Osmolyte</td>
<td>Sucrose</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Protein</td>
<td>HSA</td>
<td>HSA</td>
</tr>
</tbody>
</table>
The $\Phi$-global® Blastocyst Fast Freeze® and Thawing Kits are based on the S3 vitrification system developed by Dr. James Stachecki and his colleagues at the Tyho-Galileo Research Laboratory, Livingston, New Jersey. As described in Stachecki et al. (2008a; 2008b), the S3 system is a robust, simple, and highly effective approach to the rapid cryopreservation of human blastocysts. The $\Phi$-global® Blastocyst Fast Freeze® and Thawing Kits contain the same cryoprotectants (glycerol and ethylene glycol), in exactly the same concentrations, and employ the same procedures described by Stachecki et al. (2008a; 2008b). However, all of the three freezing and five thawing solutions have exactly the same concentrations of salts, energy substrates, amino acids and other components present in $\Phi$-global® w/ HEPES. Consequently, the stress in moving the blastocyst from culture to the freezing solutions, between the freezing solutions and thawing solutions, and from the final thawing solution back into culture, is reduced as much as possible. The $\Phi$-global® Blastocyst Fast Freeze® and Thawing Kits have been shown to be simple to use and effective for cryopreservation of human blastocysts (Lopes et al., 2015; Reed et al., 2015).

The $\Phi$-global® DMSO Blastocyst Vitrification and Warming Kits are based on the solutions described by Kuwayama et al. (2005). The cryoprotectants, DMSO and ethylene glycol, and their concentrations are exactly as described by Kuwayama et al. (2005). However, both of the vitrification solutions and the three warming solutions have exactly the same concentrations of salts, energy substrates, amino acids and other components present in $\Phi$-global® w/ HEPES, in order to reduce the stress on the blastocyst as much as possible.

H. The $\Phi$-global® Family Unified Approach to Human Embryo Culture

As noted above, when used together, the $\Phi$-global® family of ART media have been designed to minimize the stress of changing from one medium to another throughout ART procedures from oocyte collection to transfer. Figure 42 shows the time course of the use of the $\Phi$-global® family of media from oocyte collection on Day 0 through to transfer or cryopreservation on Day 5-6.

I. Conclusions

- Use of the $\Phi$-global® family of ART media allows the embryologist to maintain, as closely as possible, the same chemical environment throughout all stages of gamete and embryo handling and culture, and thereby minimize the stress on the embryo.
- The inherent developmental potential of the embryo can be best maintained by minimizing stress.

Acknowledgements

My sincere thanks to Dr. Thomas B. Pool of the Fertility Center of San Antonio, San Antonio, TX, USA who graciously provided his unpublished data, and to Drs Klaus Wiemer, Jacques Cohen, Catherine Racowsky, and Mina Alikani for their constructive comments and suggestions.

References

Fig. 42. The time-course of culture and handling of human oocytes and embryos in the global® family of ART media from oocyte collection on Day 0 through to blastocyst transfer or cryopreservation on Day 5 or 6.

*Must be supplemented with protein before use, typically at 5-10 mg protein/ml.


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From the Desk of Our Scientific Team

Questions about global® Medium answered by the Team

How can global®, a single medium, fully support both early cleavage of human embryos, and subsequent development to the blastocyst stage?

It has been suggested that the chemical environment of the oviduct is different from that of the uterus and therefore human embryos must be sequentially cultured in (at least) two different media to reflect the difference in the in-vivo environments. However, it is very doubtful that the measurements of the composition of oviduct and uterine fluids reflect the micro-environment of the embryo in vivo. Moreover, the environment in culture is physically very different from that in the reproductive tract, and embryo culture media must be designed to optimize embryo development in culture. Moving the embryo to a second medium is a stress upon the embryo. Many clinical studies have shown that single media, most notably global®, support the development of human embryos as well or better than do sequential culture media systems.

Why does global® medium contain glucose?

The concern about glucose in embryo culture medium originated from the observation that the cleavage of hamster embryos was inhibited by glucose when present in the medium with phosphate. This has shown not to be the case for a variety of media, and a variety of species. If fact, some source of glucose is absolutely required for development of the embryo at all stages. It was for this reason that glucose was included in the basic set of constituents used in the simplex optimization development of KSOM-AA, the medium upon which global® is based.

Why does global® medium contain Phenol Red?

Phenol Red is a pH indicator and is included in global® to serve as a rapid visual check that the CO₂-bicarbonate buffer system is functioning. However, the exact pH should be systematically monitored with a pH meter (see below). It has been suggested that Phenol Red may be toxic to embryos but there is absolutely no scientific evidence to support that suggestion. The one study that specifically examined the effect of Phenol Red, showed that it had no effect on the development of hamster embryos.

What CO₂ concentration should be used with global® medium?

In most cases, a 5-6% concentration of CO₂ in the incubator will produce a pH of 7.2 to 7.4 in protein-supplemented global® medium. However, the exact concentration of CO₂ required to produce the optimum pH of 7.3 depends on several factors, including the altitude and the characteristics of the protein supplementation. Consequently, we strongly recommend that each laboratory determine and use the exact concentration of CO₂ that is required to produce a pH of 7.3 in protein-supplemented global® medium.

Why does global® medium become a darker red after the bottle is opened?

During manufacture, global® is bubbled with CO₂. Each time the bottle is opened, CO₂ escapes from the medium into the head space and from there into the atmosphere. Consequently, the medium in the bottle becomes slightly more basic and the Phenol Red indication becomes a darker red. This has no effect on the medium. When the medium is placed in a CO₂ incubator, it absorbs the CO₂ and the proper pH is established.

Why is the shelf-life of LifeGlobal® culture media 10 weeks from the date of manufacture?

Some of components of embryo culture media, including the amino acids and pyruvate, are subject to oxidation or spontaneous degradation. Consequently, we limit the shelf-life of our embryo culture media to ensure the best performance possible. Moreover, we manufacture our media at least once per month, so that fresh media are always available to you.
μDrop GPS® Dish

Embryo Culture Dish for Human IVF
Safe, Effective and Easy-to-use

“The Micro GPS dish evolved from the input of dozens of scientists and engineers. It is the most versatile and ingenious culture dish ever developed. It combines so many new features. It is the strongest and safest Petri dish for culturing embryos. The bottom underneath each GPS location is so thin, you can see the embryo smile.”

New Design Features

- Precise 20 µl micro-wells with GPS® feature for rapid location and visualization
- Enhanced optics
- Better orientation and identification
- EmbryoAddress™ helps to quickly identify and track embryos
- 32 mm inner ‘oil ring’ for VOC protection
- Uses up to 75% less oil than a conventional 60 mm dish
- Fill the outer wells with oil for better temperature stability out of the incubator
- Works well with both oil-overlay and oil-underlay methods
- Designed to ensure safe embryo culturing
- For use with both standard and mini incubators

General Features of GPS® Dishware

- Improved safety (no droplet collapsing or mixing)
- Better pH control
- GPS® microwells protect against sudden movements
- Raised lid promotes gas exchange, prevents contamination, and stops oil seals from forming
- Non-toxic medical grade, non-pyrogenic olystyrene
- Better ergonomics than a 35 mm dish
- CE Certified and ISO 13485 Certified
- 1-cell MEA and < 0.03 LAL Tested

New Breathable Packaging for all GPS® Dishware

- Reduces off-gassing time
- Less VOCs introduced into the laboratory
- Tested and validated to maintain sterility for the entire 5-year shelf life of the dish

The working surface and well bottoms

The working surfaces (bottom) of all wells of the μDrop GPS® dish is sloped to create the GPS® location for all the specimens within these wells. This makes locating them quicker, easier to handle and observe, and the ability to return specimens to their controlled environment sooner.

○ = egg or embryo
The leading scientifically based and clinically proven ‘Uninterrupted Single Solution Culture Medium®’.

‘Uninterrupted Time-Lapse Culture Medium®’

- The First leading scientifically based ‘Single Solution Medium®’.
- Proven to work with any type of VOC controlled environment as a continuous culture.
- Over 500 Independent Publications using global® medium.
- Over 15 years of Consistent Superior Results worldwide.

Let the Embryos Choose!®
"We perform the majority of our embryo cryopreservation at the blastocyst stage and have found the global® Blastocyst Fast Freeze® system to be the simplest, most efficacious and cost-effective method we have tried. As a result, it has been in use exclusively in our laboratory for over a year and a half and the survival rate upon warming is in excess of 99% with stellar pregnancy rates. That the system employs 0.25 cc straws and larger volumes made it extremely simple to learn for the entire technical staff and has resulted in dramatic cost reductions since more expensive cryopreservation devices are not required. Additionally, it has been the perfect cryopreservation adjunct to trophectoderm biopsy and provides consistently high survival and pregnancy rates in conjunction with PGS/PGD. I have recommended the system to a number of my colleagues; none have been disappointed."

Dr. Thomas Pool, PhD of The Fertility Center of San Antonio, rpool@fertilitySA.com
(No commercial ties with this company.)