

Comparative development of human 1PN and 3PN day 5 blastocysts cultured in Global® and synthetic Synbios® media.

Yousef Al Helou¹

¹IVF Laboratory, Fakih IVF, Abu Dhabi, United Arab Emirates

Abstract

This study was undertaken to determine the efficacy of the latest formulation of the synthetic protein-free Synbios® (SM, Malaysia) embryo culture medium (ECM) in comparison to Total Global® protein-containing medium (GM, USA) on the developmental competence of human 1PN and 3PN embryos. The oocytes were inseminated by ICSI, equally apportioned to either one of the two ECM and subsequently cultured in the Embryoscope®. Zygotes with 1PN and 3PN were followed up until days 5-7. The rates and qualities of day 5 blastocysts generated in both media were statistically compared using the modified statistics-enabled blastocyst grading method described by Ali (2014) which is a modification of the Gardner and Schoolcraft (1999) blastocyst grading system. The qualities of blastocysts that developed from 1PN and 3PN zygotes in the two media were statistically similar for all parameters tested. The quality of blastocysts generated in the synthetic Synbios medium was marginally superior but this was statistically insignificant. This finding suggest that the synthetic Synbios® protein-free medium is comparable in efficacy to contemporary human ECM (GM) supplemented with serum proteins. The synthetic medium also overcame and eliminated the risks such as transmission of pathogens, prions, harmful proteins, donor DNA/RNA, etc, which could affect the health of the baby, mother and healthcare workers, prevent corruption of the embryonic genome thus preserving the purity of the lineage. It also prevented batch to batch variation in the quality of medium manufactured maintaining product consistency during manufacturing. In conclusion, the synthetic Synbios® medium appears as efficacious as contemporary protein-containing media. It is safe. It prevents transmission of disease and hazardous undeclared proteins which could harm patient, babies and healthcare workers. It also prevents batch variation and transmission of contaminant donor RNA/DNA thus preventing corruption of the embryonic genome thereby preserving the purity of lineage of the progeny.

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Correspondence: Yousef Al Helou; **Email:** alhelou@yahoo.com

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Introduction

About 70 years ago Wesley K. Whitten formulated the first efficacious embryo culture medium (ECM; Whitten, 1956,1957), and supplemented it with albumin for culture of mouse embryos. Many years later, in human IVF, the ECM was/is supplemented with donor human serum albumin (HSA). However, HSA carries risk of transmission of pathogens, prions, harmful proteins, donor DNA/RNA, etc, which could affect the health of the baby, mother and healthcare workers (Dyrlund et al, 2014; Kemmann,1998; Truyen et al., 1995; van Os et

al.1991). Importantly, there is evidence (Vilella et al., 2015) to suggest that the DNA/RNA strands present as in HSA as contaminants could crossover with the embryonic genome, generating embryos with genetic material from more than two parents, which is not acceptable in some cultures. The first efficacious synthetic ECM for human cleavage-stage embryos with viable pregnancies (40-50%) with livebirths in the human was described by Ali (a student of WK Whitten) who began communicating his work on the synthetic medium beginning from

1997 (Ali, 1997; Ali, 2000; Ali et al. 2000; Ali, 2004).

This synthetic medium which is devoid of added proteins supported fertilization after insemination by both conventional IVF (cIVF) as well as ICSI, with development of viable embryos in vitro. This synthetic medium appears to eliminate almost all risks associated with the use of protein-containing ECM.

The risks associated with the use of donor protein supplements in IVF media include: disease transmission (van Os et al., 1991; Kemmann, 1998), undeclared protein contaminants in human serum albumin (HSA) and its derivatives [Dyrlund et al., 2014; Morbeck et al., 2014], possible crossover (Vilella et al. 2015) of contaminant donor genetic materials with that of the embryonic genome; batch to batch variation in the quality of medium manufactured (Barnes et al., 1980), short shelf-life and a lack of compliance with cultural norms and practices such issues related to cultural norms of cleanliness, taboos (halal/haram status) and, veganism and vegetarianism and so forth. For instance, caste Hindus may prefer to use media devoid of donor protein media, whereas Muslims and Jews may be concerned about the halal-haram or Kosher status respectively. The synthetic ECM appear to eliminate these risks.

The pregnancies and livebirths from human embryos generated by the synthetic ECM reported previously was from cleavage stage embryos in the late 1990 and early 2000's. It is well documented that in the early 2000's blastocyst transfer phased out transfer of cleavage stage embryos.

The synthetic medium at that time was meant to support development of cleavage stage embryos, not blastocysts. The synthetic ECM was not investigated pending further development to enhance its ability to support blastocyst culture due to circumstances that precluded work in that direction until recently. The originator of the synthetic ECM was this author's mentor. This author obtained approval to determine whether the latest formulation of the synthetic ECM could support development of human blastocysts. This study was undertaken to determine the efficacy of the latest formulation

of the synthetic protein-free Synbios® medium (SM, Malaysia) in comparison to the Global® protein-containing medium (GM, USA) on the developmental competence of human 1PN and 3PN embryos.

Materials and methods

The ECM used were the Synbios® medium (SM, Malaysia) and the control Total Global® protein-containing medium (GM, USA). After ICSI, the oocytes were cultured in Global medium, after fertilization check, the 1PN and 3PN zygotes were equally apportioned to one of the two media and subsequently cultured in the Embryoscope®. Zygotes with 1PN and 3PN were carefully followed up until days 5-7 in the Embryoscope®.

The rates and qualities of day 5 blastocysts from 1PN and 3PN zygotes generated in both media were statistically compared using the modified statistics-enabled blastocyst grading method originally described by Ali in the SOP at the Fertility Clinic of University of Malaya (2014). The Ali blastocyst scoring method is a modification of the Gardner and Schoolcraft blastocyst grading system (Gardner and Schoolcraft, 1999) which scored for cavitation and blastulation, the quality of blastocyst, trophectoderm and inner cell mass (ICM). The Ali modification was recently published in this issue of the JRBBS by Ali and coworkers (Ali et al., 2025).

The qualities of blastocysts that developed from 1PN and 3PN embryos (Tables 1-3) in the two media were graded by Ali's modification (Ali et al., 2025) of the Gardner and Schoolcraft blastocyst scoring system. The data were subjected to statistical analysis to bring out the salient features in the development of the blastocysts in both the ECM used. This was performed so that the difference in the qualities of blastocysts that developed in the two media could be compared.

Results

The qualities of blastocysts that developed from 1PN zygotes (Table 1) in the two media were statistically similar for all parameters tested.

Table 1: Comparison of development of 1PN blastocysts in Global and Synbios® media

Description	Global media	Synbios media	P value
% Development of Total 1PN Day 5 blastulation (Includes: Cavitation/Early blastocyst/Blastocyst)	65.5 (36/55)	70.1 (39/55)	p= 0.6822; NS
% Development of Day 5 Blastocyst Development	41.8 (23/55)	43.6 (24/55)	p= 1.000; NS
% Development Total Day 5 EBL Dev	23.6 (13/55)	27.3 (15/55)	p=0.8267; NS
Cumululative Score for total blastulation [Volume+ICM+Trophect (V+I+T)] Key: 12=Excellent; 9=Good; 6=average; 3=poor	9.4783	9.7917	p=0.2534; NS
Summarized grade score (V+I+T / 3) Grading: 4 = excellent; 3=Good; 2= Average; 1=poor	3.1587	3.2646	p=0.2486; NS

Table 2: Comparison of development of 3PN blastocysts in Global and Synbios® media

Description	Global media	Synbios media	P value
% Development of Total 3PN Day 5 blastulation (Includes: Cavitation/Early blastocyst/Blastocyst)	65.5 (36/55)	60.0 (33/55)	p=0.6933; NS
% Development of Day 5 Blastocyst Development	41.8 (23/55)	38.2 (21/55)	p=0.8457; NS
% Development Total Day 5 EBL Dev	23.6 (13/55)	21.8 (12/55)	p=1.000; NS
Cumululative Score for total blastulation [Volume+ICM+Trophect (V+I+T)] Key: 12=Excellent; 9=Good; 6=average; 3=poor	9.6522	9.9048	p=0.4278; NS
Summarized grade score (V+I+T / 3) Grading: 4 = excellent; 3=Good; 2= Average; 1=poor	3.1968	3.3014	p=0.4240; NS

Table 3: Comparison of development of 1PN and 3PN blastocysts in Global and Synbios® media

Description	Global media	Synbios media	P value
% Development of Total 1PN and 3PN Day 5 blastulation (Includes: Cavitation/Early blastocyst/Blastocyst)	65.5 (72/110)	65.5 (72/110)	p= 1.000; NS
% Development of Day 5 Blastocyst Development	41.8 (46/110)	40.9 (45/110)	p= 1.000; NS
% Development Total Day 5 EBL Dev	23.6 (26/110)	24.5 (27/110)	p= 1.000; NS
Cumululative Score for total blastulation [Volume+ICM+Trophect (V+I+T)] Key: 12=Excellent; 9=Good; 6=average; 3=poor	9.5652	9.8444	p=0.2649; NS
Summarized grade score (V+I+T / 3) Grading: 4 = excellent; 3=Good; 2= Average; 1=poor	3.1773	3.2818	p= 0.2601; NS

EBL=Early blastocyst; ICM= inner cell mass; T=Trophectoderm; V= Volume

Likewise, the qualities of blastocysts that developed from 3PN zygotes (Table 2) were also statistically similar in all respects.

When the combined data (Table 3) of blastocysts generated from 1PN and 3PN zygotes were statistically analyzed the outcome was again statistically similar such that there was no difference between the two ECMs with regard to the qualities of blastocysts generated.

In general, the overall quality of the blastocysts generated in the synthetic Synbios medium was marginally superior but this was statistically insignificant.

Discussion

The synthetic Synbios® protein-free medium is comparable in efficacy to contemporary human ECM (GM) supplemented with serum proteins with reference to the rates and qualities of blastocysts generated. The synthetic medium also overcame and eliminated the risks associated with the use of medium containing donor proteins and preserved the purity of the progeny.

Cleavage stage embryos generated in the synthetic medium has resulted in clinical pregnancy rates of about 50% with live births (1-4) similar to or better than protein-containing media. It is anticipated the Synbios® medium similarly is capable of generating viable blastocysts in the human as well.

This modified method of blastocyst grading enabled the statistical comparison of the qualities of blastocysts that developed in both Synbios® and the control Total Global® media. This modification is similar to that previously proposed by Rehman and coworkers (Rehman et al., 2007) but differed in the way the following parameters of blastocyst expansion, ICM and trophectoderm respectively were scored numerically. The modified blastocyst grading method proposed by Ali scores each parameter separately as follows. The blastocyst is graded 4 (excellent), 3 (Good), 2 (average) and 1 (poor) [similar to that of cleavage stage embryos (Ali et al., 2000)].



Image 1: A hatching day 5 blastocyst generated in the synthetic Synbios® medium

In conclusion, the synthetic Synbios® medium appears as efficacious as contemporary protein-containing media. It is safe. It prevents transmission of disease and hazardous undeclared proteins which could harm patient, babies and healthcare workers. It also prevents batch variation and transmission of contaminant donor RNA/DNA thus preventing corruption of the embryonic genome thereby preserving the purity of lineage of the progeny.

Reference

- Ali J. Standard Operating Procedures. Univ. Malaya Fertil Clinic, University of Malaya, Kuala Lumpur, Malaysia, 2014.
- Ali J, Shahata MA, Al-Natsha SD. Formulation of a protein-free medium for human assisted reproduction. Human Reproduction. 2000 Jan 1;15(1):145-56.
- Ali J, Shahata MA, Al-Natsha SD. Formulation of a protein-free medium for human assisted reproduction. Human Reproduction. 2000 Jan 1;15(1):145-56.
- Ali J. Formulation of a protein-free culture system for the culture of human embryos: preliminary findings and pregnancies. Proc. 16th Ann Sci Meet Fertil Soc Australia, 2-4 Dec 1997
- Ali J. Investigation into the nutrient requirement of the human embryos: Successful formulation and clinical trial of a novel protein-

free embryo culture medium. *Emirates Med J*. 2000;18:195-202.

Barnes D, Sato G. Methods for growth of cultured cells in serum free medium. *Anal Biochem* 1980; 102(2): 255-270.

Dyrlund TF, Kirkegaard K, Poulsen ET, Sanggaard KW, Hindkjær JJ, Kjems J, et al. Unconditioned commercial embryo culture media contain a large variety of non-declared proteins: a comprehensive proteomics analysis. *Hum Reprod* 2014; 29(11): 2421-2430.

Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. Jansen R, Mortimer D. (Editors). *Toward reproductive certainty: fertility and genetics beyond 1999: the plenary proceedings of the 11th World Congress on In Vitro Fertilization and Human Reproductive Genetics* Parthenon, Pearl River, NY, 1999; 378-388

Gardner DK, Schoolcraft WB. In: Jansen R, Mortimer D, editors. London: Parthenon Publishing; 1999. p. 378–88

Kemmann E. Creutzfeldt-Jakob disease (CJD) and assisted reproductive technology (ART). Quantification of risks as part of informed consent. *Hum Reprod* 1998; 13(7): 1777.

Morbeck D, Paczkowski M, Fredrickson J, Krisher R, Hoff H, Baumann N, et al. Composition of protein supplements used for human embryo culture. *J Assist Reprod Genet* 2014; 31(12): 1703-1711.

Rehman KS, Bukulmez O, Langley M, Carr BR, Nackley AC, Doody KM, Doody KJ. Late stages of embryo progression are a much better predictor of clinical pregnancy than early cleavage in intracytoplasmic sperm injection and in vitro fertilization cycles with blastocyst-stage transfer. *Fertil Steril*. 2007 May;87(5):1041-52. doi: 10.1016/j.fertnstert.2006.11.014.

Truyen U, Parrish CR, Harder TC, Kaaden OR. There is nothing permanent except change. The emergence of new virus diseases. *Veterinary microbiology*. 1995 Feb 1;43(2-3):103-22.

van Os HC, Drogendijk AC, Fetter WP, Heijntink RA, Zeilmaker GH. The influence of contamination of culture medium with hepatitis B virus on the outcome of in vitro fertilization pregnancies. *Am J Obstet Gynecol* 1991; 165(1): 152-159.

Vilella F, Moreno-Moya JM, Balaguer N, Grasso A, Herrero M, Martínez S, Marcilla A, Simón C. Hsa-miR-30d, secreted by the human endometrium, is taken up by the pre-implantation embryo and might modify its transcriptome. *Development*. 2015; 142(18): 3210-21. doi: 10.1242/dev.124289.

Whitten WK. Culture of tubal ova. *Nature*;1956: 177:96

Whitten WK. Culture of tubal ova. *Nature* 1957;179:1-1082