Quality of sibling cleavage-stage embryos generated in commercial embryo culture media with and without added serum proteins or serum products

Hasnidar A. Tarmizi[1], Nuguelis Razali[1], Mathi Arasu[2], Sharifah Mahfudzah[1], Siti Khadijah Idris[1], Syairah Hanafiah[1], Agilan Arjunan[1], Magendra Ramalingam[3], Ghofraan A. Ata'Allah[1], Mukhri Hamdan[1]

[1]Department of Obstetrics and Gynecology, University of Malaya Medical Center, 59100 Kuala Lumpur, Malaysia.
[2]Unit G-2 ,Ground Floor , The Podium Tower 3 , UOA Business Park , No 1 , Jalan Pengaturcara U /51A Seksyen U1 , 40150 Shah Alam, Selangor, Malaysia
[3]Department of Obstetrics and Gynaecology, Tengku Ampuan Rahimah General Hospital, Klang, Selangor, Malaysia

Abstract

Objective
The objective of this study is to elucidate and determine the impact of the absence of serum proteins in embryo culture medium (ECM) on quality of embryos and pregnancies generated.
Materials and methods
Retrospective analysis of laboratory data was undertaken on the quality of sibling embryos and pregnancies generated in the synthetic chemically defined protein-free medium (PFM) from Cellcura®, Norway and the control Sage®Medium (SM) from Cooper Surgical, USA. The parameters investigated were: fertilization rate (FR), zygote arrest rate (ZAR), mean blastomere number (MBN), mean embryo grade (MEG). [Embryos graded 4 = excellent, 3=good,, 2=average, 1=poor].
Results
Day 2 sibling embryo MBN and MEG for PFM and SM was 4.4 vs 3.9, p=0.4691; 3.2 vs 3.0, p=0.0405 respectively. On day 3 the mean blastomere numbers and embryo grades for PFM and SM were 6.6 vs 6.1, p=0.2247; 3.2 vs 2.9, p=0.4318, respectively. Preliminary positive blood test for the PFM appears to be about 57.1% (16/28) for all age groups combined.
Discussion
Sibling embryo study in this small study in a newly established fertility center comparing outcome of sibling human embryo development in PFM and SM suggest the efficacy of both media to be similar in generating quality embryos albeit lower in SM. The pregnancy rate for the PFM appears to be excellent. The PFM is the only synthetic ECM available that is also certified Halal. It eliminates risk of disease transmission, and is safe. Due to its chemically-defined nature it offers batch to batch consistency in ART.
Conclusion
The synthetic PFM medium is equivalent to contemporary ECM in efficacy with excellent pregnancy rates. It eliminates risk of disease transmission, batch variation, is safe, completely chemically defined, and culturally permissible to some communities. Use of two media simultaneously will help avoid failure of treatment due to sub-optimal culture medium quality.

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Correspondence: Hasnidar Ahmad tarmizi; **Email:** hasnidar@ummc.edu.my
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Introduction

The embryo culture media (ECM) normally contains donor serum proteins or human serum albumin (HSA), which is well recognized to cause batch to batch variation in ECM

production affecting quality of embryos generated. ECM containing HSA normally has a short expiry period of 12-20 weeks. HSA-supplemented ECM carry the risk of harboring infectious agents and could transmit hazardous disease through protein-bound pathogenic agents such as prions and viruses (Van Os et al., 1991; Kemmann 1998; Ali et al., 2000; Ali 2004). Disease transmission due to tainted serum protein-supplemented embryo culture media has been reported (Van Os et al., 1991; Kemmann, 1998) and is in part because of the fact that proteins cannot be sterilized with absolute certainly (Truyen et al., 1998). Serum proteins can also be embryotoxic (Miller et al., 1995 and Fein et al., 1995). These are the disadvantages of using HSA-supplemented ECM. In view of these issues research effort led to the development of a synthetic ECM (Ali, 1997; Ali et al., 2000; Ali, 2001; Ali, 2004) which overcame the disadvantages of ECM containing HSA.

Of considerable interest is the finding that DNA fragments present in the vicinity of the embryo, such as in the uterine fluids, could be passed on to and be expressed in the embryo (Johnson, 2015; Tonkin, 2015). It follows that donor DNA and RNA strands present in HSA could contaminate the ECM and subsequently be passed on to and be expressed in the cultured embryo such that the embryo would then carry the genetic material from more than two parents. If this is proven conclusively to be true, the use of HSA in ECM would become unacceptable to some cultures that advocate the preservation of the genetic purity of the progeny and prohibits third party involvement in reproduction. The work of Vilella and coworkers has demonstrated maternal endometrial miRNAs act as transcriptomic modifiers of the preimplantation embryo (Vilella et al., 2015; Balaguer et al., 2018).

The objective of this study is to elucidate and compare the impact of commercial synthetic protein-free and protein-supplemented ECM on quality of cleavage stage embryos generated, and pregnancies elicited. The original work on the synthetic protein-free ECM were performed elsewhere (Ali, 1997; Ali et al., 2000; Ali, 2001; Ali, 2004) using media prepared in-house. The commercial synthetic media obtained from Cellcura® ASA, Norway, used in the present study is of the same formulation.

Materials and methods

Retrospective analysis of laboratory data was undertaken on the quality of sibling embryos and pregnancies generated in the synthetic chemically defined protein-free medium (PFM) from Cellcura®Medium, Norway, and the control Sage® Medium (SM) from Cooper Surgical, USA. The parameters investigated were: fertilization rate (FR), cleavage stage mean blastomere number (MBN) and the respective mean embryo grade (MEG). [Embryos graded 4 = excellent, 3=good, 2=average, 1=poor] on days 2 and 3. The embryos were transferred on day 3. The data from routine cIVF and ICSI performed eight months after the above sibling study was analyzed to compare differences between the outcomes of both studies.

Results

Laboratory data was analyzed retrospectively to determine differences between quality of sibling embryos and pregnancies generated in the PFM and the SM. There were 30 patients included in this study. Of these, 19 were below 35 years of age. While 9 were 36 to 40 years, and 2 were 41 to 42 years of age. The fertilization rate for combined cIVF and ICSI was higher for the PFM (75.8%) and somewhat lower for the SM (60.9%) but the difference was not statistically significant. Day 2 sibling embryo MBN and MEG for PFM and SM was 4.4 vs 3.9, p=0.4691; 3.2 vs 3.0, p=0.0405 respectively. On day 3 the MBN and MEG for PFM and SM were 6.6 vs 6.1, p=0.2247; 3.2 vs 2.9, p=0.4318, respectively. The positive pregnancy blood test was 53.3%. The clinical pregnancy rate (inclusive of sac & fetal heart beat) for the PFM was 43.3% (13/30) for all age (28 to 42yrs) groups combined (Table 1). There is no data for SM with regard to pregnancy rate because the lower grades and quality of embryos generated in the SM excluded its selection for embryo transfer.

The data from routine cIVF and ICSI of some cases performed eight months later using the protein-containing Irvine® medium (IR; Irvine Scientific, USA) revealed no differences statistically between the fertilization and quality of embryos generated in the IM and PFM media. The fertilization rate between the IR and SM were statistically similar but quality of

embryos generated in the SM was somewhat lower in quality statistically compared to the IM media. The proportion of positive pregnancy blood tests was lower in the IR medium (40%) compared to the PFM medium (53.3%) but this was not statistically different.

Overall the quality of embryos generated in the synthetic protein-free medium appears similar to the contemporary protein-containing media. This is also evident in the proportion of pregnancies generated in the media investigated.

Table 1: Quality of sibling embryos generated in the synthetic protein-free Cellcura® and protein-containing Sage® media

Description	Sibling Study		
	Cellcura® PFM Med	**Sage® Medium**	**Statistical Significance**
Day 1			
Fertilization Rate	75.8%	60.9%	NS
(Fertilized/Total	50/66	(42/69)	
Day 2			
Mean blastomere number	4.4	3.9	p=0.4691
Mean embryo grade	3.2	3	p=0.0405
Day 3			
Mean blastomere number	6.6	6.1	p=0.2247;
Mean embryo grade	3.2	2.9	p=0.4318
Pregnancies per ET (All age groups, 9/30 patients were >30yrs of age)			
Positive blood test	53.3% (16/30)	N/A*	N/A
Clinical Preg Rate (Sac & FHB)	43.3% (13/30	N/A	N/A

Table 2: Quality of non-sibling embryos generated in the Irvine®, Cellcura® and Sage® media

Description	**Non-sibling comparison between culture media 8 months later**		
Culture Media	**Irvine®**	**Cellcura® PFM**	**Sage®**
Fertilization rate	91&92%	NS	NS
	(61/67, ICSI)& (24/29, cIVF)		
Day 2			
Mean blastomere number	4.3	NS	NS
Mean embryo grade	3.5	NS	S
Day 2			
Mean blastomere number	7.1	NS	S
Mean embryo grade	3.1	NS	NS
Pregnancy rate per ET			
	40.0% (6/15)	NS	N/A.

Discussion

This retrospective sibling embryo study was undertaken in a newly established fertility center by a team of workers all of whom except for a single worker had no prior experience in IVF work. Consequently, in the early months of their operation, the fertilization rate for both media was somewhat less than that previously reported for a similar study (Ali et al., 2000; Ali, 2001; 2004). Nevertheless, the findings of the comparative performance of both the PFM and SM media are valid because both media were handled without bias in any manner. The team members acquired sufficient skills within 8 months of operation such that their fertilization rate had improved significantly to 91% and 92%. In general the quality of embryos generated in the PFM medium was almost similar to the SM medium. The differences between the PFM and SM were only discernible on day 2 for the embryo grade. This sibling study also revealed the importance of using two culture media which appears safer than single medium. The comparison between PFM and IR media with regard to fertilization rate, embryo quality and pregnancy rate were statistically similar. Whereas, the quality of embryos generated in the SM medium were somewhat poorer compared the IR medium.

The use of two culture media simultaneously may help patients avoid failure that could occur due to compromised media quality (Ali et al., 2014) but is expensive. Oftentimes we experience setbacks (poor culture conditions) when quality of culture medium is compromised because of (i) sub-optimal handling during long distance transport, (ii) batch to batch variation during manufacturing, (iii) breakdown of cold chain, and (iv) defective manufacturing. These issues are well recognized. It is imperative two embryo culture media are used simultaneously for sibling embryo culture to avoid treatment failure that could occur as a direct consequence of sub-optimal quality of a given embryo culture medium.

Conclusion

Sibling human embryo development in PFM and SM suggest the efficacy of both media to be similar albeit slightly lower in SM in generating quality embryos. The pregnancy rate for the PFM appears excellent. The PFM is the only synthetic ECM available. It eliminates risk of disease transmission, is safe, completely chemically defined, and culturally permissible to some communities. Due to its chemically-defined nature it offers the most advanced batch to batch functional consistency in ART. Use of two media simultaneously will help avoid failure of treatment due to sub-optimal culture medium quality.

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