BRIEF COMMUNICATION

Significance of using two embryo culture media to ensure cycle success

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Abstract

Introduction
Quality issues concerning the efficacy of embryo culture media to generate viable embryos recur from time to time. Embryo culture media (CM) could be damaged due to: (i) a break in the cold chain (ii) manufacturing defects (iii) media deterioration during handling by border security forces/local agents/distributors or (iv) batch variation. Dependence on a single culture media could therefore be disadvantageous.
Objective
To confirm previous study that using two different culture media ensures that the cycle succeeds to the embryo transfer stage.
Methods
This is a retrospective investigation comparing the outcome of various parameters of laboratory IVF procedures on sibling oocytes when two different media were utilized in each treatment cycle. The media used were Medium A = Irvine Scientific, USA; Medium B = Life Global, USA and Medium C = Origio, Denmark. ET were performed on days 2 or 3 and remaining embryos cultured and cryopreserved at blastocyst stage.
Results
There were subtle but insignificant differences between Medium A and Medium B. The mean grade for day 2 embryos was the only parameter with a significant outcome for medium B compared to medium A (3.4±0.1 vs 3.0±0.2; p=0.0203). The fertilization rate was significantly higher for medium C compared to medium A (67.0±6.7vs84.5±4.9; p= 0.0087). All other parameters were statistically similar for medium A and C. Blastocyst formation rates from leftover embryos after ET were statistically comparable between the media although the pregnancy rates were numerically different.
Discussion and Conclusion
The present study shows there are differences between the media but this could not be statistically demonstrated conclusively due to the small sample size for most parameters tested with the exception of a few. The present findings demonstrate that using two media is highly recommended to ensure cycle treatment success.

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Introduction

Making sure the embryo culture medium (CM) is reliable to support embryonic development is a crucial step in ART treatment. It was previously communicated that two media should be used in laboratory IVF procedures because the dependence on a single culture medium may be

disadvantageous (Ali et al., 2014). If the CM is sub-optimal or is damaged the treatment cycle will fail. Cycle failure has serious consequences for the patients and may reflect poorly on the service provider. The reason for this precautionary measure was because of the potential for the CM to be damaged in transit during transport from manufacturer to end user. This could occur due to the inadvertent occurrences of the following negative events during transport such as: (i) breakdown in cold chain; (ii) possible defects during manufacture; (iii) deterioration of quality of media due to handling by border security forces or by local agents/distributors or due to (iv) batch variation (Ali et al.,2014). Batch variation in particular is a well-recognized problem confronting the IVF laboratory worker (Sunde et al., 2013) and appears to occur sporadically (see EmbryoMail Dated 21,22 and 23 February 2024).

The present retrospective investigation is intended to confirm the previous report of Ali and coworkers (Ali et al., 2014) whether the use of two CMs per treatment cycle is worth the extra expenditure to improve and or to ensure the success of the treatment cycle. We report here our findings on the utilization of two CM per treatment cycle on the survival and viability of cultured embryos up to the blastocyst in vitro.

Materials and methods

This is a retrospective investigation comparing the outcome of various parameters of laboratory IVF procedures on sibling oocytes when two different media were utilized in each treatment cycle. The media used were Medium A = Irvine Scientific, USA; Medium B = Life Global, USA and Medium C = Origio, Denmark. Standard procedures were employed. The sibling oocytes were equally apportioned between media A and B or Media A and C. Embryo transfer was performed on days 2 or 3, and the remaining embryos were cultured and cryopreserved at blastocyst stage. Embryos were graded: 4 = excellent, 3=good, 2=average, 1=poor).

Results

There were subtle but insignificant differences between Medium A and Medium B or Medium A and C for the majority of the parameters investigated in Tables 1-2 with two notable exceptions. For instance, the mean blastomere number on day 2 was higher in Medium B compared to Medium A. Likewise the fertilization rate was significantly higher for Medium C.

Discussion

The present study shows the 3 media tested supported embryo development. However, numerical and minor statistical differences were noted between them. Overall there was at least one parameter in each of the above two investigations which noted significant superior outcomes for Media B and C over medium A. These two differences were with regards to significantly higher day 2 mean embryo grade in medium B and significantly higher fertilization rate in medium C. In general, however, the differences between the media tested were mostly not significant which is probably because of the small sample size which appears to have confounded the outcome, unlike the previous report of Ali and co-workers (Ali et al., 2014). Ali and co-workers demonstrated significant differences between the Cellcura synthetic medium (Cellcura, Norway) and Sage medium (Sage, USA) with regard to laboratory ART procedures. They noted significantly superior outcome with the Cellcura synthetic medium.

In the present study the pregnancy rates were numerically vastly different between the media but were not statistically significantly different due to the small sample size. The limitation of the present study is its small sample size. The comparison would likely have been more meaningful with a more pronounced demonstration of differences between the media tested if the sample size was larger. Overall however it can be inferred that differences between the media exist.

These differences could be due to batch-to-batch variation or due to defects that could have occurred during production or handling, such as disruption in cold-chain. It appears reasonable to conclude that the present findings and that of the previous report of Ali and co-workers (Ali et al., 2014) suggest using two media is highly recommended to ensure cycle success in the event the quality of one media is compromised for reasons stated previously. The present findings appear to confirm previous findings. The

Table 1: Summary of differences between Media A and B

Medium Description	Medium A	Medium B	p-Value
Fertilisation Rate	85.6±3.5	80.1±5.9	0.4186
Zygote Arrest Rate	4.7±3.5	5.8±4.5	0.8313
D2 Mean Blastomere Nos.	4.8±0.5	4.6±0.5	0.8303
D2 Mean Embryo Grade	3.0±0.2	3.4±0.1	**0.0203** (S)
D3 Mean Blastomere No.	8.5±0.4	8.0±0.5	0.6385
D3 Mean Embryo Grade	3.5±0.1	3.2±0.9	0.1126
% Blastocyst from D2 Leftover Embs.	66.7% (6/9)	45.5% (5/11)	0.6196
% Blastocyst from D3 Leftover Embs.	41.5% (17/41)	20.0% (9/45)	0.0532

D2 = day 2; D3 = Day 3

Table 2: Summary of differences between Media A and C

Description	Medium A	Medium C	P Value
Fertilisation Rate	67.0±6.7	84.5±4.9	**0.0087 (S)**
Zygote Arrest Rate	14.4±6.8	3.7±3.7	0.2776
D2 Mean Blastomere No.	3.5±0.3	3.8±0.3	0.2509
D2 Mean Embryo Grade	3.1±0.2	3.3±0.1	0.1556
D3 Mean Blastomere No.	9.8±0.7	8.8±0.7	0.3083
D3 Mean Embryo Grade	3.3±0.1	3.2±0.1	0.5200
% Blastocyst from D2 Leftover Embs.	16.7% (2/12)	37.5% (6/16)	0.4337
% Blastocyst from D3 Leftover Embs.	32.0% (8/25)	55.6% (15/27)	0.1520

D2 = day 2; D3 = Day 3

Table 3: Pregnancy rate elicited by embryos generated in culture medium A or B, and from combined and from embryos generated in A and B combined

%Total Preg	Preg From A+B	Preg From A Only	Preg From B Only
12/24 (50.0%)	5/9 (55.6%)	6/12 (50.0%)	1/3 (33.3%)

Preg = Pregnancy

Table 4: Pregnancy rate elicited by embryos generated in culture medium A or C, and from embryos generated in A and C combined

%Total Preg	Preg From A+C	Preg From A Only	Preg From C Only
5/18 (27.8%)	2/5 (40.0%)	2/9 (22.2%)	1/4 (25.0%)

Preg = Pregnancy

use of two media per treatment cycle on sibling oocytes is a risk-management strategy that can be practiced to avoid cycle failure which could occur due to a less optimal culture medium. It is very well documented cycles that fail to reach the embryo transfer stage or fail to achieve pregnancy can have devastating emotional consequences for the affected patients (Boivin et al., 1998; Cipoletta and Faccio, 2013; Holter et al., 2021; Karaca et al., 2016; Rockliff et al., 2014; Verhaak et al., 2005, 2007; Volgsten et al., 2010). Cycle failure must be avoided even if it meant extra expenditure on media purchases. Some IVF clinics have been utilizing two culture media per treatment cycle since the early 2000s successfully with no appreciable cycle failure (Ali et al., 2014).

Conclusion

In conclusion, it appears preferable to use two media per treatment cycle as a risk management strategy to avoid cycle failure due to the unlikely but possible propensity for the culture medium to be damaged during manufacture or transit from manufacturer to the end user.

References

Ali J, Razali N, Arasu M, Tarmizi HA, Mahfudzah S, Arjunan A, Ata'Allah GA, Ramalingam M, Rashid MM, Hanafiah S, Mohammad H, Omar SZ, Mat Adenan NA. Quality of sibling cleavage-stage embryos generated in commercial embryo culture media with and without added serum proteins or serum products. 2. Use of back-up medium, 3. Efficacy of ART in new clinic. Proceedings of the 5th Congress of the Asia Pacific Initiative on Reproduction (ASPIRE 2014), Brisbane, Australia, 4- 6 April 2014, Abst. FC117. pg:114

Boivin J, Andersson L, Skoog-Svanberg A, Hjelmstedt A, Collins A, Bergh T. Psychological reactions during in-vitro fertilization: similar response pattern in husbands and wives. Hum Reprod 1998;13:3262–3267

Cipolletta S, Faccio E. Time experience during the assisted reproductive journey: a phenomenological analysis of Italian couples' narratives. J Reprod Infant Psychol 2013;31:285–298

Holter H, Bergh C, Gejervall AL. Lost and lonely: a qualitative study of women's experiences of no embryo transfer owing to non-fertilization or poor embryo quality. Hum Reprod Open. 2021 Jan 19;2021(1):hoaa062. doi: 10.1093/hropen/hoaa062.

Karaca N, Karabulut A, Ozkan S, Aktun H, Orengul F, Yilmaz R, Ates S, Batmaz G. Effect of IVF failure on quality of life and emotional status in infertile couples. Eur J Obstet Gynecol Reprod Biol 2016;206:158–163.

Rockliff HE, Lightman SL, Rhidian E, Buchanan H, Gordon U, Vedhara K. A systematic review of psychosocial factors associated with emotional adjustment in in vitro fertilization patients. Hum Reprod Update 2014;20:594–613.

Sunde A, Balaban B. The assisted reproductive technology laboratory: toward evidence-based practice? Fertil Steril. 2013;100(2):310-318.

Verhaak CM, Smeenk JMJ, Evers AWM, Kremer JAM, Kraaimaat FW, Braat DDM. Women's emotional adjustment to IVF: a systematic review of 25 years of research. Hum Reprod Update 2007;13:27–36.

Verhaak CM, Smeenk JMJ, van Minnen A, Kremer JAM, Kraaimaat FW. A longitudinal, prospective study on emotional adjustment before, during and after consecutive fertility treatment cycles. Hum Reprod 2005;20:2253–2260.

Volgsten H, Skoog Svanberg A, Ekselius L, Lundkvist Ö, Sundström Poromaa I. Risk factors for psychiatric disorders in infertile women and men undergoing in vitro fertilization treatment. Fertil Steril 2010;93:1088–1096