Embryo culture media update

Details about this paper

Area(s) of strategy this paper relates to:	The best care – effective and ethical care for everyone
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Annexes	None

Output from this paper

For information or recommendation?	For recommendation
Recommendation:	Members are asked to: • consider the progress of research (since June 2019) into the effects of components in culture media used for IVF treatment; • advise the Executive if they are aware of any other recent developments and; • advise what, if anything, needs to be communicated to the MHRA who are responsible for regulating the composition and safety of culture media used in the UK
Resource implications:	None
Implementation date:	N/A
Communication(s):	None
Organisational risk:	Low

1. Introduction

1.1. Clinical in vitro fertilisation (IVF) systems aim to imitate the conditions an embryo would encounter in vivo. This means it is important to optimise the culture environment of embryos during IVF treatment. The components of embryo culture media, therefore, require scrutiny to ensure that risks are minimised, embryo stress is avoided, and embryo health is maintained.

1.2. Although generally considered to be safe based on past and current experience, uncertainties remain about the effects of embryo culture media. The concentrations of components such as growth factors, amino acids, energy substrates and antibiotics could impact fertilisation rate, early embryo development and clinical outcomes for children conceived by assisted reproduction. Different media can influence many outcomes including fertilisation rate and clinical outcome.

1.3. Concerns about the components of embryo culture media, how they are regulated, and their potential effects have been discussed by the SCAAC previously. At its meeting in:

1.3.1. June 2019, SCAAC noted that one study found no difference in cardiovascular development in 9-year-old children, who were born following IVF or intracytoplasmic sperm injection (ICSI) treatment when two different culture media were used. SCAAC also commented that the use of using data driven technologies in IVF would be difficult if the contents of embryo culture are still largely unknown.

1.3.2. In June 2017, SCAAC discussed research questioning a possible association between type of culture media and birthweight

1.3.3. Also, in 2017, SCAAC discussed the possible association between culture media and imprinting disorders based on mice studies. This is particularly concerning as the precise composition of culture media is not disclosed by the manufacturers to those purchasing and using the product.

1.4. Culture media acts as a surrogate for maternal nutrition for the first few days, therefore it would be important to know concentration of nutrients such as glucose and amino acids in the media.

1.5. New research in this area is monitored periodically to inform members of the potential impact of culture media composition on embryonic development and SCAAC's discussions on this research are passed on to the Medicines and Healthcare Products Regulatory Agency (MHRA) for information. The research highlighted in this paper has been published between June 2019 and January 2021.

2. Current regulation

2.1. Since 1 January 2021, in response to the end of the transition period after EU Exit, there have been several changes, introduced through secondary legislation, to how medical devices are placed on the market in Great Britain (England, Wales and Scotland). In the UK, all medical devices, including human embryo culture media used for treatment purposes, placed on the Great Britain market need to be registered with the MHRA and be approved by UK Approved Bodies. Following assessment, the approved body will issue relevant certification allowing manufacturers to place UKCA marking on their products and to place them on the market in Great Britain. It is not within the remit of the HFEA to regulate the composition and safety of culture media.

2.2. Prior to this, human embryo culture media used for treatment purposes had to be CE marked by a UK Notified Body recognised by the EU. The MHRA designated UK Notified Bodies to assess manufacturers for quality and safety, and these bodies were audited by MHRA within the UK under the European Medical Devices Directive 93/42/EEC[1]. Although the UKCA mark will be available for use in Great Britain from 1 January 2021, CE marking will continue to be needed for devices placed on the Northern Ireland market and EU rules will need to be met. CE marked devices will also be accepted on the Great Britain market until 30 June 2023.

2.3. From January 2021 for Great Britain, activities that can be undertaken by an approved body to assess whether manufacturers and their medical devices meet the requirements were set out in the Medical Devices Regulations 2002 , these include:

2.3.1. an assessment of the manufacturer's quality system, including design

2.3.2. assess the full design dossier relating to each type of product to ensure that they meet the requirements

2.3.3. assess the full technical information relating to each type of product and carry out appropriate testing of a representative sample of production to ensure that it meets the requirements

2.3.4. either test every unit or every batch of product to ensure that they are meeting the requirements before the manufacturer can place them onto the market

2.3.5. production and product quality assurance

2.3.6. unannounced audits of manufacturers

3. Research

Culture media comparisons

3.1. Reed et al investigated the viscosities of media used for human embryo transfer and the possible effect of viscosity as it relates to interactions between transfer media and uterine fluid. The study observed a relatively narrow distribution of viscosities across several transfer media (G1-Plus, G2-Plus, G-TL, 1-Step, Global Total, Global Total HEPES, and Sperm Wash Medium) despite the various commercial or in-house modifications. The data did however demonstrate the difference between the viscosities of embryo transfer media and the assumed viscosity of uterine fluid. The authors highlighted that embryo transfer media may be well-suited for IVF, but additional evaluation of all variables, e.g. media viscosity in the context of embryo transfer, adds to the knowledge base that should be available to practitioners.

3.2. A cross sectional study by Castillo et al surveyed 46 UK IVF clinics. Information regarding culture medium type, incubator type, and oxygen level used in ART between January 2011 and December 2013 was collated. The survey responses were merged with recorded treatment and outcome data held in the HFEA Register up to the end of 2014. There were statistically significant differences in live birth weight (LBR) between the eight culture media systems analysed; however, none of the embryo culture factors showed statistically significant associations with birth weight.

[1] https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=%20CONSLEG:1993L0042:20071011:en:PDF

The study did note a very strong effect of clinic site on both LBR and birth weight, therefore treatment practices and clinic site may have masked the effect of culture conditions.

3.3. A multi-centre randomised controlled trial (RCT) in the Netherlands carried out by Mulder et al investigated if there is a difference in DNA methylation status of imprinted genes in human placenta derived from IVF conceptions exposed to human tubal fluid (HTF) (n=43) versus G5 culture (n=54) medium. Placenta samples from 69 naturally conceived live births were collected during 2008-2013 in the Netherlands as reference material. Results showed no statistically significant differences in the mean DNA methylation status of any of the 34 imprinted differentially methylated regions in placentas derived from IVF conceptions cultured in HTF or G5 culture medium. It has often been postulated, but has yet to be rigorously tested, that there is an association between culture media and imprinted genes which brings about the effects on pregnancy, birth, and child development in humans. Since the study did not detect any statistically significant effects of embryo culture conditions on methylation status of imprinted genes in the placenta, this suggests that other unexplored mechanisms may underlie these effects.

3.4. A randomised control trial by Rose et al investigated if Embryogen®/BlastGen™ culture medium improved live birth rates compared with standard Cleavage/Blastocyst sequential culture medium for women undergoing IVF and ICSI with poor prognosis. A total of 100 couples undergoing IVF/ICSI were included in the study. A significant reduction in day-5 embryo outcome parameters was found using Embryogen®/BlastGen™ compared with standard medium, and insufficient evidence of a difference in pregnancy outcomes. Taking into consideration the small samples size, study limitations and strict inclusion criteria of this single-centre study, they found that further research is needed to determine the efficacy of Embryogen®/BlastGen™ medium in couples undergoing IVF/ICSI.

3.5. Fabozzi et al randomised sibling oocytes for culture in the novel Geri-medium (n = 631 oocytes) or continuous single culture medium (CSCM, n = 643 oocytes) to investigate the difference in blastulation rate per cohort of inseminated oocytes. They found that blastulation rate among cohorts of sibling oocytes cultured in the same incubator is a fast, reliable and comprehensive performance indicator to validate novel commercially available culture medium. The media tested were considered similarly efficient. They found that differences in blastocyst morphology and developmental timings warrant further investigation, although euploidy and ongoing implantation rates were similar.

3.6. A multicentre cohort study by Castillo et al studied whether IVF treatment and laboratory factors affect singleton birthweight (BW). They reported that BWs of IVF-conceived singleton babies are increasing with time, but could not identify the specific treatment factors responsible. The study reports that no significant associations of birth outcomes with IVF embryo culture parameters were seen independent of clinic or time, including embryo culture medium, incubator type or oxygen level, although small differences cannot be ruled out.

3.7. Desai et al published a prospective randomised study which evaluated the efficacy of two different IVF culture media for blastocyst development, pregnancy, and live birth rate on 10,768 sibling pronucleate embryos. Global (GB) medium (used without refreshment) and G-TL medium (designed specifically for culture in time-lapse incubators) were compared. The study concluded that uninterrupted culture in a time-lapse incubator without medium refreshment was well supported by both media tested. Differences in morphokinetics did not necessarily dictate the superiority of one media over the other. Both pregnancy and LBR were not significantly influenced

by choice of culture medium. No difference was noted in blastocyst euploidy rates between the two media: GB 34.7% (275/793) and G-TL 33.3% (209/627).

3.8. A study by Barberet et al compared the epigenetic profiles of 57 children, aged between seven and eight years old, according to the mode of conception (ie ART compared with naturally), the type of embryo culture medium used (ie global medium (LifeGlobal) and single step medium (Irvine Scientific)) and the mode of in vitro fertilisation (ie IVF versus ICSI). The study concluded that significant differences in the DNA methylation of imprinted genes or transposon families were reported between ART and naturally conceived children, but there was no difference between culture media.

3.9. Togola et al assessed 17 plastic consumables and 18 cell culture and ART media for the presence of bisphenols (BPS) which has already been reported to impair oocyte quality at nanomolar concentrations. The study found that while the plastic consumables did not release BPS under routine conditions, 16 of the 18 cell culture and ART media assessed contained BPS. Six media exhibited BPS concentrations higher than 1 nM and reached up to 6.7 nM (1693 ng/l). The study highlighted that a wider implication of the findings is that the presence of BPS in ART media, at a similar concentration range, could contribute to a decrease in the ART success rate.

Self-spent culture medium versus fresh medium

3.10. To explore the role of autocrine factors in embryo self-spent culture media, a study by Wu et al prospectively compared embryo transfers with self-spent culture medium and fresh medium on clinical pregnancy outcomes. A total of 318 fresh IVF/ICSI cycles were randomly allocated into two subgroups based on their transfer media. The study found that implantation rates, clinical pregnancy rates and live birth rates in the transfer group using self-spent medium instead of new pre-equilibrated culture medium were slightly improved but without statistical significance. In addition, biochemical pregnancy rate was found to be significantly decreased after transfer using self-spent medium for day 3 embryos compared with new pre-equilibrated culture media. The authors acknowledged that large sample size studies are still needed to confirm these observations.

Sequential versus single media

3.11. Stimpfel et al published a retrospective study which analysed the outcome of IVF/ICSI cycles (n=172) with regard to different types of culture media used to culture embryos, namely sequential and two types of single step continuous embryo culture media. Results indicated that continuous media can be equivalent to sequential media and could help lower the workload in busy IVF labs without impairing the clinical results. However, the authors stated that caution is needed because this study is limited by its retrospective design. To confirm the results, especially in terms of live birth rates and perinatal outcome, a prospective study is needed with a higher number of included couples.

Effects of supplementation

3.12. Gardner et al carried out a study to investigate if the inclusion of three antioxidants (A3), acetyl-l-carnitine (ALC), N-acetyl-l-cysteine (NAC) and alpha-lipoic acid (ALA) improve human embryo development and pregnancy potential. A total of 1563 metaphase II oocytes from 133 patients in two IVF centres were included and day 3 embryos and day 5/6 blastocysts quality were assessed. The study concluded that the presence of antioxidants during IVF and embryo culture for patients aged 35-40 years resulted in a significant increase in implantation and pregnancy rate. Supplementation of antioxidants to IVF and culture media may therefore improve the viability of human embryos in assisted reproductive technologies, plausibly through the reduction of oxidative stress.

3.13. Armstrong et al assessed the available evidence from RCTs on the effectiveness and safety of granulocyte macrophage colony-stimulating factor (GM-CSF) supplemented culture media, in women or couples undergoing assisted reproduction. Due to the very low to low quality of the evidence, the author's concluded that claims from marketing information that GM-CSF has a positive effect on pregnancy rates are not supported by the available evidence; further well-designed, properly powered RCTs are needed to lend certainty to the evidence.

3.14. Heymann et al published an update of a Cochrane Review first published in the Cochrane Library (2010, Issue 7) to determine whether adding adherence compounds such as hyaluronic acid (HA) to embryo transfer media could improve pregnancy outcomes, including improving LBR and decreasing miscarriage, in women undergoing assisted reproduction. The authors concluded that moderate-quality evidence shows improved clinical pregnancy and LBRs with the addition of HA as an adherence compound in embryo transfer media. Low-quality evidence suggests that adding HA may slightly decrease miscarriage rates when only studies at low risk of bias were included in the analysis, but the results were inconclusive. HA had no clear effect on the rate of total adverse events.

3.15. An RCT by Fawzy et al evaluated the influence of integration of granulocyte-macrophage colony-stimulating factor, heparin-binding epidermal growth factor-like growth factor, and leukaemia inhibitory factor into culture media on human embryo development after ICSI. The study, which included 443 ICSI cycles, concluded that inclusion of cytokines into human embryo culture media showed improvement in embryological and clinical outcomes after ICSI. However, they found the long-term effect of cytokine enrichment of a medium is still unclear and warrants further studies with longitudinal follow-up.

3.16. Hernández et al investigated whether culture media enriched in 4-OH-E2 could improve the quality and implantation rate of embryos obtained in vitro, using both in vitro and in vivo models. They also analysed its effects on the epidermal growth factor-binding (EGF-binding) capability of the embryos. Results showed that the presence of 4-OH-E2 in the culture media of embryos during the morula to blastocyst transition increases embryo quality and attachment to endometrial cells in vitro. Results also showed that 4-OH-E2 can improve viable pregnancy rates of mouse embryos produced in vitro, reaching success rates that are similar to those from embryos obtained directly from the uterus. 4-OH-E2 improved the embryos' ability to bind EGF, which could be responsible for the increased embryo implantation potential observed. Therefore, suggesting that 4-OH-E2 is a strong candidate molecule to supplement human IVF culture media in order to improve embryo implantation. However, they found that further research is required before these findings can be translated with efficacy and safety to fertility clinics.

3.17. Fujii et al carried out a proteome-wide analysis of distal tubal lavage specimens collected from 26 healthy women undergoing open microtubal anastomosis surgery to investigate if there are phase-specific changes in the early secretory (ES) phase human tubal lavage proteome that can inform and potentially optimise IVF culture media. Comparison of the ES and menstrual phase human tubal lavage proteomes revealed 74 differentially expressed proteins with enrichment of pathways and biological processes involved in the regulation of carbohydrate metabolism, oxidative stress and cell survival. The adapter-regulator protein 14-3-3 zeta was among the most significantly increased in the ES phase. Supplementation of embryo culture media with 14-3-3 zeta at concentrations tested did not significantly improve the murine blastocyst development.

4. Conclusions

4.1. Since SCAAC last considered embryo culture media in 2019, research in this area has continued to progress, however, the impact of changes in culture media composition for early embryo development and the long-term health effects of children conceived by ART remains unclear.

4.2. Possible associations have been identified between the type of culture media used and birthweight, imprinting disorders, pregnancy rate and LBR. This has prompted some debate within the sector on whether manufacturers should be more transparent in reporting the composition of their culture media.

4.3. Additional research is required to further explore the relationship between embryo culture media and LBR and longer-term health outcomes in children born from ART.

5. Recommendations

5.1. Members are asked to:

- consider the progress of research (since June 2019) into the effects of components in culture media used for IVF treatment;
- advise the Executive if they are aware of any other recent developments and;
- advise what, if anything, needs to be communicated to the MHRA

6. References

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