Embryo culture media update

Strategic delivery:	☒ Setting standards	☐ Increasing and informing choice	☐ Demonstrating efficiency economy and value
Details:			
Meeting	Scientific and Clinical Advances Advisory Committee (SCAAC)		
Agenda item	5		
Paper number	SCAAC(10/15)01		
Meeting date	21 October 2015		
Author	Sarah Testori, Scientific and Clinical Policy Manager		
Output:			
For information or decision?	For decision		
Recommendation	Members are asked to: • consider the progress of research (since February 2014) into the effects of the components in culture media used for IVF treatment; • advise the Executive if they are aware of any other recent developments; and • reflect on their views to date and identify what needs to be communicated to the MHRA.		
Resource implications	None		
Implementation date	None		
Communication(s)	None		
Organisational risk	☒ Low	☐ Medium	☐ High
Annexes	None		

1. Introduction

1.1. Clinical in vitro fertilisation (IVF) systems aim to imitate the conditions an embryo would encounter *in vivo*, and as such optimising the culture environment during IVF treatment is fundamental. The components of embryo culture media, therefore, require close scrutiny to ensure that gamete and embryo stress and risk is minimised, and embryo health is improved.

1.2. Although generally considered to be safe based on past and current experience, uncertainties remain about the effects of embryo culture media. Varying concentrations of components such as growth factors, amino acids, energy substrates and antibiotics may potentially impact early embryo development, and the long-term health of ART children.

1.3. The body of literature surrounding components of embryo culture media currently suggests that suboptimal culture conditions have the potential to affect embryo viability after transfer, and potentially the health of ART children. Researchers have noted that culture media conditions can result in delayed cell division (Bowman & McLaren 1970) and increased cell death (Brison & Schultz 1997). Furthermore, studies have shown that the environment for preimplantation development can affect the expression and imprinting of key genes (Doherty et al. 2000; Fauque et al. 2007), which can result in chromosomal defects and abnormal embryos, and may act as markers of abnormal health in newborns. A number of recent studies have also suggested that embryo culture can induce changes to epigenetic marks[1] in human embryos (Katari et al. 2009; Zhang et al. 2010) and such modifications have been shown to affect development and future disease risk (Kelsey 2007). Finally, several recent papers have reported correlations between culture media and birthweight (Dumoulin et al. 2010; Eskild et al. 2013; Nelissen et al. 2012) which may have consequences for long-term child health and the early onset of adult diseases (Barker 1986). With such a spectrum of potential for adverse effects on embryo development and potentially health outcomes, it is important to consider the composition and balance of the constituents involved in culture media.

1.4. Concerns surrounding embryo culture media components, its potential effects and its regulation have been previously discussed by the Scientific and Clinical Advances Advisory Committee (SCAAC). The careful consideration of changes to and the introduction of new technologies have also been stressed (Brison et al. 2013; Harper et al. 2012). SCAAC has agreed that new research should be monitored

[1] Epigenetic marks are molecular modifications that regulate gene expression and genome function, can lead to heritable changes in gene expression without changes in DNA sequence.

periodically to inform Members of the potential impact of culture media compositions on embryonic development and that discussion surrounding these findings should be passed to the MHRA for information. This paper presents an update summary of recent studies, looking at the impact of varying components in culture media.

Current regulation

1.5. In the UK, human embryo culture media (used for treatment purposes) must be CE marked by a Notified Body. Notified Bodies assess manufacturers for quality and safety, and these bodies are in turn assessed by the Medicines and Healthcare products Regulatory Agency (MHRA) within the UK under the European Medical Devices Directive 93/42/EC.[2] It is therefore not currently within the remit of the HFEA to regulate the composition and safety of culture media.

1.6. The MHRA currently classifies culture media used for treatment purposes as a high risk class III medical device. Therefore, manufacturers are required to note and justify any changes to culture media composition in their technical documentation. Manufacturers are also expected to ensure post-market surveillance is carried out to monitor the long-term safety of culture media, which is reviewed by a Notified Body.

1.7. Ongoing updates have been provided to SCAAC on research progress regarding culture media and their components, and relevant information has been shared with the MHRA as part of the Executive's joint-working strategy and regulatory remit. This paper is a further update and summarises key research findings between February 2014 and October 2015.

2. Research

Effects of culture conditions on birth weight

2.1. In 2010 Dumoulin et al. published the first paper to find an association between culture media type and birthweight. Following on from this a number of other studies investigating the effect of culture media on birthweight have been published with conflicting results, with some studies observing similar results to Dumoulin et al. (Eskild et al. 2013; Nelissen et al. 2012), while others found no association (Carrasco et al. 2013; Lin et al. 2013).

[2] Council Directive 93/42/EEC of 14 June 1993 concerning medical devices: http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31993L0042:EN:HTML

2.2. Since the committee was last updated on embryo culture media, there have been a number of studies further examining the effect of culture conditions and birthweight. Three recent studies have compared the effect of two different types of culture media on birthweight, finding no correlation between the two. In their 2014 paper Lemmen et al. retrospectively compared the effect on birthweights of Cook (n=974), Medicult EmbryoAssist (with (n=147) or without (n=204) added GM-CSF[3]) with that of the general Danish population (n=106842). The study found no significant effect of these culture media on either the crude nor adjusted birthweight distributions and concluded that birthweights of children born after assisted reproduction follows the same distribution as naturally conceived children (Lemmen et al. 2014).

2.3. Similarly, Wunder et al. (2014) sought to investigate if two distinct, commercially available embryo culture media have a different effect on birthweight and length of singleton infants conceived after ICSI. The authors analysed length, gestational age, and birthweight of 525 singleton pregnancies, in which either Vitrolife (n = 352) or Cook (n = 173) were used, in a cohort study based at the University Hospital, Lausanne, Switzerland. The study did not find significant differences in birthweight between the two groups and the authors concluded that the Vitrolife and Cook do not differentially affect birthweight, in contrast to the results published by Dumoulin et al (2010) comparing the same two media, albiet with a smaller sample size (Wunder et al. 2014).

2.4. Finally, in their study De Vos et al. (2015) carried out a large retrospective analysis including all singleton live births after transferring fresh day three or day five embryos in IVF and ICSI cycles performed between April 2004 and December 2009 at the Centre for Reproductive Medicine, Ziekenhuis University, Brussels, and comparing the effect of two different sequential embryo culture media: Medicult (n = 1388) and Vitrolife (n = 710). Maternal age, maternal and paternal BMI, maternal parity[4], maternal smoking, main cause of infertility, cycle rank, stimulation protocol, method of fertilisation (IVF or ICSI), time in culture and number of embryos transferred were taken into account. The authors observed no significant differences in mean singleton birthweight between the two culture media (De Vos et al. 2015).

2.5. There have also been a number of recent studies which have examined the link between birthweight and other variations to culture media conditions. In 2014 Zhu et al. published two papers investigating whether there is any association between the protein source used to supplement culture media, or the culture period, and birthweight. The authors carried

[3] Granulocyte macrophage colony-stimulating factor (GM-CSF) is a supplement often added to culture media
[4] The number of times a women has carried a fetus to viable gestational age.

out a retrospective analysis of neonatal birthweights, and included 1097 singletons born from fresh embryo transfer cycles at the Centre for Reproductive Medicine, Peking University Third Hospital, with 489 singletons born from G1 v5 culture medium (Vitrolife), which was supplemented with 5 mg/ml pharmaceutical (human serum albumin) HSA solution before use and 608 from G1-PLUS v5[5] media (Vitrolife), which is ready-to-use and includes 5mg/ml HSA. The authors observed that birthweights of singletons, adjusted for gestational age and gender (Z-score), were significantly higher from embryos cultured in G1-PLUS v5 than from those cultured in supplemented G1 v5 (0.28+1.12 versus 0.09+1.15, respectively; P = 0.04), and more large for gestational age (LGA) babies were born from G1-PLUS v5 culture compared with G1 v5 (16.8 versus 12.1%, respectively; P = 0.03), demonstrating for the first time that protein source can affect birthweight in humans (Zhu, Li, et al. 2014).

2.6. In their second study, Zhu et al. (2014) sought to investigate whether prolonged in vitro culture influences newborn birthweight by comparing embryos cultured for three or five days, in a retrospective analysis of 2929 singletons. The number of singletons after day 3 transfer was 2833 and the number of singletons after blastocyst transfer (day 5–6) was 96. The authors observed that the Z scores for singletons after blastocyst transfer were significantly higher than singletons after day three transfer (0.347 versus 0.029 respectively, P = 0.016) (Zhu, Lin, et al. 2014). However the study has significant flaws which limit the conclusions that can be drawn. Primarily, the centre mainly offers blastocyst culture to patients who have had unsuccessful IVF cycles or patients with uterine malformations, which is likely to be the cause of the large discrepancy in sample size between the two groups and is highly likely to introduce a selection bias into the study. In addition the media used for embryo culture was not controlled in the study introducing another possible confounding factor.

2.7. In 2015, the Dumoulin group published a study to examine whether the age of culture medium affects IVF outcome (S. H. M. Kleijkers et al. 2015). The authors noted that it has been demonstrated that culture media can deteriorate during storage (Stewart-Savage & Bavister 1988; Weathersbee et al. 1995; Hossain et al. 2010) and postulated that this suggests that the capacity of culture media to support optimal embryo development decreases over time. The authors used data on outcome of 1832 IVF/ICSI cycles with fresh embryo transfer, performed in the period 2008–2012 to evaluate the association of fertilisation rate, embryonic development, ongoing pregnancy and birthweight of singletons with age of the culture medium (Vitrolife AB G-1 PLUS v5). The age of culture

[5] G1-PLUS is ready-to-use and includes 5 mg/ml of HSA

media had no significant effect on fertilisation rate, early cleavage rate, or ongoing pregnancy, however, birthweight of newborns was inversely associated with age of the medium (β = 3.6 g, SE: 1.5g, P = 0.021), after controlling for possible confounders. The authors concluded that their data indicates a difference of 234g in birthweight of newborns for media with an age difference of 65 days.

2.8. In a second paper from the Dumoulin lab Kleijkers et al. looked at the effect of culture media on post-natal weight in an expansion of their previous report comparing the effect of Cook and Vitrolife on birthweight (Dumoulin et al. 2010). In their study a total of 1432 IVF treatment cycles with fresh embryo transfer were randomly allocated to have all embryos cultured in medium from Vitrolife AB (n = 715) or from Cook (n = 717). Two years after delivery, questionnaires were sent to the parents of all children requesting data about weight, height and head circumference around 1, 2, 3, 4, 6, 7.5, 9, 11, 14,18 and 24 months of age. The study found that in vitro culture of human embryos in medium from Cook resulted in singletons with a lower weight during the first two years of life compared with singletons born after embryo culture in medium from Vitrolife, indicating that the effect of culture medium on fetal development and birthweight persists during the first two years of life. The authors concluded that their data suggests that the human embryo is sensitive to its very early environment and that the culture medium used in IVF may have lasting consequences, this warrants further monitoring of the long-term growth, development and health of IVF children (Kleijkers et al. 2014).

2.9. In a final publication from the Dumoulin group, Zandstra et al. (2015), sought to investigate the association between birthweight and culture media by reviewing all the available literature reporting on a relationship between culture medium and birthweight in human studies and a selection of animal studies. Eleven studies were included in the review; five found significant differences in birthweight when offspring born after culture in different media were compared, while the remaining six found no difference. The authors noted that the studies carried out comparisons of different culture media from one another making meta-analysis impossible. In addition to this, multiple other factors (such as changes in conditons, technical procedures, stimulation protocols and altered populations characteristics) may influence the results (Zandstra et al. 2015). This serves to highlight the need for prospective randomised clincical trials (RCTs) to confirm the effect of culture media on birth weight.

Effects of single and sequential embryo culture media on embryo development

2.10. In a recent study Hardarson et al. (2015) sought to examine whether a culture medium that allows undisturbed culture supports human embryo development to the blastocyst stage equivalently to a well-established sequential media. The authors conducted a randomized, double-blinded sibling trial with 128 patients, with 1,356 zygotes randomly allocated into two study arms to compare embryo development on a time-lapse system using a single-step medium or sequential media. Using a main outcome measure of percentage of good-quality blastocysts on day five, the authors found that single-step culture medium supports blastocyst development equivalently to established sequential media (Hardarson et al. 2015).

Effects of conventional versus closed incubation on embryo development

2.11. Time-lapse imaging incubators are rapidly being introduced into IVF laboratories worldwide. In their recent study, Park et al. (2015) carried out a RCT (n=364 patients) to determine whether culture in a closed system result in an increased number of good quality embryos (GQE) on day two compared with culture in a conventional system. Their data showed no significant differences were found in the number of four-cell embryos, implantation-, pregnancy- or ongoing pregnancy rates. A significantly higher miscarriage rate was found in the time lapse imaging group compared with the control group (33.3 and 10.2%, P = 0.01) (Park et al. 2014).

Effect of hyaluronan enrichment on IVF outcomes

2.12. Hyaluronic acid is an adherence compound and its inclusion in culture media has been well studied (Chronopoulou & Harper 2015). It is present in the endometrium at concentrations that vary according to the day of the cycle (Salamonsen et al. 2001), its receptors are detected in the endometrium and the preimplantation embryo (Knudson & Knudson 1993), and it is commonly added as a supplement to embryo culture media. In their recent study, Fancsovits et al. (2015) conducted a prospective randomized study to investigate the effect of hyaluronan-enriched embryo transfer media on the outcome of in vitro fertilisation and embryo transfer (IVF-ET) treatments. The authors included a total of 581 IVF-ET cycles in the study, with 290 in the hyaluronan (HA) group and 291 in the control group. The authors found no significant difference in clinical pregnancy rate (42.4 vs. 39.2 %), implantation rate (23.3 vs. 23.2 %), delivery rate (31.0 vs. 29.2 %), and number of live births (111 vs. 110) between the groups. However, birth weight was significantly higher in the HA group than in the control group (3,018 ± 598 g vs. 2,724

± 698 g, P = 0.001). Their results therefore do not suggest that hyaluronan enrichment of the embryo transfer media has any beneficial effect on IVF outcome (Fancsovits et al. 2015).

Effect of GM-CSF enrichment on IVF outcomes

2.13. Granulocyte macrophage colony-stimulating factor (GM-CSF) is one of the cytokines playing an important role in reproductive function. In a recent study, Tevkin et al. (2014) compared the effect of EmbryoGen medium containing GM-CSF with a standard combination of medium (ISM1 + VA). Their data showed that fertilisation rate, embryo culture and transfer to patients with previous unsuccessful attempts increases clinical pregnancy rate compared to the control group 39.1 versus 27.8%, respectively. It was noted that the implantation rate (IR) (on seven weeks' gestation) and progressive clinical pregnancy rate (on 12 weeks' gestation) were significantly higher in embryos cultured in EmbryoGen medium compared with the control (20.4 and 17.4% versus 11.6 and 9.1%, respectively) (Tevkin et al. 2014).

Effects of culture media on sex

2.14. In their study, Zhu et al. (2013) sought to investigate the effect of culture media on the proportion of males and females at birth. The study was a retrospective analysis including 4411 singletons born from fresh embryo IVF or ICSI transfer cycles, with embryos cultured in either G5™, (Vitrolife); Global (IVF Online), Quinn's Advantage Medium (SAGE), and G5™ PLUS (Vitrolife). The percentage of males at birth was comparable within the IVF group for all media, however, within the ICSI group, the percentage of male babies in cycles using G5™ (56.1%) was statistically significantly higher than in cycles that used Global (47.2%; P = 0.003), G5™ PLUS (47.7%; P = 0.005) or Quinn's media (45.0%; P = 0.009). The authors concluded that their data suggests that human embryogenesis responds differently to different culture media (Zhu et al. 2015).

Effects of culture media on ectopic pregnancy

2.15. In a recent study, Lin et al. (2015) explored the effect of type of media used to culture embryos for IVF on the incidence of ectopic pregnancy, in a retrospective analysis involving 23,481 women who underwent IVF-ET cycles with embryos cultured in either G5™, Global, or G5™ PLUS. The authors reported that 23,481 fresh transfer cycles, 364 patients were diagnosed with ectopic pregnancy . The ectopic pregnancy to clinical pregnancy rate was 3.01% in the G5 group, 3.89% in the G5 Plus group, and 4.04% in the Global group. After adjusting for confounding factors, the authors found that the incidence of ectopic pregnancy was significantly associated with the G5 Plus and Global media (Lin et al. 2015).

Effects of culture media on gene expression

2.16. As previously discussed, several human studies have shown an effect of culture medium on embryo development, pregnancy outcome and birthweight. However, the underlying mechanisms in human embryos are still unknown. In animal models of human development, it has been demonstrated that culture of preimplantation embryos in vitro affects gene expression (Khosla et al. 2001; Fernández-Gonzalez et al. 2004; Saadeldin et al. 2011; Schwarzer et al. 2012).

2.17. In a recent study, Kleijkers et al. (2015) sought to determine whether gene expression in human preimplantation embryos is affected by the medium used for embryo culture in vitro during IVF treatment. In the study, women were randomly assigned to two culture medium groups (G5 or human tubal fluid (HTF)), with data on embryonic development collected for all embryos. Ten blastocysts each from the G5 and HTF study groups, matched for fertilisation method, maternal age and blastocyst quality, were selected and their mRNA was isolated and amplified. Embryos were examined individually for genome-wide gene expression by microarray and PathVisio was used to identify the pathways that showed a culture medium-dependent activity. The authors found that expression of 951 genes differed significantly (P = 0.01) between the G5 and HTF groups. Eighteen pathways, involved in apoptosis, metabolism, protein processing and cell-cycle regulation, showed a significant overrepresentation of differentially expressed genes. The DNA replication, G1 to S cell-cycle control and oxidative phosphorylation pathways were up-regulated in the G5 group compared with the HTF group, which was in agreement with the morphological assessment of all 1527 embryos which showed that embryos consisted of more cells on day two and three in the G5 group when compared with the HTF group. Furthermore, the implantation rate was significantly higher in the G5 group compared with the HTF group (26.7% versus 14.7%, P = 0.002) after transfer on the second or the third day after fertilization (Sander H.M. Kleijkers et al. 2015).

Component analysis

2.18. The composition of modern culture media is complex, and while most companies disclose information on some of the components present in their media, they do not disclose concentration or the full composition for commercial reasons (Chronopoulou & Harper 2015). This confounds attempts to identify the totality of secreted proteins (secretome) produced by embryos during culture as any information on proteins present in the media before culture are required to act as the baseline reference. Furthermore, embryo toxic factors have been identified that result in poor embryo quality and impaired developmental growth (Groebe et al. 2010).

In order for a proper assessment of safety to be made it is important to know the complete composition of the embryo culture media, and ideally it would be advisable to test all factors for their positive and negative effects on embryo development and health effects for the offspring.

2.19. In their study Dyrlund et al. (2014) sought to identify and quantify non-declared proteins present in media used for human embryo culture in order to provide a protein reference set, using advanced mass spectroscopy. The authors included eight commercial media in there study: G-PLUS and G-2 PLUS G5 Series (Vitrolife), Sydney IVF Cleavage Medium and Sydney IVF Blastocyst Medium (Cook Medical) and EmbryoAssist, BlastAssist, Sequential Cleav and Sequential Blast (ORIGIO). The authors identified a total of 110 proteins other than HSA, with the average HSA content found to be 94% (92–97%) of total protein. Other individual proteins accounted for up to 4.7% of the total protein. Analysis of purified HSA strongly suggests that these non-declared proteins are introduced to the media when the albumin is added. Geno ontology analysis showed that many of these proteins have roles in defence pathways, such as the innate immune response and inflammatory response. Their results showed that the HSA added to IVF media contained many other proteins and that the amount varies from batch to batch. The authors noted that these variations would be problematic when attempting to identify proteins derived from the embryos, and suggested that it is important that the medium used in the experimental and control groups is from the same batch when studying the embryo secretome. They went on to comment that the proteins present in media could potentially influence embryonic development, gestation age, birthweight and perhaps have subsequent effects on health of the offspring (Dyrlund et al. 2014).

Effect of culture medium on IVF success rates

2.20. In 2013, Mantikou et al conducted a systematic review of 22 randomized controlled trials (RCTs), on the effect of culture media on IVF/ICSI success rates (live birth rates, health of babies born, ongoing pregnancies, clinical pregnancies, miscarriages, multiple pregnancies, implantation rate, cryopreservation rate, embryo quality and fertilisation rate). The review evaluated 31 different media comparisons. However, conventional meta-analysis was not possible for any of the outcomes as nearly all trials compared different culture media. Only four of the studies included live birth rate as an outcome measure. Only one of the studies (Nelissen et al. 2012), observed a significant effect of culture media on live birth rate, with embryos cultured G3 media (Vitrolife) giving significantly more live births than those cultured in Sydney media IVF. With regards to pregnancy rate, the authors observed a difference of more than 5% (RD ≥ 0.05) between the culture media in the majority of

the studies. The authors commented that this indicated the clinical relevance of culture media for IVF/ICSI success rates. However, any conclusions should be tempered by the overall low quality of the studies included. Most studies had methodological limitations, such as weak randomization protocol, randomization of eggs and embryos rather than women, small sample size and absence of a power calculation. In addition, none of the included studies explicitly reported on other factors during embryo culture that could influence IVF/ICSI success rates, such as the number of embryos per drop and culture dish and embryo produced factors (Hoelker et al. 2010). The authors concluded that the existing data, especially on ongoing pregnancies and live births, are insufficient to allow the selection of the best culture medium for IVF/ICSI and more rigorously designed RCTs are necessary for both currently used culture media as well as newly introduced culture media (Mantikou et al. 2013).

Animal studies

2.21. So far the studies presented in this paper have focused on the effect of culture conditions in human IVF. However, research using animal models has been pivotal in the development of human IVF, and as such below are brief summaries of recent research investigating the effect of culture conditions in animal systems.

2.22. In a recent study investigating the roles of vascular endothelial growth factor (VEGF) during early embryo development and implantation in mouse, Binder et al. (2014) found that VEGF plays key roles during mouse preimplantation embryo development, with beneficial effects on time to cavitation, blastocyst cell number and outgrowth, as well as implantation rate and fetal limb development. The authors concluded that there is potential for improvement of clinical IVF outcomes by the addition of VEGF to human embryo culture media, but that this needs further investigation (Binder et al. 2014).

2.23. Myo-inositol (myoIns) has a positive role in mammalian development and human reproduction. In their recent paper, Colazingari et al. (2014) evaluated the hypothesis that the inclusion of myoIns in human embryo culture media would produce an increase in embryo quality in IVF cycles, using the mouse embryo assay. The authors found that the presence of myoIns resulted in both an increase in proliferation activity and developmental rate of in vitro cultured early mouse embryos, and concluded that this represented a substantial improvement of culture conditions and may identify myoIns as an important supplement for human embryo preimplantation culture (Colazingari et al. 2014).

2.24. In a recent study, McPherson et al. (2014) sought to determine whether supplementation of embryo culture media with a substrate to stimulate

mitochondrial activity improves embryo viability and pregnancy establishment in aged mice. Their data demonstrated that the addition of dichloroacetic acid (DCA) to embryo culture media improves mitochondrial output in embryos produced from aged mice. The authors commented that although DCA itself may be of limited therapeutic value in a clinical setting due to its low threshold of dosage and high toxicity, their study does suggest that the addition of a physiological-based mitochondrial stimulator to embryo culture media for aged women may potentially improve IVF outcomes (McPherson et al. 2014).

2.25. In a recent study, Schulte et al. (2015) investigated whether apoptosis is responsible for cell loss in mouse preimplantation embryos after exposure to different human culture media. The authors found that culture medium–dependent decline in total cell count and the developmental restriction in embryos cultured in innovative sequential medium1/ Blast Assist and human tubal fluid/MultiBlast were related to processes affecting cell proliferation rather than apoptosis (Schulte et al. 2015).

2.26. In their study, Li et al. (2014) investigated the developmental competence, reaction oxygen species (ROS) level, and apoptosis index when glutathione (GSH) or cysteine was supplemented into the in vitro culture medium for ICSI-derived porcine embryos. Their results indicated that GSH or cysteine can improve the developmental competence of porcine ICSI-derived embryos by reducing intracellular ROS level and the apoptosis index (Li et al. 2014).

2.27. During IVF, co-incubation of eggs and sperm generates high free radical levels surrounding growing zygotes which may impair subsequent embryo viability. Melatonin eliminates a wide variety of free radicals and Cheuquemán et al. (2014) sought to improve bovine in vitro embryo production by adding melatonin to in vitro fertilisation (IVF) media. The authors found that IVF media with 1mmol melatonin is deleterious for embryo development, and in lower concentrations, it modulated sperm functionality, but had no effects on embryo production (Cheuquemán et al. 2014).

2.28. In their study, Castillo-Martín et al. (2014) sought to determine the effect of l-ascorbic acid on embryo quality and gene expression of porcine blastocysts after supplementations of in vitro culture medium and/or vitrification-warming media. Their data demonstrated that supplementing culture and/or vitrification media with l-ascorbic acid enhances survival rates of porcine blastocysts (Castillo-Martín et al. 2014).

2.29. In their study, Sakurai et al. (2015) evaluated the effect of knockout serum replacement (KSR), a substitute for serum or albumin, on the viability and development of porcine blastocysts. Their data

demonstrated that the addition of KSR to porcine blastocyst medium enhanced the in vitro viability of porcine blastocysts (Sakurai et al. 2015).

2.30. Conjugated linoleic acid (CLA) isomers can affect the lipid profile and signalling of cells and thereby alter their function. In their paper, Absalón-Medina et al. (2014) conducted a series of experiments to test the effects of CLA cis-9,trans-11 and CLA trans-10,cis-12 in vitro. The authors found that supplementation with either CLA isomer did not improve embryo production, but inclusion of CLA cis-9,trans-11 before vitrification improved the quality of bovine IVF embryos after rewarming and culture (Absalón-Medina et al. 2014).

2.31. Lipid accumulated in embryos produced in vitro has been linked to reductions in both quality and post-cryopreservation viability. In their study, (Ghanem et al. (2014) investigated the influence of lipid-reducing chemicals on embryo development, quality, and postcryopreservation viability in cow. The authors concluded that the addition of two lipid metabolism regulators (phenazine ethosulfate and L-carnitine) improved embryo quality and cryotolerance, but embryo development rate and downstream lipid metabolism-regulating genes were more influenced with L-carnitine supplementation (Ghanem et al. 2014).

2.32. Plasminogen activators/Plasmin system plays pivotal role in regulating reproductive functions of mammals. In a recent study, Krania et al. (2014) examined the effects of modification of culture medium with the addition of tissue-type plasminogen activator (t-PA), on bovine embryo development and quality. Their data suggests that excessive t-PA content in the IVF media, suppresses blastocyst formation rate, possibly due to induction of apoptotic phenomena (Krania et al. 2014).

3. Conclusion

3.1. Although numerous studies relating to the impact of culture media have been published over recent years, the research remains inconclusive. A large proportion of the available data is limited to retrospective studies, with all their inherent limitations.

3.2. More rigorously designed, large-scaled, RCTs are needed to make firm conclusions on the impact of culture media and these should investigate the effects of culture media on a range of endpoints including long-term health outcomes. To aid in the assessment of the effect of commercially available media, companies should provide explicit information concerning the precise concentration of each media product. This is in agreement with the conclusions of a recent Association of Clinical Embryologist (ACE) consensus meeting report (Bolton et al. 2014).

3.3. It should also be noted that a range of chemical and physical factors may also impact on embryo development and IVF outcomes (for a recent review see Wale & Gardner 2015) and further studies exploring the impact of the wider culture environment should also continue. Only by conducting such research will we ensure that culture media is both safe for use and contains components optimal for embryo, fetal and long-term development.

4. Recommendations

4.1. Members are asked to:

- consider the progress of research (since February 2014) into the effects of the components in culture media used for IVF treatment;
- advise the Executive if they are aware of any other recent developments; and
- reflect on their views to date and identify what needs to be communicated to the MHRA.

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