IVF culture media: past, present and future

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BACKGROUND: The advances in the world of IVF during the last decades have been rapid and impressive and culture media play a major role in this success. Until the 1980s fertility centers made their media in house. Nowadays, there are numerous commercially available culture media that contain various components including nutrients, vitamins and growth factors. This review goes through the past, present and future of IVF culture media and explores their composition and quality assessment.

METHODS: A computerized search was performed in PubMed regarding IVF culture media including results from 1929 until March 2014. Information was gathered from the websites of companies who market culture media, advertising material, instructions for use and certificates of analysis. The regulation regarding IVF media mainly in the European Union (EU) but also in non-European countries was explored.

RESULTS: The keyword ‘IVF culture media’ gave 923 results in PubMed and ‘embryo culture media’ 12 068 results dating from 1912 until March 2014, depicting the increased scientific activity in this field. The commercialization of IVF culture media has increased the standards bringing a great variety of options into clinical practice. However, it has led to reduced transparency and comparisons of brand names that do not facilitate the scientific dialogue. Furthermore, there is some evidence suggesting that suboptimal culture conditions could cause long-term reprogramming in...
the embryo as the periconception period is particularly susceptible to epigenetic alterations. IVF media are now classified as class III medical devices and only CE (Conformité Européenne)-marked media should be used in the EU.

**CONCLUSION:** The CE marking of IVF culture media is a significant development in the field. However, the quality and efficiency of culture media should be monitored closely. Well-designed randomized controlled trials, large epidemiological studies and full transparency should be the next steps. Reliable, standardized models assessing multiple end-points and post-implantation development should replace the mouse embryo assay. Structured long-term follow-up of children conceived by assisted reproduction technologies and traceability are of paramount importance.

**Key words:** culture media / CE marking / embryo culture / IVF / mouse embryo assay

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**Introduction**

Since the birth of Louise Brown, the first child born as a result of IVF (Steptoe and Edwards, 1978), the advances in the world of assisted reproduction have been rapid and impressive and the future holds even more. The number of children conceived by assisted reproduction technologies (ART) has reached 5 million as calculated by the International Committee for monitoring Assisted Reproduction Technologies (ICMART) and presented in European Society of Human Reproduction and Embryology (ESHRE) (Adamson et al., 2012). New techniques are introduced at such a rate that they are often incorporated into clinical practice without solid proof of their benefit and safety (Harper et al., 2012). IVF success rates are high and the results have tempted some in the scientific community to wonder if its performance can actually be better than nature (Vajta et al., 2010). The great improvement in culture conditions and especially IVF culture media is an important part of this success. The commercialization of culture media has created competition, has increased the standards and has brought into clinical practice a variety of options. In contrast to media produced in house, there are now strict manufacturing and quality assessment requirements, improved batch-to-batch consistency and less contamination (Karamalegos and Bolton, 1999; Quinn, 2004).

There is accumulating evidence showing that culture conditions are important for the IVF outcome and have an impact on pre and post-implantation development and possibly the future health of the offspring (Dumoulin et al., 2010; Nelissen et al., 2012, 2013; El Hajj and Haaf, 2013; Mantikou et al., 2013). However, the evidence for a role of the composition of IVF culture media in these outcomes is often insufficient and controversial. The current media are mostly designed based on data from animal studies which are not always transferable in human embryology (Harper et al., 2012). Also, the strong financial aspect of this field has led to a lack of transparency regarding composition, which does not facilitate the scientific effort to refine IVF culture media and reach a consensus (Biggers, 2000). IVF media are now classified as class III medical devices in the European Union (EU) under the remit of the Medicines and Healthcare products Regulatory Agency (MHRA) and all media used should now be CE (Conformité Européenne) marked.

**Challenges in the optimization of ART culture media**

The ideal steps that should be followed before the introduction of any new technique or culture media in the IVF laboratory have been described by Harper et al. (2012). In comparison to culture media for mouse embryos, media for human embryos are not truly optimized and probably will never be. In order to optimize basic culture parameters for mice, Brinster used several thousand mouse embryos (Brinster, 1963, 1965a, b, c, d). To achieve similar experiments in humans, the number of human embryos would be very high and numerous ethical issues would be raised. Quality assessment is challenging since numerous parameters influence the outcome (manipulation of gametes and embryos, ovarian stimulation protocols, subfertility factors, genetic background of parents) and there is no ‘gold standard’ quality assessment method since the mouse embryo assay (MEA) has its own limitations and is not standardized (Quinn and Horstman, 1998).

**Methods**

This review goes through the past, present and future of IVF culture media and explores their composition and quality assessment. The history of IVF culture media is summarized with references to embryo metabolism. Controversies are highlighted and the logic behind current media formulations is discussed. The major culture media companies and their products are presented along with the information they provide to IVF units and the public through their websites and publications.

A computerized search was performed in PubMed regarding IVF culture media including results from 1929 until March 2014. The keywords and combinations used included: ‘IVF culture media’, ‘embryo culture media’, ‘in vitro fertilization’, ‘IVF conceived children’, ‘mouse embryo assay’, ‘metabolism’ and ‘embryo’, ‘growth factors’ and ‘embryo culture’, ‘epigenetics’ and ‘embryo culture’, ‘IVF culture media’ and ‘quality assessment’. Information was gathered from the culture media companies’ websites, advertising material, instructions for use (IFU) and certificates of analysis. The European Medicines Agency, European Commission and MHRA websites were explored to investigate current legislation regarding the use of IVF media in the EU and the regulation in non-European countries was also investigated. Results relevant to IVF culture media for human embryos were included but not for media for gamete handling, cryopreservation, PGD or culture oils and supplements. The keyword ‘IVF culture media’ gave 923 results in PubMed and ‘embryo culture media’ 12,068 results dating from 1912 until March 2014, depicting the increased scientific activity in this field.

**The past: development of IVF culture media**

A remarkable scientific journey led to the development of the current complex IVF culture media. From the first tissue culture medium based on blood serum that was developed in University College London (UCL), England, UK and was able to support in vitro a beating frog heart (Ringer, 1882) we have reached an era where culture media contain up to 80 components including nutrients, vitamins and growth
factors (GFs) (Supplementary data, Table S1). The development of culture media for human embryos is the result of many years of laborious animal research. Some of the early culture media and their composition are summarized in Supplementary data, Table SII.

Landmarks of mammalian embryo culture

After Lewis and Gregory studied the development of rabbit embryos in vitro in plasma (1929), Kühl (1941) was the first to culture mouse embryos on a blood clot and Chang (1947) successfully cultured and stored rabbit embryos conceived in vivo at low temperature and then transferred them to recipients achieving live births. Chang (1959) successfully fertilized and cultured rabbit eggs in vitro using autologous serum and 42% of the embryos resulted in healthy offspring. Hammond (1949) managed to recover and incubate 4-cell and 8-cell mouse embryos to blastocyst stage in a solution produced daily based on Krebs-Ringers bicarbonate that contained egg white (Krebs and Henseleit, 1932). The efforts continued and the 8-cell mouse embryos now developed into blastocysts in a medium with just nine ingredients including glucose, water and egg white (Whitten, 1956). The birth of healthy offspring in mice after embryo culture was achieved using a formula containing Krebs-Ringers bicarbonate, glucose and bovine serum albumin (BSA) (McLaren and Biggers, 1958). The in vitro culture of mouse embryos from the 1-cell stage was achieved in a chemically defined medium (Biggers et al., 1962). At the time the embryos were cultured in organ cultures with Fallopian tube cells. No large-scale experiments had been performed to study the effect of culture conditions on the embryos. Brinster directly studied the effect of individual components and observed their interactions. Antibiotics and BSA were included in the basic formulation of salts, along with lactic acid neutralized with sodium hydroxide and ionized water. Thousands of mouse embryos were needed in order to complete a series of experiments that led to the optimization of basic parameters such as pH, osmolality, energy substrates, amino acids (AAs) and albumin (Brinster, 1963, 1965a, b, c). Brinster’s experiments also demonstrated the changing needs of the embryo according to its stage of development; pyruvate for the 2-cell embryo, glucose and malate for the 8-cell stage (Brinster, 1965b, d). An optimized culture medium (BMOC-2) was developed for 2-cell mouse embryos and set the basis for a general culture solution for mammalian embryos (Brinster, 1965c, d, 1968). Whitten and Biggers (1968) achieved the first cleavage divisions (1-cell to 2-cell stage) completely in vitro but co-culture with Fallopian tube cells was necessary for these 2-cell embryos to further develop to blastocysts. Brinster and Biggers (1965) fertilized and cultured mouse eggs within the ampulla of Fallopian tubes that were retrieved from superfertilized females. The in vitro culture of mouse embryos from 1-cell stage to blastocyst without co-cultures was achieved by Whitten and Biggers (1968) and the first IVF and embryo culture for mice was reported in a modified Krebs-Ringers bicarbonate solution containing sodium lactate, sodium pyruvate, glucose, bovine serum, streptomycin and penicillin (Whittingham, 1968). BMOC-2 was the basis for other media such as M16 (Whittingham, 1971) but the 2-cell block remained a challenge as the in vitro developing embryos arrested at that stage (Goddard and Pratt, 1983). Some strains were excluded from this block (Whitten and Biggers, 1967). Different groups adopted different approaches to this phenomenon. Abramczuk et al. (1977) highlighted the beneficial role of EDTA in the culture of the blocking strains. Cross and Brinster (1973) studied the effect of the lactate to pyruvate ratio for the 1-cell mouse embryos. The 2-cell block was later attributed to the presence of glucose and phosphate (Schini and Bivester, 1988; Chatot et al., 1989).

A success story for human IVF

The IVF and culture of human eggs was proposed by Rock and Menkin (1944). The eggs were collected from ovarian tissue obtained after laparotomy close to the time of ovulation and were cultured in human serum. Edwards and colleagues achieved major breakthroughs in the world of IVF. Human eggs were retrieved with laparoscopy and were in vitro matured and fertilized in a medium that contained Tyrode’s solution (Tyrode, 1910), BSA and penicillin with the addition of sodium pyruvate, phenol red and an increased concentration of bicarbonate (Edwards et al., 1969; Steptoe and Edwards, 1970). The team also managed to cultivate human embryos up to the blastocyst stage using Ham’s F10 with human or fetal calf serum (Steptoe et al., 1971). The embryos from the first IVF cycles were cultured in Earle’s simple salt solution with pyruvate supplemented with the patient’s serum, and the first IVF baby was born (Steptoe and Edwards, 1978). Edwards et al. also used Ham’s F10 with homologous human serum or BSA (Edwards et al., 1980; Edwards, 1981). Ham’s F10 supplemented with 20% fetal calf serum was also used for embryo culture by the Australian group that reported the first IVF pregnancy which resulted in early miscarriage (De Kretser et al., 1973). The first IVF baby to be born in Australia was Candice Reed (Lopata et al., 1980). At the same time, Mohr and Trounson (1980) focused on culture media and used the mouse model for the quality assessment of culture conditions. Ménézo et al. (1984) questioned the necessity of whole serum since its omission in the B3 medium, that contained albumin and an array of AAs, did not have an effect on embryo development or pregnancy rates (PR). The B3 medium was a modification of the B2 medium which was designed for bovine embryos based on the composition of their reproductive fluids and serum (Ménézo, 1976). At the time there was full transparency regarding composition and the media were made in house. The Ménézo and Quinn groups set the foundation for the commercially available culture media specialized for human IVF (Ménézo et al., 1984; Quinn et al., 1985). The B2 medium was widely used for the first IVF cycles in France and was known as the ‘French medium’ (Cohen et al., 2005).

Embryo-somatic cell co-culture was an important chapter in the history of embryo culture as initially, co-cultures were essential to achieve development in vitro. Mouse embryos co-cultured with Fallopian tube cells were able to develop in vitro even from the zygote stage (Biggers et al., 1962). Also, when arrested embryos were transferred to the ampulla of Fallopian tubes they resumed their developmental capacity (Whittingham and Biggers, 1967). The co-culture of human embryos with fetal bovine uterine fibroblasts or human tubal cells appeared to be superior to conventional embryo culture, improving embryo morphology, implantation and PR (Bongso et al., 1989; Wiemer et al., 1989a, b). Since then, various studies have addressed the issue using various feeder cell lines (human tubal, endometrial and ovarian cancer cell lines, human autologous endometrial cells, bovine tubal and endometrial cells, monkey renal cells) in different cases, such as prior to embryo freezing (Jayot et al., 1995; Tucker et al., 1995) and for patients with recurrent IVF failures or poor quality embryos (Wiemer et al., 1996; Desai et al., 2008). The results were favorable for co-cultures after randomization in some studies (Wiemer et al., 1989a, 1993; Morgan et al., 1995;
Ben-Chetrit et al., 1996; Parikh et al., 2006). However, others failed to prove their benefit (Sakkas et al., 1994; Tucker et al., 1995; Hu et al., 1998). The beneficial effects of co-culture were not species-specific or tissue-specific and included GF production, pH and gas regulation and clearance of deleterious products (Bongso et al., 1991; Bavister, 1992; Barmat et al., 1997). A hormonal effect was excluded after using prepubertal cells in animal studies and non-genital tract-derived cells in humans (Ménézo et al., 1989, 1990). A recent meta-analysis found co-cultures beneficial for implantation rate (IR), clinical pregnancy rate (CPR) and ongoing pregnancy rate (OPR) (Kattal et al., 2008). Despite their initial success, co-cultures with animal-derived feeder cell lines are considered too risky and technically challenging.

The research activity continued, including attempts to omit protein from the media formulation (Caro and Trounson, 1986), to assess the need for serum (Feichtinger et al., 1986; Parmaud et al., 1987) and to select a superior medium in the effort to improve success rates (Muggleston Harris et al., 1990; Staessen et al., 1994) while the blastocyst stage transfer became a feasible option (Gardner and Lane, 1997). Human amniotic fluid was also tested as a culture medium (Gianaroli et al., 1986). Finally, the search for the ideal formula followed two paths; the ‘back to nature’ and the simplex optimization strategy (Quinn et al., 1985; Lawitts and Biggers, 1991; Leese, 1998).

The ‘back to nature’ strategy studied reproductive fluids and the composition of the media was developed accordingly. The analysis of the Fallopian tube secretions (Borland et al., 1980) and of uterine fluid led to synthetic ovudict fluid for sheep (Tervo et al., 1972), mouse tubal fluid (Gardner and Leese, 1990), human tubal fluid (HTF) (Quinn et al., 1985) and synthetic tubal fluid for humans (Mortimer, 1986). However, the analysis of biological fluids is incomplete and laborious, gives information of unknown importance and could be influenced by various parameters (Leese, 2002; Summers and Biggers, 2003). The HTF was found to be only slightly similar to actual tubal fluid (Summers and Biggers, 2003). On the contrary, the simplex optimization strategy used sophisticated software to assess multiple components and their interactions simultaneously (Spendley et al., 1962; Lawitts and Biggers, 1991). This was applied for mouse embryos and gave the optimized medium SOM (simplex optimization medium), later supplemented with potassium (KSOM) (Lawitts and Biggers 1991, 1993). This approach identified the composition and concentration that gave maximum response but not necessarily the best results (Summers and Biggers, 2003). Human culture media could only be based on these results, as similar experiments with human embryos would not be possible.

In the first few decades of human IVF, the majority of IVF cycles transferred embryos at the cleavage stage as the media used at the time was poor at supporting blastocyst development. It was recognized that the environment around the embryo changes naturally during its journey through the reproductive system, which led to the development of sequential media. Sequential media contain different ingredients during different days of culture (Chatot et al., 1989; Gardner, 1994; Gardner and Lane, 1997; Hentemann and Bertheussen, 2009). Gardner et al. (1996) collected uterine and tubal fluid at different stages of the menstrual cycle and demonstrated the cyclical changing concentrations of lactate and glucose. These observations contributed to the composition of G1 and G2 media (Barnes et al., 1995; Gardner et al., 1996). Also, the metabolic needs of the embryo change at different stages as demonstrated for glucose and pyruvate which can affect development (Conaghan et al., 1993; Gardner, 1998). Other teams argue, however, that the change in culture conditions creates extra stress for the embryo and in vitro conditions should not be regulated according to the in vivo paradigm. Interestingly, the stress to the embryo or gametes in the right form and timing may not always be harmful (Isom et al., 2009). The debate is ongoing without consistent results (Macklon et al., 2002; Reed et al., 2009; Sepúlveda et al., 2009; Paternot et al., 2010; Vajta et al., 2010; Wirleitner et al., 2010; Khoury et al., 2012; Quinn, 2012). Sequential media have captured the imagination and are widely used but there is no solid evidence for their superiority (Basile et al., 2013; Summers et al., 2013). Also, as reports emerge regarding the benefit of time-lapse imaging as a method for embryo selection (Meseguer et al., 2011, 2012b), the popularity of the more convenient single step media is increasing.

Glucose: essential or deleterious?

Schini and Bavister (1988) attributed the 2-cell block to glucose and phosphate. Chatot et al. (1989) was the first to overcome the block removing glucose from the medium during the early stages while keeping phosphate and adding glutamine (CZB medium). Quinn (1995) modified the HTF medium to produce a glucose- and phosphate-free medium that contained EDTA and glutamine and yielded improved results in mouse and human embryos. Pool (2004) developed P1, another modification of HTF, without glucose and phosphate containing citrate and taurine instead of EDTA and glutamine. The KSOM medium (Lawitts and Biggers, 1993) had low levels of glucose and phosphate and both the KSOM and the CZB included EDTA. A proposed explanation for the beneficial role of EDTA is that it blocks one of the enzymes of the glycolytic pathway and therefore prevents the inappropriate use of glucose (Abramczuk et al., 1977; Lane and Gardner, 2001). However, the toxicity of glucose for the early embryo is controversial (Lawitts and Biggers., 1992). Barak et al. (1998) concluded that glucose is not deleterious for the early human embryo. On the contrary, the presence of glucose or fructose is essential (Ludwig et al., 2001) and AAs were considered key to overcome the 2-cell block possibly by regulating the reactive oxygen species (ROS) (Gardner and Lane, 1996). A large RCT showed no difference in PR after the culture of pronucleate human embryos in media lacking glucose or containing glucose at a concentration of 5.5 mmol/l (Coates et al., 1999). However, in this study and that by Conaghan et al. (1993), the embryo quality was impaired. Also, high levels of glucose were associated with increased apoptosis and favored the female sex in bovine (Jiménez et al., 2003). Nevertheless, a medium containing 4.7 mmol/l glucose supported human embryo development to blastocyst as efficiently as sequential media or media renewal in a randomized trial (Macklon et al., 2002). A retrospective study by Michaeli et al. (2011) compared three culture media with different concentrations of glucose (P1 that is glucose free, ISM1 which contains 1 mmol/l of glucose and Universal IVF medium containing 5.55 mmol/l glucose). The results demonstrated that high glucose concentrations did not affect the embryo quality but resulted in a higher polyploid fertilization and affected cleavage rate while ISM1 had a beneficial effect on embryo development. Therefore, it has been suggested that glucose is not inhibitory for the early embryo at concentrations that mimic the in vivo conditions, avoiding extremes (Bavister, 1999; Ménézo et al., 2013). Glucose-free media are still advertised for the early stages of embryo culture even though the inhibitory role of glucose is no longer a dogma and the content of phosphate during Days 1–3 varies from zero to high concentration (Quinn, 2004).
Which AAs to include and when?

Simple media lacking AAs were able to support the development of mouse embryos to blastocyst stage (Whitten, 1956; Whitten and Biggers, 1968). However, the importance of AAs became evident as research activity was increased around the embryo and its environment (Chatot et al., 1989; Lane and Gardner, 1997a). AAs, pyruvate and lactate were recognized as preferred energy resources for the early embryo over glucose (Brinster, 1965d; Biggers et al., 1967). Lawitts and Biggers (1992) noticed that glutamine alleviated the inhibitory effect of NaCl when its concentration was high in the medium. Thus, they concluded that AAs act as organic osmolytes counteracting the deleterious effect of high levels of inorganic ions. This was confirmed for glycine and betaine (van Winkle et al., 1990; Biggers et al., 1993). Except from their obvious role as protein precursors, AAs function as pH buffers and metabolism regulators (Gardner and Lane, 1997). Besides, mammalian embryos have AA transporters (Van Winkle, 1988) and AAs are detected in uterine and tubal fluids of animals and humans (Cassilén, 1987; Gardner and Leese, 1990). Devreker et al. (2001) randomized human embryos in three media with and without AAs and concluded that AA supplementation increases blastocyst cell number, however the use of the Cook Sydney IVF media in this study was criticized (Mortimer, 2001). Due to the complex nature of embryo metabolism and the interaction between components, it is still unclear which specific AAs are necessary or harmful to the embryo (Summers and Biggers, 2003).

Experiments in mouse embryos demonstrated that non-essential AAs are important during early embryo development while essential and non-essential AAs should be included in the medium after the 8- to 16-cell stage, and the G1/2 media were formulated accordingly. G1 contained only non-essential AAs but all AAs were included in G2 (Barnes et al., 1995; Lane and Gardner, 1997b). These media were then modified and finally, after other versions, the GIII series of media was developed. This sequential use of AAs was considered beneficial for embryo development and PR (Gardner and Lane, 1998; Jones et al., 1998). However, this theory is highly controversial (Summers and Biggers, 2003; Ménézo et al., 2013). The inclusion of all AAs at a lower concentration throughout embryo culture was shown to be beneficial for embryo development in monocultures (Ho et al., 1995). There is also evidence suggesting that lack of sulfur AAs, such as methionine, is associated with monozygotic twinning in humans (Cassuto et al., 2003). Methionine is related to glutathione, hypotaurine and taurine synthesis and imprinting (Ménézo et al., 2013).

The use of AAs, and especially glutamine, in the media is associated with ammonium accumulation (Lane and Gardner, 1994, 2007; Zander et al., 2006). A single human embryo in culture for 4 days can produce 100 μM of ammonium and the AA breakdown adds further to this which can affect the developmental capacity and gene expression (Gardner et al., 2001, 2013; Virant Klun et al., 2006). The initial approaches were either to reduce the concentration of AAs or to renew the medium (Gardner and Lane, 1993; Devreker and Hardy, 1997; Lane et al., 2001; Gardner et al., 2013). However, others argue that ammonium accumulation is not that significant and is pH and temperature dependent and studies should avoid using stable forms of exogenous ammonium which could bias the results (Summers and Biggers, 2003; Ménézo et al., 2013). The use of the stable l-alanyl-l-glutamine or glycyl-l-glutamine instead of glutamine was adopted in commercial media although this does not eliminate the contribution from other AAs. There are few relevant studies showing that this strategy may be beneficial (Biggers et al., 2004; Summers et al., 2005). Kleijkers et al. (2013) found ammonium accumulation in Vitrolife G1 Plus but this did not have an effect on embryo development, PRs or birthweight.

According to the quiet embryo hypothesis, embryos with low metabolic activity, especially AA turnover, have greater viability (Leese, 2002; Brison et al., 2004). However, this theory has been questioned especially when 5% oxygen is used (Gardner and Wale, 2013).

Growth factors

GFs evaluated as supplements to human IVF culture media include epidermal growth factor (EGF) (Khamisi et al., 1996), insulin-like growth factor I (IGFI) (Lighten et al., 1998; Spanos et al., 2000), brain-derived neurotrophic factor (Anderson et al., 2010), the combination of the three (Yu et al., 2012), leukemia inhibitory factor (LIF) (Dunglison et al., 1996), platelet-derived growth factor (Lopata and Oliva, 1993), heparin-binding epidermal growth factor (HB-EGF) (Martin et al., 1998), platelet activation factor (PAF) which was found to increase PR (O’Neill et al., 1989) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Ziebe et al., 2013). The results are optimistic showing accelerated development, better quality, increased blastocyst rate, increased cell number, earlier hatching, increased hCG and IR (Richter, 2008). GFs play a key role in the beneficial effect of co-cultures, which are shown to increase the embryonic expression of GF ligands (Liu et al., 1999), and of embryo culture in groups through their cross-talk (O’Neill, 2008). GFs are present in vivo, act on embryo receptors, they are anti-apoptotic and increase development rates. However, the same principles that render GFs beneficial are reasons for caution. Apoptosis is a normal phenomenon during embryo development (Hardy et al., 1989), which not only serves as an essential parameter of embryogenesis but also as an extreme repair mechanism to deal with abnormal cells (Alson and Sarraf, 1992; Hardy and Spanos, 2002). Even though development rate is used as an end-point to evaluate culture conditions, faster is not necessarily better. The faster developing mouse embryos presented loss of genomic imprinting raising safety issues for media that promote fast growth (Market Velker et al., 2012). Also GFs, if not well regulated, can have adverse effects on development and have been associated with large offspring syndrome (LOS) (Young et al., 2001). Furthermore, embryos produce GFs creating a closed loop of autocrine signaling (O’Neill, 2008) and the addition of a single GF could disturb the balance (Ménézo et al., 2013). Finally, after embryo culture with LIF, IGF-1 and HB-EGF, distinct deviations were noticed in the expression of various cell fate genes (Kimber et al., 2008).

GM-CSF is expressed in the female genital tract (Dudley et al., 1990; Zhao and Chegini, 1994; Giacomini et al., 1995; Zhao et al., 1995) and placenta (Berkowitz et al., 1990). It has receptors on human embryos and acts by suppressing apoptosis (Sjöblom et al., 2002). GM-CSF was reduced in patients with recurrent miscarriages (Perricone et al., 2003). It inhibits the expression of genes involved in apoptotic and stress response pathways in mouse embryos (Chin et al., 2009). Its significance has been documented for mouse embryo development, implantation and placentation (Wegmann et al., 1989; Robertson and Seamark, 1992; Sjöblom et al., 2005; Robertson, 2007). GM-CSF-knockout mice had increased miscarriages, intrauterine growth...
restriction, impaired placentation and fetal malformations and the offspring presented impaired growth and higher post-natal mortality (Robertson et al., 1999). The results were more evident for males and for GM-CSF-deficient embryos. Media supplemented with GM-CSF appeared to be safe for human embryos in a multicenter double-blinded RCT as no significant difference in embryo chromosomal constitution was found after fluorescence in situ hybridization (FISH) (Agerholm et al., 2010). The 86 oocytes in this trial were fertilized by the same chromosomally normal sperm donor. The results agree with a mouse study that showed no increase in aneuploidy or mosaicism for embryos cultured in GM-CSF (Elaimi et al., 2012). Embryo culture with GM-CSF appeared to be beneficial since it enhanced the development rate of frozen-thawed embryos in two different culture systems (Sjöblom et al., 1999) and yielded improved PRs in a study by Kim et al. (2001). The RCT by Shapiro et al. (2003) showed increased blastocyst cell number and more expanded blastocysts after GM-CSF supplementation but no difference in PR. In a multicenter double-blinded RCT supported by ORIGIO, 14 fertility units and more than 1300 women participated (Zbie et al., 2013). Supplementation of culture media with GM-CSF showed a significant increase in live birth rate (LBR) in a subgroup of patients with at least one previous miscarriage but only in low human serum albumin media. Also, GM-CSF supplementation appeared beneficial for LBR in a retrospective study for patients with previous miscarriages by Mignini Renzini et al. (2013). A pilot study by Sfountsou et al. (2013) showed a trend toward increased PR with GM-CSF-supplemented media for patients with previous unsuccessful IVF cycles. Well-powered, properly randomized trials assessing LBR and long-term follow-up of the offspring are needed to determine the benefit and safety of GM-CSF-supplemented media for the general IVF population and these particular patient groups (Siristatidis et al., 2013).

Hyaluronic acid

Hyaluronic acid is an adherence compound and its inclusion in culture media has been well studied. The macromolecule is present in the endometrium at concentrations that vary according to the day of the cycle (Salamonsen et al., 2001) and its receptors are detected in the endometrium and the preimplantation embryo (Knutson and Knudson, 1993; Campbell et al., 1995). A Cochrane review concluded that hyaluronic supplementation appeared to be safe and significant benefit was documented for PR but not LBR (Bontekoe et al., 2010). A recent update on this Cochrane review including 16 RCTs concluded that the addition of hyaluronic acid to embryo transfer medium yielded improved LBR. However, only six trials reported on LBR and the obtained evidence was of moderate quality (Bontekoe et al., 2014). Balaban et al. (2011) found significantly increased LBR for the hyaluronan group in a follow-up study for participants of a previous RCT that compared EmbryoGlue to G2 version 3 medium for embryo transfer (both Vitrolife media) (Urman et al., 2008). Large RCTs are needed to draw solid conclusions for the benefit in LBR, which is where the interest lies for both patients and clinicians.

The present: where are we now?

Up until the late 1980s, embryologists were still making their own culture media in house, with limited quality control. The competitive nature of the field saw the first commercial media produced around this time (Medicult from Denmark). The development of this field over the past 30 years has led to a huge commercial market making IVF culture media, freezing media, biopsy media, etc. There are eight major companies whose culture media are used in the majority of studies. The list includes Cook Medical, Cooper Surgical that produces both SAGE and ORIGIO media, FertiPro, Gynemed, Gynotec, Irvine Scientific, IVF online and Vitrolife. Other companies that currently produce IVF culture media are Genea Biomedx (previously Sydney IVF), InVitroCare and Kitazato. The main companies and the culture media they produce along with their quality assessment information, IFU and components disclosed in their websites are included in supplementary data, Tables S1–S5. Supplementary data, Table SII presents the basic early culture media; the simplicity and transparency of their composition is evident. In contrast, supplementary data, Table SIII presents some of the most commonly used modern culture media (1+2 media from each company are included). Their composition is far more complex but the details not revealed.

Different companies highlight different features through their advertising messages, emphasizing both quality and success rates. Commercial media contain salts, energy substrates, serum supplements, AAs, buffer solutions, antibiotics, vitamins, nucleotides, GFs, and other reagents such as trace elements, nuclease inhibitors, etc. (Supplementary data, Table S1). Some of these reagents have unclear functions. The formulations vary and most components are used at different concentrations in the different media, illustrating the lack of consensus. Even for the most basic parameters of embryo culture (use of AAs and glucose, monoculture or sequential culture, temperature, gas composition) the different groups agree to disagree (Vajta et al., 2010). The components are disclosed by most companies but concentrations are not disclosed (Supplementary data, Table SIII). Some of these certificates are available online. For all companies it is documented that the human source materials used are found to be non-reactive to antibodies for Hepatitis C, human immunodeficiency virus and hepatitis B surface antigen. In addition, the donors have been screened for Creutzfeldt-Jakob disease but all human source material should be treated as if it were capable of transmitting infection as the possibility of transmitting infective agents, including unknown or emerging viruses, cannot be totally excluded. In the medical safety data sheet (MSDS) all companies state that they shall not be held liable for any damage resulting from handling or from contact with the product.

Quality assessment

CE marking

By definition a ‘medication’ should be used on or in the human body. In the UK the embryo is not legally considered a human being therefore culture media do not strictly comply with this definition. However, they may have an effect on the endometrium and contain substances classified as medicinal products. In 2008, 30 years after Louise Brown was born, IVF culture media and utensils were classified as class III Medical...
Devices in the EU under the Directive 93/42/EEC as amended, as they are (indirectly) used for human beings by promulgating pregnancy. The CE mark obtained its form in 1993 and indicates that the manufacturer complies with the EU regulations. The Human Fertilisation and Embryology Authority produced a paper regarding CE marking and its significance in 2013. The CE marking process is described in Supplementary data, Table SV and the information the companies provide online regarding quality control (QC) vary (Supplementary data, Table SV). After an adjustment period all IVF culture media in the EU should now be CE marked.

Disclosure of the full composition, quality assessment, post-market surveillance, well-defined reporting and investigating systems for adverse incidents, and traceability are important pylons of CE marking. A constant cross-talking should, therefore, be established between the culture media companies and the IVF units regarding the IVF outcome, the health of the ART conceived offspring and the recording of possible adverse effects or suboptimal performance of the media but is this truly the case in everyday clinical practice? Also, the full composition of the medium is hardly ever disclosed for commercial reasons and the need for traceability of the culture conditions is not always documented. Only Cook and ORIGIO have included this information in their IFU. Finally, it is unclear exactly what information the notifying bodies request as some components are still used ‘based on experience’.

Outside Europe

The Food and Drug Administration (FDA) (as per Code of Federal Regulations Title 21, volume 8, revised in April 2013) classifies IVF culture media as class II Medical Devices (special controls) since they come in direct contact with human gametes or embryos. Their quality assessment should involve the MEA, endotoxin testing, sterilization validation, design specifications, labeling requirements, biocompatibility testing and clinical testing. Special controls also include post-market surveillance.

In Australia, IVF culture media are classified as class III Medical Devices under the Therapeutic Goods Administration. According to the guidance all components and raw materials should be clearly identified and the results of the MEA should be provided in the labeling. Other requirements include sterility and stability testing, pH, osmolality and endotoxin testing. Also, biocompatibility testing for the individual components and the raw materials is required as well as cytotoxicity and embryotoxicity testing. Finally, clinical data on the safety and efficiency of the product are requested according to the Australian Regulatory Guidelines for Medical Devices. It should be highlighted that the guidance mentions that genotoxicity tests should also be considered to determine potential developmental toxicity; this could include the bacterial gene reverse mutation assay, in vitro mammalian cell chromosomal aberration assay and in vivo rodent bone marrow micronucleus assay.

In China, culture media require the approval of the China Food and Drug Administration (CFDA) (Good manufacturing practice for drugs, 2010 revision) as laboratory reagents. In Japan, there is no clear regulation around IVF media and culture media are not classified as pharmaceuticals or medical devices.

Mouse embryo assay

The QC of commercial media includes pH, osmolality, sterility, endotoxin tests and the MEA (Ackerman et al., 1984, 1985; Byers et al., 2006). The medium can usually pass the MEA if it supports development of ≥80% of mouse embryos to blastocyst stage. The MEA was found valid in some studies (van den Bergh et al., 1996; Gardner et al., 2005). However, its use has been heavily criticized (Fleming et al., 1987; Dumoulin et al., 1991; Fleetham et al., 1993) as it is only really testing toxicity and certainly not testing formulations for human IVF. The mouse model should be used with caution as it presents obvious differences in reproductive physiology compared with humans (Ménézo and Hérubel, 2002) and there is no standardization of the day the culture should start, the mouse strain, number of embryos, etc. Efforts to increase its sensitivity include one cell as the starting point (Davidson et al., 1988; Scott et al., 1993), zona-free embryos (Montoro et al., 1990), the inclusion of positive controls (Dubin et al., 1995), accurate sample size calculations (Hendriks et al., 2005), assessment of multiple end-points and of blastocyst cell number (Scott et al., 1993; Gardner et al., 2005). The FDA (1998) considered MEA appropriate without specifying its design, which often ignores the basics, such as a power calculation (Punt-van der Zalm et al., 2009). Different companies use various protocols so the test cannot be considered standardized. None of the companies provides information online regarding the number of mouse embryos used.

Mouse embryos have varying sensitivities to deleterious effects depending on the strain (Dandekar and Glass, 1987; Scott et al., 1993; Dubin et al., 1995). They can even develop to blastocysts when cultured in media with tap water lacking any protein supplement (Silverman et al., 1987; George et al., 1989). Mouse embryos that lost one blastomere at the 2-cell stage and therefore had reduced inner cell mass (ICM) developed to blastocyst and achieved implantation (Papaioannou and Ebert, 1995). Mouse blastocysts developed further despite having a 10% imbalance of the founder cell lineages and responded differently to culture media compared with human embryos in a study by Schwarz et al. (2012). These findings raise questions regarding the reliability of the current MEA as guide for the suitability and safety of culture media for human embryos but currently we have no alternative.

Which is the best?

A Cochrane review has been designed to answer the question of which is the best culture medium (Youssef et al., 2009). The primary outcome should be LBR. A recent systematic review reported that the existing data are insufficient to conclude which is the best culture medium for ART (Mantikou et al., 2013). Twenty-two RCTs were included that compared 20 different IVF commercial culture media produced by 11 companies. Most media were only compared once and the design and end-points of the studies varied significantly so a meta-analysis was not possible. The authors suggested that culture media make a difference not only for success rates but also for embryo quality and development, as also stated in other reviews (Pool, 2004; Lane and Gardner, 2007). Schwarz et al. (2012) compared the effect of 13 culture protocols used in human embryology on mouse embryos fertilized in vivo. A significant difference was noticed not only in embryo development but also during the post-implantation development and litter size according to the culture medium used. As expected, however, safe conclusions for a superior culture medium for human embryo culture cannot be drawn. Also, mouse embryos responded in the opposite fashion to human embryos to the studied culture media.

Recently two culture media were compared regarding their effect on CPR, perinatal outcomes and intrauterine growth by a group in the...
Netherlands. The culture media were both sequential media from Vitrolife and Cook. In the first paper (Dumoulin et al. 2010) embryos from 826 IVF cycles were randomized through alternate allocation to culture with the Cook or the Vitrolife medium that resulted in 188 live births of singletons. Only first IVF treatments and fresh transfers were included. Dumoulin et al. (2010) concluded that the CPR and the mean birthweight for singletons were higher for the Vitrolife group (more than 200 g difference in the average birthweight). The study was not optimally randomized and there were more double embryo transfers and increased maternal weight and height and paternal weight in the Vitrolife group. Also, embryos with less blastomeres but higher morphological grade were transferred for the Cook group. However, the parents had otherwise similar characteristics, the same stimulation protocols and embryo transfer strategy were followed for both groups, confounding factors were considered and birthweight was adjusted for gestational age and sex. Although the difference was not as dramatic as in animal studies, this study was the first to demonstrate a link between the culture medium and the offspring phenotype in humans. This is particularly important since low birthweight (LBW) has been associated with susceptibility to future cardiovascular disease and type 2 diabetes (Barker, 1993, 2004) and high birthweight has been associated with overgrowth abnormalities in animals.

A later study by Nelissen et al. (2012) that included twin pregnancies, fresh and frozen transfers and women with previous failed cycles confirmed the above results for singletons and also demonstrated an effect on the perinatal outcome of twins. For singletons the Cook group had significantly increased incidence of LBW and LBW for gestational age and the average birthweight was lower compared with the Vitrolife group. A similar trend was noticed for twins along with significantly higher inter-twin mean birthweight disparity and birthweight discordance for the Cook group. Also, while five large for gestational age singletons were found in the Vitrolife group, none was reported for the Cook group. The same team reported in a retrospective study that differences attributed to culture media were detected from the second trimester of pregnancy based on the 20 weeks scan (Nelissen et al., 2013). Also, in a follow-up study they found that at 2 years of age there was still a significant difference in weight (up to 500 g) and weight gain amongst the two groups, with no difference in height and head circumference (Kleijkers et al., 2012). Furthermore, Eskild et al. (2013) found in a retrospective study that IVF culture media significantly influenced both the birthweight and the placental weight to birthweight ratio when compared with the trend from spontaneous conceptions. However, Carrasco et al. (2013) in a prospective and retrospective study found no effect of culture media on the birthweight of singletons, and three recent retrospective studies reached the same conclusion (Eaton et al., 2012; Vergouw et al., 2012; Lin et al., 2013). Carrasco et al. (2013) compared Cook, Medicult and Vitrolife media and reported that their main difference is the AA composition. The comparison of four different culture media showed that the hCG rise during early pregnancy was related to the medium used (Orasanu et al., 2006) which was also the case in the Nelissen et al. study (2013). Also, comparison between the one-step Universal IVF medium and the sequential ISM1 (ORIGIO) yielded better results for ISM1 regarding embryo quality, IR and OPR (Xella et al., 2010). An effect of culture media on embryo quality after ICSI from the second day of culture was also demonstrated by Cossiello et al. (2012). The exact composition of the compared media is not disclosed so specific components cannot be linked to the outcome. Various studies have been published exploring effects of culture media from fertilization to post-natal phenotype (Supplementary data, Table SVII).

### IVF and embryo culture: do they come without risks?

Time is the only true counselor regarding the safety of any intervention during the periconception period as it is characterized by increased epigenetic plasticity that creates a window for long-term reprogramming (El Hajj and Haaf, 2013; Steegers-Theunissen et al., 2013). There are numerous examples supporting the susceptibility of this period to epigenetic changes whose consequences may not be obvious at birth or during childhood. In the case of diethylstilbestrol the effects on the female offspring only appeared in adulthood (Li et al., 2003). Also, according to the Barker hypothesis, adult diseases have their origins in the conditions around the early stages of development (Barker, 1993, 2003). Animal studies have also demonstrated a link between the early environment and adult disease (Langley-Evans, 2006). Dietary supplementation in female mice 2 weeks prior to conception and during pregnancy and lactation had a phenotypic effect on the offspring, changing the coat color due to alterations in gene methylation (Waterland and Jirtle, 2003). Mild undernutrition of the mother during the first trimester of pregnancy, not severe enough to cause growth restriction or LBW, caused cardiovascular changes and reduced ovarian reserve for the bovine offspring (Mossa et al., 2013). Similarly, in vitro embryo culture can induce epigenetic alterations for different species highlighting the need for caution in human IVF (Grace and Sinclair, 2009; El Hajj and Haaf, 2013). Clinical practice until now shows that the in vitro culture of human embryos does not confer major adverse effects on the offspring but possible consequences in late childhood or adulthood are still to be explored keeping in mind that even the first children conceived by ART are still young. Studies reporting phenotypic and behavioral abnormalities attributed to ART for different species are summarized in Supplementary data, Tables SVIII and SIX.

### Effects of assisted conception on mammals

Animal studies have demonstrated that in vitro culture and manipulation of the early embryo can alter gene expression and influence imprinted genes affecting the phenotype of the offspring (Reik et al., 1993; Young and Fairburn, 2000; El Hajj and Haaf, 2013). It was recognized that in vitro conceived mice had reduced fetal growth (Bowman and McLaren, 1970). Also, in some studies, in vitro developed blastocysts were found to have decreased cell number in both the ICM and trophoderm and to generate a reduced number of embryonic stem cells (Tielens et al., 2006; Watkins et al., 2007). IVF and embryo culture also influences placentalation (Bertolini et al., 2004; Miles et al., 2004; Delle Piane et al., 2010). It affects post-natal growth, blood pressure (BP), cardiovascular profile and metabolism in mice (Watkins et al., 2007). Alteration of the culture medium creating a suboptimal environment leads to delayed neoromotor development, hyperactivity and impaired growth in the mouse model (Fernández-Gonzalez et al., 2004). FISH studies in bovine and murine embryos demonstrated increased chromosomal abnormalities after in vitro culture compared with in vivo (Hyttel et al., 2000; Viuff et al., 2000; Lonergan et al., 2004; Sabhnan et al., 2010). Furthermore, overgrowth abnormalities, such as LOS, are well documented in different species and attributed to epigenetic alterations due to complex media...
containing serum (Thompson et al., 1995; McEvoy et al., 1998). LOS increases mortality and causes skeletal, placental and multiple organ developmental abnormalities (Farin and Farin, 1995; Thompson et al., 1995; Sinclair et al., 1999, 2000).

Children conceived by IVF

The in vitro culture of human embryos is relatively new and the scientific community is still exploring its effects. Various parameters can influence the IVF outcome and the phenotype of the offspring, including intrinsic factors from the couple (infertility, genetic factors) and ART-related procedures (stimulation protocols, manipulation of the gametes and embryos, embryo culture).

Many follow-up studies give reassuring results for the physical and mental health and neurological outcome of IVF conceived children (Rufat et al., 1994; Olivennes et al., 1997; Bradbury and jick, 2004; Ponjaert Kristoffersen et al., 2005; Middelburg et al., 2009; Wagenaar et al., 2009, 2011; Basatemur et al., 2010; Beydoun et al., 2010; Mains et al., 2010; Schendelaar et al., 2011; Hart and Norman, 2013). However, increased risk of obstetric and perinatal complications, preterm birth, congenital defects and higher mortality has been associated with assisted conception. When compared with spontaneously conceived controls in various studies, ART children have been found to have lower birthweight, higher BP, dyslipidemia, increased cancer risk, increased risk for imprinting disorders, increased need for health care resources and the list goes on (Supplementary data, Table SIX). The vast majority of IVF conceived children are healthy and for rare disorders, such as imprinting disorders, the absolute numbers would be low even with a large increase in the risk. Also, the link between ART and imprinting disorders has been questioned in large epidemiological studies (Lidegaard et al., 2005; Bowdlin et al., 2007; Doornbos et al., 2007).

Some of the most well-designed relevant studies were conducted by Ceelen et al. (2007, 2008a, b, 2009) who followed up 233 IVF conceived children in comparison to their age- and gender-matched naturally conceived controls whose parents had fertility problems. IVF children had significantly lower weight and height in early childhood but later had a catch up period with significantly higher growth velocity associated with future cardiovascular risk (Ceelen et al., 2009). It was also reported that 8–18 year old IVF children tend to have increased total body fat (Ceelen et al., 2007), and higher fasting glucose levels and BP than their spontaneously conceived peers (Ceelen et al., 2008a). Furthermore, adolescent IVF conceived girls had advanced bone age and significantly higher levels of LH and dehydroepiandrosterone sulfate (DHEAS) with no difference in pubertal stage and age at menarche (Ceelen et al., 2008b). Miles et al. (2007) also concluded that 4–10 year old IVF children had a more favorable lipid profile, were taller and had higher levels of IGFI and IGF2. Increased mean birthweight for singletons and increased risk of preterm birth, birth defects and monozygotic twinning has been documented after blastocyst transfer compared with Day 3 transfer (Ménézo et al., 1999; Tarlatzis et al., 2002; Milki et al., 2003; Luna et al., 2007; Källén et al., 2010; Finnström et al., 2011; Zhu et al., 2014).

Epigenetics

Why is the environment during embryo culture important for embryonic development and possibly the phenotype of the offspring?

In IVF, fertilization occurs in a Petri dish and the culture medium supports the embryo from the very beginning until the transfer. The culture medium plays a very important role in IVF by supporting these first delicate steps. The first week after fertilization is characterized by precisely orchestrated events including the first mitotic divisions, zygotic genome activation, compaction, morula formation, cavitation and blastocyst formation (Cockburn and Rossant, 2010). During these stages developmental programming takes place through timed epigenetic modifications (Smith et al., 2012). The epigenome can be considered a type of software that determines how the genome (hardware) behaves (Brower, 2011). Mainly through methylation of the promoter region of genes and through other mechanisms, such as histone methylation, epigenetic changes (either inherited or acquired) can cause silencing of a specific gene permanently ceasing its expression. The genome and the epigenome are equally important for embryo development. Their interaction is responsible for the necessary changes in gene expression that accompany the passage from fertilization and zygote to blastocyst and implantation (Shi and Wu, 2009). Methylation and demethylation profiles change through the development of the mouse embryo as the cells become differentiated and the ICM that will give the fetal structures is separated from the trophoderm that will give rise to the placenta (Rougier et al., 1998; Santos et al., 2002; Santos and Dean, 2004; Morgan et al., 2005; Reik, 2007). This period is particularly sensitive and any epigenetic alterations that may give a survival advantage to the embryo facilitating its adaptation, can have irreversible consequences in gene expression and metabolic profile for the offspring and even for future generations (Thompson et al., 2002; Fleming et al., 2004; Denomme and Mann, 2012; van Montfoort et al., 2012; Feuer et al., 2013). Furthermore, metabolism is linked to epigenetics through histone modification (Rathmell and Newgard, 2009).

The most obvious example of epigenetic alteration after mouse embryo culture was the case of the ‘agouti viable yellow’ allele (Morgan et al., 2008). Furthermore, altered expression was detected after mouse embryo culture in Whitten’s medium and in KSOM with AAs for 114 genes and 24 genes, respectively (Rinaudo and Schultz, 2004). Also, suboptimal culture conditions led to impaired neuromotor development, behavioral abnormalities and anxiety in mice (Fernández-Gonzalez et al., 2004). The transgenerational adverse effects of a suboptimal culture environment, including lower weight to the time of weaning and larger organs during adulthood, were also documented by Mahsoudi et al. (2007). In another study by Calle et al. (2012), the modification of the culture medium led to the alteration of gene expression in the testis of adult mice and induced apoptosis. The adverse effects, such as subfertility, glucose intolerance and overgrowth abnormalities, for the male offspring were also passed on to next generations of male progeny. These are data from animal studies and they cannot be extrapolated to humans. However, there is evidence suggesting that embryo culture can also induce epigenetic alterations in human embryos. Katari et al. (2009) demonstrated the impact that embryo culture can have on gene expression in different tissues by studying the methylation pattern for more than 700 genes from the placenta and the cord blood of ten ART conceived children compared with spontaneously conceived controls. A lower level of promoter methylation was detected in the placenta and higher level in cord blood for some of the studied genes for the IVF conceived children. Also, placental gene expression was altered for 26 genes in three IVF pregnancies in comparison to three controls (Zhang et al., 2010).

Imprinted genes are closely linked with epigenetics. For these genes only the paternal or the maternal derived allele should be expressed.
They are particularly interesting since the methylation pattern that leads to silencing of the paternal or maternal allele should be maintained during development and is susceptible to epigenetic changes (Surani, 1998). The period that this methylation or demethylation occurs is vulnerable to errors due to environmental parameters. In humans maternal imprinting can be completed during or after fertilization (El Maarri et al., 2001) and therefore can be affected by the culture conditions. Imprinted genes are important both for embryo development and for future health (Kelsey, 2007). In animal studies different regulation of the imprinted genes H19, IGF2 and Grb10 was reported depending on whether the culture medium was supplemented with serum or not (Khosla et al., 2001; Shi and Haaf, 2002). Epigenetic alteration of the H19 and IGF2 in ruminants has been associated with LOS (van Montfoort et al., 2012). Also, culture of mouse embryos in Whitten’s medium resulted in inappropriate expression of the paternal allele for the imprinted H19 gene in comparison to culture in KSOM medium with AAs (Doherty et al., 2000). Alterations of the methylation pattern of IGF2 and H19 were also detected after mouse embryo culture in Quinn’s Advantage medium (Li et al., 2005). Naturally conceived mouse embryos were cultured in five commercial media and were compared with those cultured in Whitten medium and those that had developed in vivo. In all media groups there were epigenetic alterations for the three imprinted loci studied in comparison to the in vivo derived group, to different extents for the different media (Market-Velker et al., 2010).

Conclusion

The focus of this review was on IVF culture media. Other factors, such as incubator gases and temperature, culture oils and supplements, are equally important and can change the dynamics of how the embryo interacts with components of the medium. Characteristics of the surfaces used in ART (e.g. elasticity), of the devices used, such as Petri dishes, and other parameters, such as pH and oxygen concentration, can also affect the embryos (Swain, 2010, 2012; Vajta et al., 2010; Hentermann, 2011; Swain and Smith, 2011; Kolahi et al., 2012; Sommer et al., 2012). Interestingly, commercial culture media (depending on their composition) contribute to the creation of ROS even under conditions of low oxygen concentration (Martin-Romero et al., 2008).

The field of embryo culture is a focus of attention and research is being conducted worldwide aiming to optimize the culture conditions and increase the IVF success rates. With the introduction of new techniques, such as microfluidics and dynamic culture systems, that aim to replenish the media (Tascenko et al., 2010; Alegretti et al., 2011) we may move toward an automated, precise IVF “lab-on-a-chip” (Swain et al., 2013). From the very first steps of IVF to the robotic IVF of the future, culture media will always play a major role in the IVF laboratory. The standards of culture media that are currently produced are obviously very high and the CE marking process in the EU is a significant development in the field. However, the future can be even brighter.

We are largely still using culture media whose exact composition is unknown and this not only raises ethical issues but also deprives us of the chance of developing even better culture media after constructive comparisons. The field of IVF culture media is surrounded by numerous controversies some of which were highlighted in this review. Furthermore, there is some evidence suggesting that culture media can induce epigenetic changes affecting development and future disease risk. Well-designed studies are currently lacking but human embryos are too valuable to be used in large-scale optimization experiments. The only well-studied supplements are GM-CSF and hyaluronan but still their benefit in LBR is yet to be shown and the possibility of rescuing abnormal embryos should be considered.

Well-designed RCTs, large epidemiological studies and full transparency should be the next steps without further delays. All those involved in the field should contribute to a worldwide effort. The companies should fully disclose composition with concentrations. Reliable, standardized models assessing multiple endpoints and post-implantation development should replace the MEA. These models could be used to identify possible epigenetic dysregulations after exposure to different media. Fertility clinics should facilitate comparisons by sharing their results. They should be encouraged to only work with companies that disclose media composition and are CE marked. Embryologists should be critical of the media used, evaluate their performance in house, use them as instructed and report adverse effects. The importance of the perconception and preimplantation period for the future health of the progeny is now recognized in animal models and humans. Structured follow-up for IVF conceived individuals is of paramount importance to identify possible adverse outcomes during childhood or adulthood. Central confidential databases documenting the exact culture conditions and media used for every embryo could be created. These outcomes could, therefore, be linked to culture conditions to determine trends aiming to identify epigenetic alterations and demystify the pathways that induce them.

Supplementary data

Supplementary data are available at http://humupd.oxfordjournals.org/.

Authors’ roles

J.C.H.: conception of this paper, editing of the manuscript, final approval of the manuscript. E.C.: wrote the first draft which was edited by J.C.H.

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References


George, M.A., Braude, P.R., Johnson, M.H., Sweetnam, D.G. Quality control in the IVF laboratory: in vitro and in vivo development of mouse embryos is unaffected by the quality of water used in culture media. Hum Reprod 1989; 4:826–831.


George, M.A., Braude, P.R., Johnson, M.H., Sweetnam, D.G. Quality control in the IVF laboratory: in vitro and in vivo development of mouse embryos is unaffected by the quality of water used in culture media. Hum Reprod 1989; 4:826–831.


Zander D, Thompson J, Lane M. Perturbations in mouse embryo development and viability caused by ammonium are more severe after exposure at the cleavage stages. Biol Reprod 2006;74:288 – 294.