Human Reproduction Update, Vol.21, No.1 pp. 39–55, 2015
Advanced Access publication on July 17, 2014 doi:10.1093/humupd/dmu040

human reproduction update

IVF culture media: past, present and future

Elpiniki Chronopoulou[1,*] and Joyce C. Harper[2,3]

[1]Institute for Women's Health, University College London, 86–96 Chenies Mews, London WC1E 6HX, UK [2]UCL Centre for PG and D, Institute for Women's Health, University College London, London, UK [3]The Centre for Reproductive and Genetic Health, UCLH, London, UK

*Correspondence address. E-mail: elpiniki.chronopoulou.12@ucl.ac.uk

Submitted on October 29, 2013; resubmitted on May 5, 2014; accepted on May 12, 2014

TABLE OF CONTENTS

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 Européene)-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

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 Européene) 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 Medichines 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 SI). 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), Kuhl (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 superovulated 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 Bavister, 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 Kretzer *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 ampula 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 pre-pubertal 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; Parinaud *et al.*, 1987) and to select a superior medium in the effort to improve success rates (Muggleton 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 oviduct fluid for sheep (Tervit *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 (Casslén, 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) (Khamsi *et al.*, 1996), insulin-like growth factor I (IGF1) (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 (Alison 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 (Ziebe *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 Sfontouris *et al.* (2013), showed a trend toward increased PR with GM-CSF-supplemented medium 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-CF-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 (Knudson and Knudson, 1993; Campbell *et al.*, 1995). A Cohrane review concluded that hyaluronan supplementation appeared to be safe and significant benefit was documented for PR but not LBR (Bontekoe *et al.*, 2010). A recent update on this Cohrane 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 Supplemetary data, Tables SI–SV. 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 SI). 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) (Biggers, 2000; Carrasco *et al.*, 2013). All companies provide in their websites media specifications and IFU and most of them include relevant scientific publications. Most of the media are now CE marked and all companies use the MEA but none of them discloses in the website or printed material the number of mouse embryos used. Certificates of Analysis are available on request for every batch of culture media and for some companies samples 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 SVI 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 $\geq$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 Schwarzer *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 Cohrane 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). Schwarzer *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 trophectoderm and to generate a reduced number of embryonic stem cells (Tielens *et al.*, 2006; Watkins *et al.*, 2007). IVF and embryo culture also influences placentation (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 neuromotor 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; Sabhnani *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; Bowdin *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 IGF1 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 trophectoderm 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 maternal or the paternal 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; Hentemann, 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 (Martín-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 (Isachenko *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 since 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 end-points 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 periconception 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..

Funding

No external funding was either sought or obtained for this work.

Conflict of interest

The authors report no financial or commercial conflicts of interest.

References

Abramczuk J, Solter D, Koprowski H. The beneficial effect of EDTA on development of mouse one-cell embryos in chemically defined medium. *Dev Biol* 1977;**61**:378–383.

Ackerman SB, Taylor SP, Swanson RJ, Laurell LH. Mouse embryo culture for screening in human IVF. *Arch Androl* 1984;**12**:129–136.

Ackerman SB, Stokes GL, Swanson RJ, Taylor SP, Fenwick L. Toxicity testing for human *in vitro* fertilization programs. *J In Vitro Fert Embryo Transf* 1985;**2**:132–137.

Adamson GD, Zegers-Hochschild F, Ishihara O, Sullivan E, Mansour R, Nygren KG, Banker M, Dyer S, de Mouzon J. ICMART World Report: Preliminary 2008 Data. *Hum Reprod* 2012;**27**(Suppl 2):ii38–ii39.

Agerholm I, Loft A, Hald F, Lemmen J, Munding B, Sørensen P, ZiebeSr. Culture of human oocytes with granulocyte-macrophage colony-stimulating factor has no effect on embryonic chromosomal constitution. *Reprod Biomed Online* 2010; **20**:477–484.

Alegretti JR, Rocha AM, Barros BC, Serafini P, Motta ELA, Smith GD. Microfluidic dynamic embryo culture increases the production of top quality human embryos through reduction in embryo fragmentation. *Fertil Steril* 2011;**96**:S58–S59.

Alison MR, Sarraf CE. Apoptosis: a gene-directed programme of cell death. *J R Coll Physicians Lond* 1992;**26**:25–35.

Anderson R, Bayne RAL, Gardner J, De Sousa P. Brain-derived neurotrophic factor is a regulator of human oocyte maturation and early embryo development. *Fertil Steril* 2010;**93**:1394–1406.

Australian Government, Department of Health, Therapeutic Goods Administration. *In vitro* fertilisation (IVF) solutions. 2013. Retrieved from http://www.tga.gov.au/industry/devices-ivf-solutions.htm.

Balaban B, Yakin K, Ata B, Isiklar A, Urman B. Effect of hyaluronan-enriched transfer medium on take home baby rate after day 3 and day 5 embryo transfers: a prospective randomized study. *Hum Reprod* 2011;**26**(Suppl 1):i24–i26.

Barak Y, Goldman S, Gonen Y, Nevo Z, Bartoov B, Kogosowski A. Does glucose affect fertilization, development and pregnancy rates of human *in-vitro* fertilized oocytes? *Hum Reprod* 1998;**13**(Suppl 4):203–211.

Barker DJ. The intrauterine origins of cardiovascular disease. *Acta Paediatr Suppl* 1993; **82**(Suppl 391):93–99.

Barker DJP. The developmental origins of adult disease. *Eur J Epidemiol* 2003;**18**:733–736.

Barker DJP. The developmental origins of adult disease. *J Am Coll Nutr* 2004; **23**:588S–595S.

Barmat LI, Worrilow KC, Paynton BV. Growth factor expression by human oviduct and buffalo rat liver coculture cells. *Fertil Steril* 1997;**67**:775–779.

Barnes FL, Crombie A, Gardner DK, Kausche A, Lacham Kaplan O, Suikkari AM, Tiglias J, Wood C, Trounson AO. Blastocyst development and birth after *in-vitro* maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. *Hum Reprod* 1995;**10**:3243–3247.

Basatemur E, Shevlin M, Sutcliffe A. Growth of children conceived by IVF and ICSI up to 12 years of age. *Reprod Biomed Online* 2010;**20**:144–149.

Basile N, Morbeck D, García Velasco J, Bronet F, Meseguer M. Type of culture media does not affect embryo kinetics: a time-lapse analysis of sibling oocytes. *Hum Reprod* 2013;**28**:634–641.

Bavister BD. Co-culture for embryo development: is it really necessary? *Hum Reprod* 1992;**7**:1339–1134.

Bavister BD. Glucose and culture of human embryos. *Fertil Steril* 1999;**72**:233–234.

Ben Chetrit A, Jurisicova A, Casper RF. Coculture with ovarian cancer cell enhances human blastocyst formation *in vitro*. *Fertil Steril* 1996;**65**:664–666.

Berkowitz RS, Faris HM, Hill JA, Anderson DJ. Localization of leukocytes and cytokines in chorionic villi of normal placentas and complete hydatidiform moles. *Gynecol Oncol* 1990;**37**:396–400.

Bertolini M, Moyer AL, Mason JB, Batchelder CA, Hoffert KA, Bertolini LR, Carneiro GF, Cargill SL, Famula TR, Calvert CC *et al.* Evidence of increased substrate availability to *in vitro*-derived bovine foetuses and association with accelerated conceptus growth. *Reproduction* 2004;**128**:341–354.

Beydoun H, Sicignano N, Beydoun M, Matson D, Bocca S, Stadtmauer L, Oehninger S. A cross-sectional evaluation of the first cohort of young adults conceived by *in vitro* fertilization in the United States. *Fertil Steril* 2010;**94**:2043–2049.

Biggers JD. Ethical issues and the commercialization of embryo culture media. *Reprod Biomed Online* 2000;**1**:74–76.

Biggers JD, Gwatkin RBL, Brinster RL. Development of mouse embryos in organ cultures of fallopian tubes on a chemically defined medium. *Nature* 1962;**194**:747–749.

Biggers JD, Whittingham DG, Donahue RP. The pattern of energy metabolism in the mouse oocyte and zygote. *Proc Natl Acad Sci USA* 1967;**58**:560–567.

Biggers JD, Lawitts JA, Lechene CP. The protective action of betaine on the deleterious effects of NaCl on preimplantation mouse embryos *in vitro*. *Mol Reprod Dev* 1993; **34**:380–390.

Biggers JD, McGinnis LK, Lawitts JA. Enhanced effect of glycyl-L-glutamine on mouse preimplantation embryos *in vitro*. *Reprod Biomed Online* 2004;**9**:59–69.

Bongso A, Ng SC, Fong CY, Mok H, Ng PL, Ratnam SS. Cocultures in human assisted reproduction. Support of embryos *in vitro* and their specificity. *Ann N Y Acad Sci* 1991; **626**:438–444.

Bongso A, Soon Chye N, Sathananthan H, Lian NP, Rauff M, Ratnam S. Improved quality of human embryos when co-cultured with human ampullary cells. *Human reproduction* 1989;**4**:706–713.

Bontekoe S, Blake D, Heineman MJ, Williams EC, Johnson N. Adherence compounds in embryo transfer media for assisted reproductive technologies. *Cochrane Database Syst Rev* 2010;**7**:CD007421–CD007421.

Bontekoe S, Heineman MJ, Johnson N, Blake D. Adherence compounds in embryo transfer media for assisted reproductive technologies. *Cochrane Database Syst Rev* 2014, Issue 2. Art. No.: CD007421. doi: 10.1002/14651858.CD007421.pub3.

Borland RM, Biggers JD, Lechene CP, Taymor ML. Elemental composition of fluid in the human Fallopian tube. *J Reprod Fertil* 1980;**58**:479–482.

Bowdin S, Allen C, Kirby G, Brueton L, Afnan M, Barratt C, Kirkman Brown J, Harrison R, Maher E, Reardon W. A survey of assisted reproductive technology births and imprinting disorders. *Hum Reprod* 2007;**22**:3237–3240.

Bowman P, McLaren A. Viability and growth of mouse embryos after *in vitro* culture and fusion. *J Embryol Exp Morphol* 1970;**23**:693–704.

Bradbury B, Jick H. *In vitro* fertilization and childhood retinoblastoma. *Br J Clin Pharmacol* 2004;**58**:209–211.

Brinster RL. A Method for *in vitro* cultivation of mouse ova from two-cell to blastocyst. *Exp Cell Res* 1963;**32**:205–208.

Brinster RL. Studies on the development of mouse embryos *in vitro*. 3. The effect of fixed-nitrogen source. *J Exp Zool* 1965a;**158**:69–77.

Brinster RL. Studies on the development of mouse embryos *in vitro*. I. The effect of osmolarity and hydrogen ion concentration. *J Exp Zool* 1965b;**158**:49–57.

Brinster RL. Studies on the development of mouse embryos *in vitro*. I. The effect of osmolarity and hydrogen ion concentration. *J Exp Zool* 1965c;**158**:49–57.

Brinster RL. Studies on the development of mouse embryos *in vitro*. II. The effect of energy source. *J Exp Zool* 1965d;**158**:59–68.

Brinster RL. *In vitro* culture of mammalian embryos. *J Anim Sci* 1968;**27**(Suppl 1):1–14.

Brinster RL, Biggers JD. *In-vitro* fertilization of mouse ova within the explanted fallopian tube. *J Reprod Fertil* 1965;**10**:277–279.

Brison DR, Houghton FD, Falconer D, Roberts SA, Hawkhead J, Humpherson PG, Lieberman BA, Leese HJ. Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover. *Hum Reprod* 2004;**19**:2319–2324.

Brower V. Epigenetics: unravelling the cancer code. *Nature* 2011;**471**:S12–S13.

Byers S, Payson S, Taft R. Performance of ten inbred mouse strains following assisted reproductive technologies (ARTs). *Theriogenology* 2006;**65**:1716–1726.

Calle A, Miranda A, Fernandez Gonzalez R, Pericuesta E, Laguna R, Gutierrez Adan A. Male mice produced by *in vitro* culture have reduced fertility and transmit organomegaly and glucose intolerance to their male offspring. *Biol Reprod* 2012; **87**:34–34.

Campbell S, Swann HR, Aplin JD, Seif MW, Kimber SJ, Elstein M. CD44 is expressed throughout pre-implantation human embryo development. *Hum Reprod* 1995; **10**:425–430.

Caro CM, Trounson A. Successful fertilization, embryo development, and pregnancy in human *in vitro* fertilization (IVF) using a chemically defined culture medium containing no protein. *J In Vitro Fert Embryo Transf* 1986;**3**:215–217.

Carrasco B, Boada M, Rodríguez I, Coroleu B, Barri PN, Veiga A. Does culture medium influence offspring birth weight? *Fertil Steril*. Advance access published August 2, 2013. doi: http://dx.doi.org/10.1016/j.fertnstert.2013.07.006.

Casslén BG. Free amino acids in human uterine fluid. Possible role of high taurine concentration. *J Reprod Med* 1987;**32**:181–184.

Cassuto G, Chavrier M, Ménézo Y. Culture conditions and not prolonged culture time are responsible for monozygotic twinning in human *in vitro* fertilization. *Fertil Steril* 2003;**80**:462–463.

Ceelen M, van Weissenbruch M, Roos J, Vermeiden JPW, van Leeuwen F, Delemarre-van de Waal HA. Body composition in children and adolescents born after *in vitro* fertilization or spontaneous conception. *J Clin Endocrinol Metab* 2007; **92**:3417–3423.

Ceelen M, van Weissenbruch M, Vermeiden JPW, van Leeuwen F, Delemarre-van de Waal HA. Cardiometabolic differences in children born after *in vitro* fertilization: follow-up study. *J Clin Endocrinol Metab* 2008a;**93**:1682–1688.

Ceelen M, van Weissenbruch M, Vermeiden JPW, van Leeuwen F, Delemarre-van de Waal HA. Pubertal development in children and adolescents born after IVF and spontaneous conception. *Hum Reprod* 2008b;**23**:2791–2798.

Ceelen M, van Weissenbruch M, Prein J, Smit J, Vermeiden JPW, Spreeuwenberg M, van Leeuwen F, Delemarre-van de Waal HA. Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8–18 years of IVF children and spontaneously conceived controls born to subfertile parents. *Hum Reprod* 2009; **24**:2788–2795.

CFR – Code of Federal Regulations. 2013; Title 21, Volume 8.

Chang MC. Normal development of fertilized rabbit ova stored at low temperature for several days. *Nature* 1947;**159**:602–602.

Chang MC. Fertilization of rabbit ova *in vitro*. *Nature* 1959;**184**:466–467.

Chatot CL, Ziomek CA, Bavister BD, Lewis JL, Torres I. An improved culture medium supports development of random-bred 1-cell mouse embryos *in vitro*. *J Reprod Fertil* 1989;**86**:679–688.

Chin P, Macpherson A, Thompson J, Lane M, Robertson S. Stress response genes are suppressed in mouse preimplantation embryos by granulocyte-macrophage colony-stimulating factor (GM-CSF). *Hum Reprod* 2009;**24**:2997–3009.

Coates A, Rutherford AJ, Hunter H, Leese HJ. Glucose-free medium in human *in vitro* fertilization and embryo transfer: a large-scale, prospective, randomized clinical trial. *Fertil Steril* 1999;**72**:229–232.

Cockburn K, Rossant J. Making the blastocyst: lessons from the mouse. *J Clin Invest* 2010; **120**:995–1003.

Cohen J, Trounson A, Dawson K, Jones H, Hazekamp J, Nygren K-Gs, Hamberger L. The early days of IVF outside the UK. *Hum Reprod Update* 2005;**11**:439–459.

Conaghan J, Handyside AH, Winston RM, Leese HJ. Effects of pyruvate and glucose on the development of human preimplantation embryos *in vitro*. *J Reprod Fertil* 1993; **99**:87–95.

Cossiello RDF, Aggelis A, Faúndes D, Petta C. Morphological differences in human zygotes and embryos cultured in different media. *Zygote* 2012;**20**:399–405.

Cross PC, Brinster RL. The sensitivity of one-cell mouse embryos to pyruvate and lactate. *Exp Cell Res* 1973;**77**:57–62.

Dandekar PV, Glass RH. Development of mouse embryos *in vitro* is affected by strain and culture medium. *Gamete Res* 1987;**17**:279–285.

Davidson A, Vermesh M, Lobo RA, Paulson RJ. Mouse embryo culture as quality control for human *in vitro* fertilization: the one-cell versus the two-cell model. *Fertil Steril* 1988;**49**:516–521.

De Kretzer D, Dennis P, Hudson B, Leeton J, Lopata A, Outch K, Talbot J, Wood C. Transfer of a human zygote. *Lancet* 1973;**2**:728–729.

Delle Piane L, Lin W, Liu X, Donjacour A, Minasi P, Revelli A, Maltepe E, Rinaudo PF. Effect of the method of conception and embryo transfer procedure on mid-gestation placenta and fetal development in an IVF mouse model. *Hum Reprod* 2010; **25**:2039–2046.

Denomme M, Mann MRW. Genomic imprints as a model for the analysis of epigenetic stability during assisted reproductive technologies. *Reproduction* 2012; **144**:393–409.

Desai N, Abdelhafez F, Bedaiwy M, Goldfarb J. Live births in poor prognosis IVF patients using a novel non-contact human endometrial co-culture system. *Reprod Biomed Online* 2008;**16**:869–874.

Devreker F, Hardy K. Effects of glutamine and taurine on preimplantation development and cleavage of mouse embryos *in vitro*. *Biol Reprod* 1997;**57**:921–928.

Devreker F, Hardy K, Van den Bergh M, Vannin AS, Emiliani S, Englert Y. Amino acids promote human blastocyst development *in vitro*. *Hum Reprod* 2001;**16**:749–756.

Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod* 2000;**62**:1526–1535.

Doornbos M, Maas S, McDonnell J, Vermeiden JPW, Hennekam RCM. Infertility, assisted reproduction technologies and imprinting disturbances: a Dutch study. *Hum Reprod* 2007;**22**:2476–2480.

Dubin NH, Bornstein DR, Gong Y. Use of endotoxin as a positive (toxic) control in the mouse embryo assay. *J Assist Reprod Genet* 1995;**12**:147–152.

Dudley DJ, Mitchell MD, Creighton K, Branch DW. Lymphokine production during term human pregnancy: differences between peripheral leukocytes and decidual cells. *Am J Obstet Gynecol* 1990;**163**:1890–1893.

Dumoulin JC, Menheere PP, Evers JL, Kleukers AP, Pieters MH, Bras M, Geraedts JP. The effects of endotoxins on gametes and preimplantation embryos cultured *in vitro*. *Hum Reprod* 1991;**6**:730–734.

Dumoulin J, Land J, Van Montfoort A, Nelissen E, Coonen E, Derhaag J, Schreurs I, Dunselman G, Kester A, Geraedts J *et al.* Effect of *in vitro* culture of human embryos on birthweight of newborns. *Hum Reprod* 2010;**25**:605–612.

Dunglison GF, Barlow DH, Sargent IL. Leukaemia inhibitory factor significantly enhances the blastocyst formation rates of human embryos cultured in serum-free medium. *Hum Reprod* 1996;**11**:191–196.

Duranthon V, Watson A, Lonergan P. Preimplantation embryo programming: transcription, epigenetics, and culture environment. *Reproduction* 2008;**135**:141–150.

Eaton JL, Lieberman ES, Stearns C, Chinchilla M, Racowsky C. Embryo culture media and neonatal birthweight following IVF. *Hum Reprod* 2012;**27**:375–379.

Edwards RG. Test-tube babies, 1981. *Nature* 1981;**293**:253–256.

Edwards RG, Bavister BD, Steptoe PC. Early stages of fertilization *in vitro* of human oocytes matured *in vitro*. *Nature* 1969;**221**:632–635.

Edwards RG, Steptoe PC, Purdy JM. Establishing full-term human pregnancies using cleaving embryos grown *in vitro*. *Br J Obstet Gynaecol* 1980;**87**:737–756.

Elaimi A, Gardner K, Kistnareddy K, Harper J. The effect of GM-CSF on development and aneuploidy in murine blastocysts. *Hum Reprod* 2012;**27**:1590–1595.

El Hajj N, Haaf T. Epigenetic disturbances in *in vitro* cultured gametes and embryos: implications for human assisted reproduction. *Fertil Steril* 2013;**99**:632–641.

El Maarri O, Buiting K, Peery EG, Kroisel PM, Balaban B, Wagner K, Urman B, Heyd J, Lich C, Brannan CI *et al.* Maternal methylation imprints on human chromosome 15 are established during or after fertilization. *Nat Genet* 2001;**27**:341–344.

Eskild A, Monkerud L, Tanbo T. Birthweight and placental weight; do changes in culture media used for IVF matter? Comparisons with spontaneous pregnancies in the corresponding time periods. *Hum Reprod* 2013;**28**:3207–3214.

European Commission. Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. Retrieved from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31993L0042:EN:HTML.

European Commission. Guidance MEDDEVs. Retrieved from http://ec.europa.eu/health/medical-devices/documents/guidelines/index_en.htm.

Farin PW, Farin CE. Transfer of bovine embryos produced *in vivo* or *in vitro*: survival and fetal development. *Biol Reprod* 1995;**52**:676–682.

Feichtinger W, Kemeter P, Ménézo Y. The use of synthetic culture medium and patient serum for human *in vitro* fertilization and embryo replacement. *J In Vitro Fert Embryo Transf* 1986;**3**:87–92.

Fernández-Gonzalez RI, Moreira P, Bilbao A, Jiménez A, Pérez-Crespo M, Ramírez M, Rodriguez De Fonseca F, Pintado Bn, Gutiérrez-Adán. Long-term effect of *in vitro* culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. *Proc Natl Acad Sci USA* 2004; **101**:5880–5885.

Feuer SK, Camarano L, Rinaudo PF. ART and health: clinical outcomes and insights on molecular mechanisms from rodent studies. *Mol Hum Reprod* 2013;**19**:189–204.

Finnström O, Källén B, Lindam A, Nilsson E, Nygren K-Gs, Olausson P. Maternal and child outcome after *in vitro* fertilization—a review of 25 years of population-based data from Sweden. *Acta Obstet Gynecol Scand* 2011;**90**:494–500.

Fleetham JA, Pattinson HA, Mortimer D. The mouse embryo culture system: improving the sensitivity for use as a quality control assay for human *in vitro* fertilization. *Fertil Steril* 1993;**59**:192–196.

Fleming TP, Pratt HP, Braude PR. The use of mouse preimplantation embryos for quality control of culture reagents in human *in vitro* fertilization programs: a cautionary note. *Fertil Steril* 1987;**47**:858–860.

Fleming T, Kwong W, Porter R, Ursell E, Fesenko I, Wilkins A, Miller D, Watkins A, Eckert J. The embryo and its future. *Biol Reprod* 2004;**71**:1046–1054.

Food and Drug Administration. Obstetric and gynecologic devices; reclassification and classification of medical devices used for *in vitro* fertilization and related assisted reproduction procedures. *Fed Regist* 1998;**63**:48428–48437.

Gardner DK. Mammalian embryo culture in the absence of serum or somatic cell support. *Cell Biol Int* 1994;**18**:1163–1179.

Gardner DK. Changes in requirements and utilization of nutrients during mammalian preimplantation embryo development and their significance in embryo culture. *Theriogenology* 1998;**49**:83–102.

Gardner DK, Lane M. Amino acids and ammonium regulate mouse embryo development in culture. *Biol Reprod* 1993;**48**:377–385.

Gardner DK, Lane M. Alleviation of the '2-cell block' and development to the blastocyst of CF1 mouse embryos: role of amino acids, EDTA and physical parameters. *Hum Reprod* 1996;**11**:2703–2712.

Gardner D, Lane M. Culture and selection of viable blastocysts: a feasible proposition for human IVF? *Hum Reprod Update* 1997;**3**:367–382.

Gardner DK, Lane M. Culture of viable human blastocysts in defined sequential serum-free media. *Hum Reprod* 1998;**13**(Suppl 3):148–159.

Gardner DK, Leese HJ. Concentrations of nutrients in mouse oviduct fluid and their effects on embryo development and metabolism *in vitro*. *J Reprod Fertil* 1990; **88**:361–368.

Gardner DK, Lane M, Calderon I, Leeton J. Environment of the preimplantation human embryo *in vivo*: metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. *Fertil Steril* 1996;**65**:349–353.

Gardner DK, Lane M, Stevens J, Schoolcraft WB. Noninvasive assessment of human embryo nutrient consumption as a measure of developmental potential. *Fertil steril* 2001;**76**:1175–1180.

Gardner D, Reed L, Linck D, Sheehan C, Lane M. Quality control in human *in vitro* fertilization. *Semin Reprod Med* 2005;**23**:319–324.

Gardner DK, Hamilton R, McCallie B, Schoolcraft WB, Katz Jaffe MG. Human and mouse embryonic development, metabolism and gene expression are altered by an ammonium gradient *in vitro*. *Reproduction* 2013;**146**:49–61.

Gardner D, Wale P. Analysis of metabolism to select viable human embryos for transfer. *Fertil steril* 2013;**99**:1062–1072.

George MA, Braude PR, Johnson MH, Sweetnam DG. 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.

Giacomini G, Tabibzadeh SS, Satyaswaroop PG, Bonsi L, Vitale L, Bagnara GP, Strippoli P, Jasonni VM. Epithelial cells are the major source of biologically active granulocyte macrophage colony-stimulating factor in human endometrium. *Hum Reprod* 1995;**10**:3259–3263.

Gianaroli L, Seracchioli R, Ferraretti AP, Trounson A, Flamigni C, Bovicelli L. The successful use of human amniotic fluid for mouse embryo culture and human *in vitro* fertilization, embryo culture, and transfer. *Fertil Steril* 1986;**46**:907–913.

Goddard MJ, Pratt HP. Control of events during early cleavage of the mouse embryo: an analysis of the '2-cell block'. *J Embryol Exp Morphol* 1983;**73**:111–133.

Good Manufacturing Practice for Drugs (2010 Revision). 2011; MOH Decree No. 79.

Grace K, Sinclair K. Assisted reproductive technology, epigenetics, and long-term health: a developmental time bomb still ticking. *Semin Reprod Med* 2009; **27**:409–416.

Hammond J. Recovery and culture of tubal mouse ova. *Nature* 1949;**163**:28–28.

Hardy K, Spanos S. Growth factor expression and function in the human and mouse preimplantation embryo. *J Endocrinol* 2002;**172**:221–236.

Hardy K, Handyside AH, Winston RM. The human blastocyst: cell number, death and allocation during late preimplantation development *in vitro*. *Development* 1989; **107**:597–604.

Harper J, Cristina Magli M, Lundin K, Barratt CLR, Brison D. When and how should new technology be introduced into the IVF laboratory? *Hum Reprod* 2012; **27**:303–313.

Hart R, Norman R. The longer-term health outcomes for children born as a result of IVF treatment. Part II—Mental health and development outcomes. *Hum Reprod Update* 2013;**19**:244–250.

Hendriks JCM, Teerenstra S, Punt-Van der Zalm JPE, Wetzels AMM, Westphal JR, Borm GF. Sample size calculations for a split-cluster, beta-binomial design in the assessment of toxicity. *Stat Med* 2005;**24**:3757–3772.

Hentemann M, Bertheussen K. New media for culture to blastocyst. *Fertil Steril* 2009; **91**:878–883.

Hentemann M, Mousavi K, Bertheussen K. Differential pH in embryo culture. *Fertil Steril* 2011;**95**:1291–1294.

Ho Y, Wigglesworth K, Eppig JJ, Schultz RM. Preimplantation development of mouse embryos in KSOM: augmentation by amino acids and analysis of gene expression. *Mol Reprod Dev* 1995;**41**:232–238.

Hu Y, Maxson W, Hoffman D, Ory S, Eager S, Dupre J, Worrilow K. Co-culture with assisted hatching of human embryos using Buffalo rat liver cells. *Hum Reprod* 1998; **13**:165–168.

Human Fertilisation Embryology Authority (HFEA). Horizon scanning annual report 2009/2010. Retrieved from http://www.hfea.gov.uk/Horizon-scanning-report.html.

Human Fertilisation and Embryology Authority (HFEA). Scientific and Clinical Advances Advisory Committee Paper 2011. Retrieved from http://www.hfea.gov.uk/6348.html.

Hyttel P, Viuff D, Laurincik J, Schmidt M, Thomsen PD, Avery B, Callesen H, Rath D, Niemann H, Rosenkranz C *et al.* Risks of *in-vitro* production of cattle and swine embryos: aberrations in chromosome numbers, ribosomal RNA gene activation and perinatal physiology. *Hum Reprod* 2000;**15**(Suppl 5):87–97.

Isachenko E, Maettner R, Isachenko V, Roth S, Kreienberg R, Sterzik K. Mechanical agitation during the *in vitro* culture of human pre-implantation embryos drastically increases the pregnancy rate. *Clin Lab* 2010;**56**:569–576.

Isom SC, Lai L, Prather R, Rucker E. Heat shock of porcine zygotes immediately after oocyte activation increases viability. *Mol Reprod Dev* 2009;**76**:548–554.

Jayot S, Parneix I, Verdaguer S, Discamps G, Audebert A, Emperaire JC. Coculture of embryos on homologous endometrial cells in patients with repeated failures of implantation. *Fertil Steril* 1995;**63**:109–114.

Jiménez A, Madrid Bury N, Fernández RL, Pérez-Garnelo S, Moreira P, Pintado BN, de la Fuente J, Gutiérrez-Adán A. Hyperglycemia-induced apoptosis affects sex ratio of bovine and murine preimplantation embryos. *Mol Reprod Dev* 2003; **65**:180–187.

Jones GM, Trounson AO, Gardner DK, Kausche A, Lolatgis N, Wood C. Evolution of a culture protocol for successful blastocyst development and pregnancy. *Hum Reprod* 1998;**13**:169–177.

Källén B, Finnström O, Lindam A, Nilsson E, Nygren K-Gs, Olausson P. Cancer risk in children and young adults conceived by *in vitro* fertilization. *Pediatrics* 2010; **126**:270–276.

Karamalegos C, Bolton VN. A prospective comparison of 'in house' and commercially prepared Earle's balanced salt solution in human *in-vitro* fertilization. *Hum Reprod* 1999;**14**:1842–1846.

Katari S, Turan N, Bibikova M, Erinle O, Chalian R, Foster M, Gaughan J, Coutifaris C, Sapienza C. DNA methylation and gene expression differences in children conceived *in vitro* or *in vivo*. *Hum Mol Genet* 2009;**18**:3769–3778.

Kattal N, Cohen J, Barmat L. Role of coculture in human *in vitro* fertilization: a meta-analysis. *Fertil Steril* 2008;**90**:1069–1076.

Kelsey G. Genomic imprinting—roles and regulation in development. *Endocr Dev* 2007; **12**:99–112.

Khamsi F, Armstrong DT, Zhang X. Expression of urokinase-type plasminogen activator in human preimplantation embryos. *Mol Hum Reprod* 1996;**2**:273–276.

Khosla S, Dean W, Brown D, Reik W, Feil R. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biol Reprod* 2001;**64**:918–926.

Khoury C, Coffler M, Potter D, Frederick J, Battaglia D. Improved blastocyst development using a single step medium versus a sequential medium. *Fertil Steril* 2012;**97**:S4–S5.

Kim D, Kim M, Kang H, Lee H, Park W, Kwon H. The supplementation of granulocyte-macrophage colony-stimulating factor (GM-CSF) in culture medium improves the pregnancy rate in human ART programs. *Fertil Steril* 2001;**76**:S6.

Kimber SJ, Sneddon SF, Bloor DJ, El Bareg AM, Hawkhead JA, Metcalfe AD, Houghton FD, Leese HJ, Rutherford A, Lieberman BA *et al.* Expression of genes involved in early cell fate decisions in human embryos and their regulation by growth factors. *Reproduction* 2008;**135**:635–647.

Kleijkers SHM, Van Montfoort APA, Smits LJM, Viechtbauer W, Roseboom TJ, Nelissen ECM, Coonen E, Derhaag JG, Bastings L, Schreurs IEL *et al.* IVF culture medium affects postnatal growth in humans during the first 2 years of live. *Hum Reprod* 2012;**27**(Suppl 2):ii70–ii71.

Kleijkers SHM, Van Montfoort APA, Bekers O, Coonen E, Derhaag JG, Schreurs IEL, Evers JLH, Dumoulin JMC. Ammonium accumulates in IVF culture medium but this does not affect the birth weight of the newborn. *Hum Reprod* 2013; **28**(Suppl 1):i60–i62.

Knudson CB, Knudson W. Hyaluronan-binding proteins in development, tissue homeostasis, and disease. *FASEB J* 1993;**7**:1233–1241.

Kolahi K, Donjacour A, Liu X, Lin W, Simbulan R, Bloise E, Maltepe E, Rinaudo P. Effect of substrate stiffness on early mouse embryo development. *PLoS One* 2012; **7**:e41717–e41717.

Krebs HA, Henseleit K. Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyler's Zeitschrift für Physiol. Chemie* 1932;**210**:33–66.

Kuhl W. Untersuchungen uber die cytodynamik der furchung und fruhentwicklung des eis der weissen maus. *Abb Senchenb Naturforsch Ges* 1941;**456**:1–17.

Lane M, Gardner DK. Increase in postimplantation development of cultured mouse embryos by amino acids and induction of fetal retardation and exencephaly by ammonium ions. *J Reprod Fertil* 1994;**102**:305–312.

Lane M, Gardner DK. Differential regulation of mouse embryo development and viability by amino acids. *J Reprod Fertil* 1997a;**109**:153–164.

Lane M, Gardner DK. Nonessential amino acids and glutamine decrease the time of the first three cleavage divisions and increase compaction of mouse zygotes *in vitro*. *J Assist Reprod Genet* 1997b;**14**:398–403.

Lane M, Gardner DK. Inhibiting 3-phosphoglycerate kinase by EDTA stimulates the development of the cleavage stage mouse embryo. *Mol Reprod Dev* 2001; **60**:233–240.

Lane M, Gardner DK. Embryo culture medium: which is the best? *Best Pract Res Clin Obstet Gynaecol* 2007;**21**:83–100.

Lane M, Hooper K, Gardner DK. Effect of essential amino acids on mouse embryo viability and ammonium production. *J Assist Reprod Genet* 2001; **18**:519–525.

Langley Evans S. Developmental programming of health and disease. *Proc Nutr Soc* 2006; **65**:97–105.

Lawitts JA, Biggers JD. Optimization of mouse embryo culture media using simplex methods. *J Reprod Fertil* 1991;**91**:543–556.

Lawitts JA, Biggers JD. Joint effects of sodium chloride, glutamine, and glucose in mouse preimplantation embryo culture media. *Mol Reprod Dev* 1992;**31**:189–194.

Lawitts JA, Biggers JD. Culture of preimplantation embryos. *Methods Enzymol* 1993; **225**:153–164.

Leese HJ. Human embryo culture: back to nature. *J Assist Reprod Genet* 1998; **15**:466–468.

Leese H. Quiet please, do not disturb: a hypothesis of embryo metabolism and viability. *Bioessays* 2002;**24**:845–849.

Lewis WH, Gregory PW. Cinematographs of living developing rabbit-eggs. *Science* 1929;**69**:226–229.

Li S, Hursting S, Davis B, McLachlan J, Barrett JC. Environmental exposure, DNA methylation, and gene regulation: lessons from diethylstilbesterol-induced cancers. *Ann N Y Acad Sci* 2003;**983**:161–169.

Li T, Vu T, Ulaner G, Littman E, Ling J-Q, Chen H-L, Hu J-F, Behr B, Giudice L, Hoffman A. IVF results in de novo DNA methylation and histone methylation at an Igf2-H19 imprinting epigenetic switch. *Mol Hum Reprod* 2005;**11**:631–640.

Lidegaard O, Pinborg A, Andersen A. Imprinting diseases and IVF: Danish National IVF cohort study. *Hum Reprod* 2005;**20**:950–954.

Lighten AD, Moore GE, Winston RM, Hardy K. Routine addition of human insulin-like growth factor-I ligand could benefit clinical *in-vitro* fertilization culture. *Hum Reprod* 1998;**13**:3144–3150.

Lin S, Li M, Lian Y, Chen L, Liu P. No effect of embryo culture media on birthweight and length of newborns. *Hum Reprod* 2013;**28**:1762–1767.

Liu HC, He ZY, Mele CA, Veeck LL, Davis O, Rosenwaks Z. Human endometrial stromal cells improve embryo quality by enhancing the expression of insulin-like growth factors and their receptors in cocultured human preimplantation embryos. *Fertil Steril* 1999;**71**:361–367.

Lonergan P, Pedersen H, Rizos D, Greve T, Thomsen P, Fair T, Evans A, Boland M. Effect of the post-fertilization culture environment on the incidence of chromosome aberrations in bovine blastocysts. *Biol Reprod* 2004;**71**:1096–1100.

Lopata A, Oliva K. Chorionic gonadotrophin secretion by human blastocysts. *Hum Reprod* 1993;**8**:932–938.

Lopata A, Johnston IW, Hoult IJ, Speirs AI. Pregnancy following intrauterine implantation of an embryo obtained by *in vitro* fertilization of a preovulatory egg. *Fertil Steril* 1980;**33**:117–120.

Ludwig TE, Lane M, Bavister BD. Differential effect of hexoses on hamster embryo development in culture. *Biol Reprod* 2001;**64**:1366–1374.

Luna M, Duke M, Copperman A, Grunfeld L, Sandler B, Barritt J. Blastocyst embryo transfer is associated with a sex-ratio imbalance in favor of male offspring. *Fertil Steril* 2007;**87**:519–523.

Macklon NS, Pieters MHEC, Hassan MA, Jeucken PHM, Eijkemans MJC, Fauser BCJM. A prospective randomized comparison of sequential versus monoculture systems for *in-vitro* human blastocyst development. *Hum Reprod* 2002;**17**: 2700–2705.

Mahsoudi B, Li A, O'Neill C. Assessment of the long-term and transgenerational consequences of perturbing preimplantation embryo development in mice. *Biol Reprod* 2007;**77**:889–896.

Mains L, Zimmerman M, Blaine J, Stegmann B, Sparks A, Ansley T, Van Voorhis B. Achievement test performance in children conceived by IVF. *Hum Reprod* 2010; **25**:2605–2611.

Mantikou E, Youssef MAFM, van Wely M, van der Veen F, Al-Inany HG, Repping S, Mastenbroek S. Embryo culture media and IVF/ICSI success rates: a systematic review. *Hum Reprod Update* 2013;**19**:210–220.

Market-Velker BA, Fernandes AD, Mann MRW. Side-by-side comparison of five commercial media systems in a mouse model: suboptimal *in vitro* culture interferes with imprint maintenance. *Biol Reprod* 2010;**83**:938–950.

Market Velker B, Denomme M, Mann MRW. Loss of genomic imprinting in mouse embryos with fast rates of preimplantation development in culture. *Biol Reprod* 2012;**86**:143–143,116.

Martin KL, Barlow DH, Sargent IL. Heparin-binding epidermal growth factor significantly improves human blastocyst development and hatching in serum-free medium. *Hum Reprod* 1998;**13**:1645–1652.

Martín-Romero F, Miguel Lasobras E, Domínguez-Arroyo J, González-Carrera E, Alvarez I. Contribution of culture media to oxidative stress and its effect on human oocytes. *Reprod Biomed Online* 2008;**17**:652–661.

McEvoy TG, Sinclair KD, Broadbent PJ, Goodhand KL, Robinson JJ. Post-natal growth and development of Simmental calves derived from *in vivo* or *in vitro* embryos. *Reprod Fertil Dev* 1998;**10**:459–464.

McLaren A, Biggers JD. Successful development and birth of mice cultivated *in vitro* as early as early embryos. *Nature* 1958;**182**:877–878.

Medicines and Healthcare products Regulatory Agency (MHRA). Medical Device Alert: Medical devices in general and non-medical products (MDA/2010/001) 2010. Retrieved from http://www.mhra.gov.uk/Publications/Safetywarnings/MedicalDeviceAlerts/CON065771.

Ménézo Y. Synthetic medium for gamete survival and maturation and for culture of fertilized eggs. *C R Acad Sci Hebd Seances Acad Sci D* 1976;**282**:1967–1970.

Ménézo YJR, Hérubel Fo. Mouse and bovine models for human IVF. *Reprod Biomed Online* 2002;**4**:170–175.

Ménézo Y, Testart J, Perrone D. Serum is not necessary in human *in vitro* fertilization, early embryo culture, and transfer. *Fertil Steril* 1984;**42**:750–755.

Ménézo Y, Hamidi J, Khatchadourian C, Nardon C. The murine prepuberal oviduct supports early embryo development *in vitro*. *Dev Growth Differ* 1989; **31**:557–561.

Ménézo YJ, Guerin JF, Czyba JC. Improvement of human early embryo development *in vitro* by coculture on monolayers of Vero cells. *Biol Reprod* 1990;**42**:301–306.

Ménézo YJ, Chouteau J, Torelló J, Girard A, Veiga A. Birth weight and sex ratio after transfer at the blastocyst stage in humans. *Fertil Steril* 1999;**72**:221–224.

Ménézo Y, Lichtblau I, Elder K. New insights into human pre-implantation metabolism *in vivo* and *in vitro*. *J Assist Reprod Genet* 2013;**30**:293–303.

Meseguer M, Herrero J, Tejera A, Hilligsøe KM, Ramsing NB, Remohí J. The use of morphokinetics as a predictor of embryo implantation. *Hum Reprod* 2011; **26**:2658–2671.

Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. *Fertil Steril* 2012b; **98**:1481–1489.

Michaeli M, Rotfarb N, Karchovsky E, Ruzov O, Atamny R, Slush K, Fainaru O, Ellenbogen A. The influence of different concentrations of glucose in culture media (P1, ISM1, IVF) post insemination and ICSI outcome, in *in-vitro* fertilization treatment. *Hum Reprod* 2011;**26**(Suppl 1):i160–i202.

Middelburg KJ, Heineman MJ, Bos AF, Pereboom M, Fidler V, Hadders-Algra M. The Groningen ART cohort study: ovarian hyperstimulation and the *in vitro* procedure do not affect neurological outcome in infancy. *Hum Reprod* 2009;**24**:3119–3126.

Mignini Renzini M, Dal Canto M, Coticchio G, Comi R, Brigante C, Caliari I, Brambillasca F, Merola M, Lain M, Turchi D. Clinical efficiency and perinatal outcome of ART cycles following embryo culture in the presence of GM-CSF in patients with miscarriage or early pregnancy loss history. *Hum Reprod* 2013; **28**(Suppl 1):i139–i149.

Miles J, Farin C, Rodriguez K, Alexander J, Farin P. Angiogenesis and morphometry of bovine placentas in late gestation from embryos produced *in vivo* or *in vitro*. *Biol Reprod* 2004;**71**:1919–1926.

Miles H, Hofman P, Peek J, Harris M, Wilson D, Robinson E, Gluckman P, Cutfield W. *In vitro* fertilization improves childhood growth and metabolism. *J Clin Endocrinol Metab* 2007;**92**:3441–3445.

Milki A, Jun S, Hinckley M, Westphal L, Giudice L, Behr B. Comparison of the sex ratio with blastocyst transfer and cleavage stage transfer. *J Assist Reprod Genet* 2003; **20**:323–326.

Mohr LR, Trounson AO. The use of fluorescein diacetate to assess embryo viability in the mouse. *J Reprod Fertil* 1980;**58**:189–196.

Montoro L, Subias E, Young P, Baccaro M, Swanson J, Sueldo C. Detection of endotoxin in human *in vitro* fertilization by the zona-free mouse embryo assay. *Fertil Steril* 1990; **54**:109–112.

Morgan K, Wiemer K, Steuerwald N, Hoffman D, Maxson W, Godke R. Use of videocinematography to assess morphological qualities of conventionally cultured and cocultured embryos. *Hum Reprod* 1995;**10**:2371–2376.

Morgan H, Santos Ft, Green K, Dean W, Reik W. Epigenetic reprogramming in mammals. *Hum Mol Genet* 2005;**14** Spec No 1:R47–R58.

Morgan HD, Jin XL, Li A, Whitelaw E, O'Neill C. The culture of zygotes to the blastocyst stage changes the postnatal expression of an epigentically labile allele, agouti viable yellow, in mice. *Biol Reprod* 2008;**79**:618–623.

Mortimer D. Elaboration of a new culture medium for physiological studies on human sperm motility and capacitation. *Hum Reprod* 1986;**1**:247–250.

Mortimer D. Human blastocyst development media. *Hum Reprod* 2001;**16**:2725.

Mossa F, Carter F, Walsh S, Kenny D, Smith G, Ireland JLH, Hildebrandt T, Lonergan P, Ireland J, Evans ACO. Maternal undernutrition in cows impairs ovarian and cardiovascular systems in their offspring. *Biol Reprod* 2013;**88**:92–92.

Muggleton Harris AL, Findlay I, Whittingham DG. Improvement of the culture conditions for the development of human preimplantation embryos. *Hum Reprod* 1990;**5**:217–220.

Nelissen E, Van Montfoort A, Coonen E, Derhaag J, Geraedts J, Smits L, Land J, Evers J, Dumoulin J. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. *Hum Reprod* 2012; **27**:1966–1976.

Nelissen ECM, Van Montfoort APA, Smits LJM, Menheere PPCA, Evers JLH, Coonen E, Derhaag JG, Peeters LL, Coumans AB, Dumoulin JCM. IVF culture medium affects human intrauterine growth as early as the second trimester of pregnancy. *Hum Reprod* 2013;**28**:2067–2074.

Olivennes F, Kerbrat V, Rufat P, Blanchet V, Fanchin R, Frydman R. Follow-up of a cohort of 422 children aged 6 to 13 years conceived by *in vitro* fertilization. *Fertil Steril* 1997; **67**:284–289.

O'Neill C. The potential roles for embryotrophic ligands in preimplantation embryo development. *Hum Reprod Update* 2008;**14**:275–288.

O'Neill C, Ryan JP, Collier M, Saunders DM, Ammit AJ, Pike IL. Supplementation of *in-vitro* fertilisation culture medium with platelet activating factor. *Lancet (London, England)* 1989;**2**:769–772.

Orasanu B, Jackson K, Hornstein M, Racowsky C. Effects of culture medium on HCG concentrations and their value in predicting successful IVF outcome. *Reprod Biomed Online* 2006;**12**:590–598.

Papaioannou VE, Ebert KM. Mouse half embryos: viability and allocation of cells in the blastocyst. *Dev Dyn* 1995;**203**:393–398.

Parikh F, Nadkarni S, Naik N, Naik D, Uttamchandani S. Cumulus coculture and cumulus-aided embryo transfer increases pregnancy rates in patients undergoing *in vitro* fertilization. *Fertil Steril* 2006;**86**:839–847.

Parinaud J, Monrozies X, Reme JM, Sarramon MF, Grandjean H, Pontonnier G. Randomized trial of two media used for *in vitro* fertilization. *Eur J Obstet Gynecol Reprod Biol* 1987;**25**:203–208.

Paternot G, Debrock S, D'Hooghe T, Spiessens C. Early embryo development in a sequential versus single medium: a randomized study. *Reprod Biol Endocrinol* 2010; **8**:83–83.

Perricone R, De Carolis C, Giacomelli R, Guarino M, De Sanctis G, Fontana L. GM-CSF and pregnancy: evidence of significantly reduced blood concentrations in unexplained recurrent abortion efficiently reverted by intravenous immunoglobulin treatment. *Am J Reprod Immunol (1989)* 2003;**50**:232–237.

Ponjaert Kristoffersen I, Bonduelle M, Barnes J, Nekkebroeck J, Loft A, Wennerholm UB, Tarlatzis BC, Peters C, Hagberg BS, Berner A *et al.* International collaborative study of intracytoplasmic sperm injection-conceived, *in vitro* fertilization-conceived, and naturally conceived 5-year-old child outcomes: cognitive and motor assessments. *Pediatrics* 2005;**115**:e283–e289.

Pool T. Development of culture media for human assisted reproductive technology. *Fertil Steril* 2004;**81**:287–289.

Punt-van der Zalm JPEM, Hendriks JCM, Westphal JR, Kremer JAM, Teerenstra S, Wetzels AMM. Toxicity testing of human assisted reproduction devices using the mouse embryo assay. *Reprod Biomed Online* 2009;**18**:529–535.

Quinn P. Enhanced results in mouse and human embryo culture using a modified human tubal fluid medium lacking glucose and phosphate. *J Assist Reprod Genet* 1995; **12**:97–105.

Quinn P. The development and impact of culture media for assisted reproductive technologies. *Fertil Steril* 2004;**81**:27–29.

Quinn P. Culture systems: sequential. *Methods Mol Biol* 2012;**912**:211–230.

Quinn P, Horstman FC. Is the mouse a good model for the human with respect to the development of the preimplantation embryo *in vitro*? *Hum Reprod* 1998;**13**:173–183.

Quinn P, Kerin JF, Warnes GM. Improved pregnancy rate in human *in vitro* fertilization with the use of a medium based on the composition of human tubal fluid. *Fertil Steril* 1985;**44**:493–498.

Quinn P, Moinipanah R, Steinberg JM, Weathersbee PS. Successful human *in vitro* fertilization using a modified human tubal fluid medium lacking glucose and phosphate ions. *Fertil Steril* 1995;**63**:922–924.

Rathmell J, Newgard C. Biochemistry. A glucose-to-gene link. *Science* 2009; **324**:1021–1022.

Reed M, Hamic A, Thompson D, Caperton C. Continuous uninterrupted single medium culture without medium renewal versus sequential media culture: a sibling embryo study. *Fertil Steril* 2009;**92**:1783–1786.

Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* 2007;**447**:425–432.

Reik W, Römer I, Barton SC, Surani MA, Howlett SK, Klose J. Adult phenotype in the mouse can be affected by epigenetic events in the early embryo. *Development* 1993; **119**:933–942.

Richter K. The importance of growth factors for preimplantation embryo development and *in-vitro* culture. *Curr Opin Obstet Gynecol* 2008;**20**:292–304.

Rinaudo P, Schultz R. Effects of embryo culture on global pattern of gene expression in preimplantation mouse embryos. *Reproduction* 2004;**128**:301–311.

Ringer S. Concerning the Influence exerted by each of the Constituents of the Blood on the Contraction of the Ventricle. *J Physiol* 1882;**3**:380–393.

Robertson S. GM-CSF regulation of embryo development and pregnancy. *Cytokine Growth Factor Rev* 2007;**18**:287–298.

Robertson SA, Seamark RF. Granulocyte-macrophage colony stimulating factor (GM-CSF): one of a family of epithelial cell-derived cytokines in the preimplantation uterus. *Reprod Fertil Dev* 1992;**4**:435–448.

Robertson SA, Roberts CT, Farr KL, Dunn AR, Seamark RF. Fertility impairment in granulocyte-macrophage colony-stimulating factor-deficient mice. *Biol Reprod* 1999;**60**:251–261.

Rock J, Menkin MF. *In vitro* fertilization and cleavage of human ovarian eggs. *Science* 1944; **100**:105–107.

Rougier N, Bourc'his D, Gomes DM, Niveleau A, Plachot M, Pàldi A, Viegas Péquignot E. Chromosome methylation patterns during mammalian preimplantation development. *Genes Dev* 1998;**12**:2108–2113.

Rufat P, Olivennes F, de Mouzon J, Dehan M, Frydman R. Task force report on the outcome of pregnancies and children conceived by *in vitro* fertilization (France: 1987 to 1989). *Fertil Steril* 1994;**61**:324–330.

Sabhnani TV, Elaimi A, Sultan H, Alduraihem A, Serhal P, Harper JC. Increased incidence of mosaicism detected by FISH in murine blastocyst cultured *in vitro*. *Reprod Biomed Online* 2011;**22**:621–631.

Sakkas D, Jaquenoud N, Leppens G, Campana A. Comparison of results after *in vitro* fertilized human embryos are cultured in routine medium and in coculture on Vero cells: a randomized study. *Fertil Steril* 1994;**61**:521–525.

Salamonsen LA, Shuster S, Stern R. Distribution of hyaluronan in human endometrium across the menstrual cycle. Implications for implantation and menstruation. *Cell Tissue Res* 2001;**306**:335–340.

Santos Ft, Dean W. Epigenetic reprogramming during early development in mammals. *Reproduction* 2004;**127**:643–651.

Santos Ft, Hendrich B, Reik W, Dean W. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev Biol* 2002;**241**:172–182.

Schendelaar P, Middelburg KJ, Bos AF, Heineman MJ, Jongbloed Pereboom M, Hadders Algra M. The Groningen ART cohort study: the effects of ovarian hyperstimulation and the IVF laboratory procedures on neurological condition at 2 years. *Hum Reprod* 2011;**26**:703–712.

Schini SA, Bavister BD. Two-cell block to development of cultured hamster embryos is caused by phosphate and glucose. *Biol Reprod* 1988;**39**:1183–1192.

Schwarzer C, Esteves T, Araúzo-Bravo M, Le Gac Sv, Nordhoff V, Schlatt S, Boiani M. ART culture conditions change the probability of mouse embryo gestation through defined cellular and molecular responses. *Hum Reprod* 2012;**27**:2627–2640.

Scott LF, Sundaram SG, Smith S. The relevance and use of mouse embryo bioassays for quality control in an assisted reproductive technology program. *Fertil Steril* 1993; **60**:559–568.

Sepúlveda S, Garcia J, Arriaga E, Diaz J, Noriega Portella L, Noriega Hoces L. *In vitro* development and pregnancy outcomes for human embryos cultured in either a single medium or in a sequential media system. *Fertil Steril* 2009; **91**:1765–1770.

Sfontouris IA, Lainas GT, Anagnostara K, Kolibianakis EM, Lainas TG. Effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on pregnancy rates in patients with multiple unsuccessful IVF attempts. *Hum Reprod* 2013;**28**:(Suppl 1):i60–i62.

Shapiro BS, Richter KS, Daneshmand ST, Quinn P, Behr B. Granulocyte-macrophage colony-stimulating factor enhances human embryo development to the blastocyst stage: a randomized study. *Fertil Steril* 2003;**79**:(Suppl 2):15–16.

Shi W, Haaf T. Aberrant methylation patterns at the two-cell stage as an indicator of early developmental failure. *Mol Reprod Dev* 2002;**63**:329–334.

Shi L, Wu J. Epigenetic regulation in mammalian preimplantation embryo development. *Reprod Biol Endocrinol* 2009;**7**:59–59.

Silverman IH, Cook CL, Sanfilippo JS, Yussman MA, Schultz GS, Hilton FH. Ham's F-10 constituted with tap water supports mouse conceptus development *in vitro*. *J In Vitro Fert Embryo Transf* 1987;**4**:185–187.

Sinclair KD, McEvoy TG, Maxfield EK, Maltin CA, Young LE, Wilmut I, Broadbent PJ, Robinson JJ. Aberrant fetal growth and development after *in vitro* culture of sheep zygotes. *J Reprod Fertil* 1999;**116**:177–186.

Sinclair KD, Young LE, Wilmut I, McEvoy TG. *In-utero* overgrowth in ruminants following embryo culture: lessons from mice and a warning to men. *Hum Reprod* 2000;**15**(Suppl 5):68–86.

Siristatidis C, Vogiatzi P, Salamalekis G, Creatsa M, Vrachnis N, Glujovsky Dn, Iliodromiti Z, Chrelias C. Granulocyte macrophage colony stimulating factor supplementation in culture media for subfertile women undergoing assisted reproduction technologies: a systematic review. *Int J Endocrinol* 2013; **2013**:704967. First published February 21, 2013, doi:10.1155/2013/704967.

Sjöblom C, Wikland M, Robertson SA. Granulocyte–macrophage colony-stimulating factor promotes human blastocyst development *in vitro*. *Hum Reprod* 1999; **14**:3069–3076.

Sjöblom C, Wikland M, Robertson S. Granulocyte-macrophage colony-stimulating factor (GM-CSF) acts independently of the beta common subunit of the GM-CSF receptor to prevent inner cell mass apoptosis in human embryos. *Biol Reprod* 2002;**67**:1817–1823.

Sjöblom C, Roberts C, Wikland M, Robertson S. Granulocyte-macrophage colony-stimulating factor alleviates adverse consequences of embryo culture on fetal growth trajectory and placental morphogenesis. *Endocrinology* 2005; **146**:2142–2153.

Smith Z, Chan M, Mikkelsen T, Gu H, Gnirke A, Regev A, Meissner A. A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* 2012;**484**:339–344.

Sommer AP, Haddad MK, Fecht H-J. It is time for a change: Petri dishes weaken cells. *J Bionic Eng* 2012;**9**:353–357.

Spanos S, Becker DL, Winston RM, Hardy K. Anti-apoptotic action of insulin-like growth factor-I during human preimplantation embryo development. *Biol Reprod* 2000;**63**:1413–1420.

Spendley W, Hext GR, Himsworth FR. Sequential application of simplex designs in optimisation and evolutionary operation. *Technometrics* 1962;**4**:441–461.

Staessen C, Janssenswillen C, De Clerck E, Van Steirteghem A. Controlled comparison of commercial media for human *in-vitro* fertilization: Ménézo B2 medium versus Medi-Cult universal and BM1 medium. *Hum Reprod* 1998;**13**:2548–2554.

Steegers-Theunissen RPM, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. *Hum Reprod Update* 2013;**19**:640–655.

Steptoe PC, Edwards RG. Laparoscopic recovery of preovulatory human oocytes after priming of ovaries with gonadotrophins. *Lancet (London, England)* 1970;**1**:683–689.

Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet (London, England)* 1978;**2**:366–366.

Steptoe PC, Edwards RG, Purdy JM. Human blastocysts grown in culture. *Nature* 1971; **229**:132–133.

Summers M, Biggers J. Chemically defined media and the culture of mammalian preimplantation embryos: historical perspective and current issues. *Hum Reprod Update* 2003;**9**:557–582.

Summers MC, McGinnis LK, Lawitts JA, Biggers JD. Mouse embryo development following IVF in media containing either L-glutamine or glycyl-L-glutamine. *Hum Reprod* 2005;**20**:1364–1371.

Summers M, Bird S, Mirzai F, Thornhill A, Biggers J. Human preimplantation embryo development *in vitro*: a morphological assessment of sibling zygotes cultured in a single medium or in sequential media. *Hum Fertil* 2013;**16**:278–285.

Surani MA. Imprinting and the initiation of gene silencing in the germ line. *Cell* 1998; **93**:309–312.

Swain J. Optimizing the culture environment in the IVF laboratory: impact of pH and buffer capacity on gamete and embryo quality. *Reprod Biomed Online* 2010; **21**:6–16.

Swain JE. Is there an optimal pH for culture media used in clinical IVF? *Hum Reprod Update* 2012;**18**:333–339.

Swain JE, Smith GD. Advances in embryo culture platforms: novel approaches to improve preimplantation embryo development through modifications of the microenvironment. *Hum Reprod Update* 2011;**17**:541–557.

Swain JE, Lai D, Takayama S, Smith GD. Thinking big by thinking small: application of microfluidic technology to improve ART. *Lab Chip* 2013;**13**:1213–1224.

Tarlatzis B, Qublan H, Sanopoulou T, Zepiridis L, Grimbizis G, Bontis J. Increase in the monozygotic twinning rate after intracytoplasmic sperm injection and blastocyst stage embryo transfer. *Fertil Steril* 2002;**77**:196–198.

Tervit HR, Whittingham DG, Rowson LE. Successful culture *in vitro* of sheep and cattle ova. *J Reprod Fertil* 1972;**30**:493–497.

Thompson JG, Gardner DK, Pugh PA, McMillan WH, Tervit HR. Lamb birth weight is affected by culture system utilized during *in vitro* pre-elongation development of ovine embryos. *Biol Reprod* 1995;**53**:1385–1391.

Thompson J, Kind K, Roberts C, Robertson S, Robinson J. Epigenetic risks related to assisted reproductive technologies: short- and long-term consequences for the health of children conceived through assisted reproduction technology: more reason for caution? *Hum Reprod* 2002;**17**:2783–2786.

Thompson JGB, Mitchell M, Kind K. Embryo culture and long-term consequences. *Reprod Fertil Dev* 2007;**19**:43–52.

Tielens S, Verhasselt B, Liu J, Dhont M, Van Der Elst J, Cornelissen M. Generation of embryonic stem cell lines from mouse blastocysts developed *in vivo* and *in vitro*: relation to Oct-4 expression. *Reproduction* 2006;**132**:59–66.

Tucker MJ, Kort HI, Toledo AA, Morton PC, Wright G, Ingargiola PE, Sweitzer CL. Effect of coculture on subsequent survival and implantation of cryopreserved human embryos. *J Assist Reprod Genet* 1995;**12**:689–692.

Tyrode MV. The mode of action of some purgative salts. *Arch Int Pharmacodyn* 1910; **20**:205–223.

Urman B, Yakin K, Ata B, Isiklar A, Balaban B. Effect of hyaluronan-enriched transfer medium on implantation and pregnancy rates after day 3 and day 5 embryo transfers: a prospective randomized study. *Fertil Steril* 2008;**90**:604–612.

Utsunomiya T, Naitou T, Nagaki M. A prospective trial of blastocyst culture and transfer. *Hum Reprod* 2002;**17**:1846–1851.

Vajta Gb, Rienzi L, Cobo A, Yovich J. Embryo culture: can we perform better than nature? *Reprod Biomed Online* 2010;**20**:453–469.

van den Bergh M, Baszó I, Biramane J, Bertrand E, Devreker F, Englert Y. Quality control in IVF with mouse bioassays: a four years' experience. *J Assist Reprod Genet* 1996; **13**:733–738.

van Montfoort APA, Hanssen LLP, de Sutter P, Viville S, Geraedts JPM, de Boer P. Assisted reproduction treatment and epigenetic inheritance. *Hum Reprod Update* 2012;**18**:171–197.

Van Winkle LJ. Amino acid transport in developing animal oocytes and early conceptuses. *Biochim Biophys Acta* 1988;**947**:173–208.

Van Winkle LJ, Haghighat N, Campione AL. Glycine protects preimplantation mouse conceptuses from a detrimental effect on development of the inorganic ions in oviductal fluid. *J Exp Zool* 1990;**253**:215–219.

Vergouw C, Kostelijk EH, Doejaaren E, Hompes PGA, Lambalk C, Schats R. The influence of the type of embryo culture medium on neonatal birthweight after single embryo transfer in IVF. *Hum Reprod* 2012;**27**:2619–2626.

Virant Klun I, Tomazevic T, Vrtacnik Bokal E, Vogler A, Krsnik M, Meden Vrtovec H. Increased ammonium in culture medium reduces the development of human embryos to the blastocyst stage. *Fertil Steril* 2006;**85**:526–528.

Viuff D, Greve T, Avery B, Hyttel P, Brockhoff PB, Thomsen PD. Chromosome aberrations in *in vitro*-produced bovine embryos at days 2–5 post-insemination. *Biol Reprod* 2000;**63**:1143–1148.

Wagenaar K, van Weissenbruch MM, Knol DL, Cohen Kettenis PT, Delemarre-van de Waal HA, Huisman J. Information processing, attention and visual-motor function of adolescents born after *in vitro* fertilization compared with spontaneous conception. *Hum Reprod* 2009;**24**:913–921.

Wagenaar K, van Weissenbruch M, van Leeuwen F, Cohen Kettenis P, Delemarre-van de Waal HA, Schats R, Huisman J. Self-reported behavioral and socioemotional functioning of 11- to 18-year-old adolescents conceived by *in vitro* fertilization. *Fertil Steril* 2011;**95**:611–616.

Waterland R, Jirtle R. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 2003;**23**:5293–5300.

Watkins A, Platt D, Papenbrock T, Wilkins A, Eckert J, Kwong W, Osmond C, Hanson M, Fleming T. Mouse embryo culture induces changes in postnatal phenotype including raised systolic blood pressure. *Proc Natl Acad Sci USA* 2007; **104**:5449–5454.

Wegmann TG, Athanassakis I, Guilbert L, Branch D, Dy M, Menu E, Chaouat G. The role of M-CSF and GM-CSF in fostering placental growth, fetal growth, and fetal survival. *Transplant Proc* 1989;**21**:566–568.

Whitten WK. Culture of tubal mouse ova. *Nature* 1956;**177**:96–96.

Whitten WK, Biggers JD. Complete development *in vitro* of the pre-implantation stages of the mouse in a simple chemically defined medium. *J Reprod Fertil* 1968; **17**:399–401.

Whittingham DG. Fertilization of mouse eggs *in vitro*. *Nature* 1968;**220**:592–593.

Whittingham DG. Culture of mouse ova. *J Reprod Fertil Suppl* 1971;**14**:7–21.

Whittingham DG, Biggers JD. Fallopian tube and early cleavage in the mouse. *Nature* 1967;**213**:942–943.

Wiemer KE, Cohen J, Amborski GF, Wright G, Wiker S, Munyakazi L, Godke RA. *In-vitro* development and implantation of human embryos following culture on fetal bovine uterine fibroblast cells. *Hum Reprod* 1989a;**4**:595–600.

Wiemer KE, Cohen J, Wiker SR, Malter HE, Wright G, Godke RA. Coculture of human zygotes on fetal bovine uterine fibroblasts: embryonic morphology and implantation. *Fertil Steril* 1989b;**52**:503–508.

Wiemer KE, Hoffman DI, Maxson WS, Eager S, Muhlberger B, Fiore I, Cuervo M. Embryonic morphology and rate of implantation of human embryos following co-culture on bovine oviductal epithelial cells. *Hum Reprod* 1993;**8**:97–101.

Wiemer KE, Garrisi J, Steuerwald N, Alikani M, Reing AM, Ferrara TA, Noyes N, Cohen J. Beneficial aspects of co-culture with assisted hatching when applied to multiple-failure *in-vitro* fertilization patients. *Hum Reprod* 1996;**11**:2429–2433.

Wirleitner B, Vanderzwalmen P, Stecher A, Zech MH, Zintz M, Zech NH. Individual demands of human embryos on IVF culture medium: influence on blastocyst development and pregnancy outcome. *Reprod Biomed Online* 2010; **21**:776–782.

Xella S, Marsella T, Tagliasacchi D, Giulini S, La Marca A, Tirelli A, Volpe A. Embryo quality and implantation rate in two different culture media: ISM1 versus Universal IVF Medium. *Fertil Steril* 2010;**93**:1859–1863.

Young LE, Fairburn HR. Improving the safety of embryo technologies: possible role of genomic imprinting. *Theriogenology* 2000;**53**:627–648.

Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, Broadbent PJ, Robinson JJ, Wilmut I, Sinclair KD. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. *Nat Genet* 2001; **27**:153–154.

Youssef MFM, van Wely M, Al-Inany H, Van der Veen F, Repping S. Culture media for human preimplantation embryos in assisted reproductive technology cycles. *Cochrane Database Syst Rev* 2009; Issue 3. Art. No.: CD007876. doi: 10.1002/14651858.CD007876.

Yu Y, Yan J, Li M, Yan L, Zhao Y, Lian Y, Li R, Liu P, Qiao J. Effects of combined epidermal growth factor, brain-derived neurotrophic factor and insulin-like growth factor-1 on human oocyte maturation and early fertilized and cloned embryo development. *Hum Reprod* 2012;**27**:2146–2159.

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.

Zhang Y, Cui Y, Zhou Z, Sha J, Li Y, Liu J. Altered global gene expressions of human placentae subjected to assisted reproductive technology treatments. *Placenta* 2010;**31**:251–258.

Zhao Y, Chegini N. Human fallopian tube expresses granulocyte-macrophage colony stimulating factor (GM-CSF) and GM-CSF alpha and beta receptors and contain immunoreactive GM-CSF protein. *J Clin Endocrinol Metab* 1994;**79**:662–665.

Zhao Y, Rong H, Chegini N. Expression and selective cellular localization of granulocyte-macrophage colony-stimulating factor (GM-CSF) and GM-CSF alpha and beta receptor messenger ribonucleic acid and protein in human ovarian tissue. *Biol Reprod* 1995;**53**:923–930.

Zhu J, Lin S, Li M, Chen L, Lian Y, Liu P, Qiao J. Effect of *in vitro* culture period on birthweight of singleton newborns. *Hum Reprod*. 2014. Advance Access published January 8, 2014, doi:10.1093/humrep/det460.

Ziebe S, Loft A, Povlsen BB, Erb K, Agerholm I, Aasted M, Gabrielsen A, Hnida C, Zobel DP, Munding B *et al.* A randomized clinical trial to evaluate the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) in embryo culture medium for *in vitro* fertilization. *Fertil Steril* 2013;**99**:1600–1609.e1602.