Title

Culture conditions in the IVF laboratory: state of the ART and possible new directions.

Journal

Journal of Assisted Reproduction and Genetics, 40(11)

Authors

Sciorio, Romualdo
Rinaudo, Paolo

Publication Date

2023-11-01

DOI

10.1007/s10815-023-02934-5

Peer reviewed

Journal of Assisted Reproduction and Genetics (2023) 40:2591–2607
https://doi.org/10.1007/s10815-023-02934-5

ASSISTED REPRODUCTION TECHNOLOGIES

Culture conditions in the IVF laboratory: state of the ART and possible new directions

Romualdo Sciorio[1] · **Paolo Rinaudo**[2]

Received: 9 May 2023 / Accepted: 6 September 2023 / Published online: 19 September 2023

Abstract

In the last four decades, the assisted reproductive technology (ART) field has witnessed advances, resulting in improving pregnancy rates and diminishing complications, in particular reduced incidence of multiple births. These improvements are secondary to advanced knowledge on embryonic physiology and metabolism, resulting in the ability to design new and improved culture conditions. Indeed, the incubator represents only a surrogate of the oviduct and uterus, and the culture conditions are only imitating the physiological environment of the female reproductive tract. In vivo, the embryo travels through a dynamic and changing environment from the oviduct to the uterus, while in vitro, the embryo is cultured in a static fashion. Importantly, while culture media play a critical role in optimising embryo development, a large host of additional factors are equally important. Additional potential variables, including but not limited to pH, temperature, osmolality, gas concentrations and light exposure need to be carefully controlled to prevent stress and permit optimal implantation potential. This manuscript will provide an overview of how different current culture conditions may affect oocyte and embryo viability with particular focus on human literature.

Keywords Culture media · Single-step media · Sequential media · pH · Osmolality · Temperature · Oxygen tension · Oil overlay · Culture condition · Assisted reproductive technology (ART)

Introduction

The essential role of embryo culture is to create a permissive environment to maximize the health and growth potential of embryos while minimizing any probable iatrogenic stress. Since the early days of IVF in the 1970s, several modifications to the culture system have been introduced to enhance the number of good-quality embryos available for transfer [1, 2]. As a result, currently, IVF laboratories culture embryos to the blastocyst stage with high efficiency, enabling elective single-embryo transfer (eSET), with the net result that multiple pregnancies have decreased while maintaining high pregnancy rates [3, 4]. However, despite these improvements, culture conditions are unlikely to mirror the physiologic condition encountered in vivo by an embryo. There are concerns that suboptimal culture conditions might impair embryo development and compromise its viability and implantation potential. In fact, in vitro culture requires several steps that could increase embryonic stress, including not only the use of different culture media, but also the use of plastic dishes and consumables that are in contact with gametes and embryos, different oxygen concentrations, temperature, pH and osmolality. All these factors may play a critical role in embryo development. Therefore, constant improvement of culture techniques to minimize embryonic stress is a necessary on-going venture. The embryology laboratory needs to assure and provide optimal culture conditions to support the growth of healthy embryos with a high potential to implant.

Search methods

In this manuscript, we aim to provide a commentary and comprehensive review of the current literature relating to the application of human culture system in ART, and whether

✉ Romualdo Sciorio
sciorioromualdo@hotmail.com

[1] Fertility Medicine and Gynaecological Endocrinology Unit, Department Woman-Mother-Child, CHUV-Lausanne University Hospital, 1011 Lausanne, Switzerland

[2] Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, CA, USA

un-physiologic conditions and culture stress could have an impact on embryo development and viability and affect the chance to obtain a healthy pregnancy to term. Relevant studies were identified in the English-language literature using PubMed search terms related to the focus of the review, including features that are known to affect embryo evolution, including pH, osmolality, temperature, oxygen tension, and oil overlay. In addition, it also briefly analysed the culture media composition, single-step and sequential media; the use of amino acids and macromolecules and finally, some novel microscopy applications that might become critical and can be used in the future to select or deselect human gametes and embryos in ART program. All relevant publications until January 2023 were critically evaluated and discussed.

A primer of early preimplantation embryo development

In vivo, between days 1 and 3 of the embryo development, the embryo travels into the oviduct, while at days 4 and 5 (compaction and blastocyst formation), the embryo reaches the uterine cavity. In the early days of ART, it was common to use simple culture media and subsequent transfer of cleavage stage embryos on days 2 or 3, when embryos are only four to eight cells [1]. The mammalian embryo prior to embryonic genome activation (EGA) is transcriptionally silent and uses mainly maternal mRNA for its needs [5]. EGA in human embryos occurs after 3 cleavage divisions, between the 4- and 8-cell stages (Fig. 1). Braude and his colleagues in 1988 established the time of EGA in human embryos and observed that distinct aspects of protein synthesis correlated to transcriptional activation [6]. At these early stages, embryos display a predilection for pyruvate/lactate as energy sources, while after EGA, they switch to glucose-based metabolism [2, 7, 8]. Following EGA, the embryo undergoes compaction, the blastomeres adhere with neighbouring cells to form a cluster called the 'morula'. This is the first change in radial symmetry. Next, the morula will create a cavity (the blastocoel) secondary to the secretion of fluid that raises the salt concentration within the embryo, attracting water by osmosis [9]. The continuous expansion of the blastocyst will enlarge the cavity. This process, together with the secretion of lysine by trophectodermal (TE) cells, will result in the zona pellucida (ZP) thinning followed by the hatching process [1]. The blastocyst is at this stage composed of the inner cell mass (ICM) and surrounding TE cells. The ICM further develops into early epiblast, containing pluripotent cells that are able to give rise to all the tissues of the foetus, while the TE is responsible to form the placenta. In humans, implantation occurs at approximately day 7 of development. Failure of implantation, which might be secondary to poor embryo development and/or poor uterine receptivity [10] is a main roadblock to hamper ART success. Potential factors that might be involved in embryo development and viability are linked to media composition. The introduction of new generation media has represented a major breakthrough in ART and has allowed scientists to grow human embryos easily beyond the cleavage stage [1, 11]. In

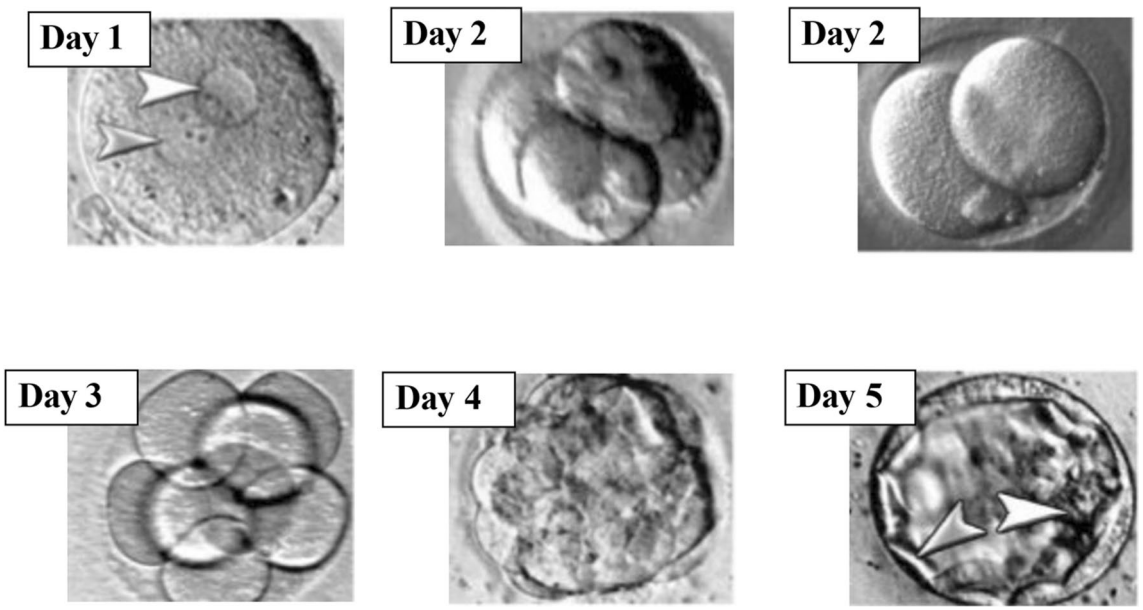

Fig. 1 Early stages of human embryo development. Images of human embryo development from day 1 to day 5. Following fertilization, embryos undergo a series of mitotic cell divisions. Arrowheads in day 1 indicate pronuclei formation. On day 4, the embryo compacts, resulting in the formation of the 'morula' a groups of blastomeres. On day 5, the embryo become a 'blastocyst', a fluid-filled structure formed of the inner cell mass (white arrowhead) and trophectoderm (grey arrowhead)

the late 1990s with the consistency achieved from these commercially produced media, it became more common to culture embryos to the blastocyst stage, resulting in an improvement in pregnancy rate and reduction of multiple pregnancies with the transfer of single blastocyst [1, 2, 12–17].

The historical landscape of embryo culture media: sequential and single-step media

The early attempt of IVF and culture of human eggs were performed in 1940s by Rock and Menkin [18]. They collected eggs at the approximate time of ovulation in patients undergoing laparotomy for different medical conditions. They adapted animal protocols to fertilize human oocytes, but their attempts were not successful. Next, Landrum Shuttle at Columbia University claimed to have fertilized human eggs from one patient in 1973, but the resulting embryos were discarded in an infamous case that had large media attention [19]. The first real breakthrough of in vitro fertilization was reported by Edwards and Steptoe [20] with the birth of Louise Brown. In the early days of IVF, embryologists prepared culture media 'in-house', occasionally introducing variables that could increase or decrease effectiveness, such as adding patients' serum to the media. These media were based on simple culture media supplemented with bovine serum albumin (BSA), penicillin and the addition of sodium pyruvate, phenol red and bicarbonate [21, 22]. Earle's simple salt solution medium with pyruvate supplementation and patient's serum was used for the IVF that resulted in the birth of Lousie Brown [20]. Only in the early 1980s, commercial media became available. Their formulation was mainly based on the modification of Earle's balanced salt solution with the addition of maternal serum [23, 24]. Progress occurred when Menezo and colleagues proposed the supplementation of amino acids to the balanced salt solution media [25]. A different media specifically formulated to culture human embryos was the human tubal fluid (HTF) designed by Patrick Quinn [11]. Improved knowledge of embryo metabolism with the transition from pyruvate/lactate during the cleavage stage, to glucose-based metabolism following EGA [2–4] and a better understanding of the physiological changes occurring in the oviduct and uterus [8], resulted in the development of a novel culture media called "sequential" media [12]. These media are based on the "back-to-nature" concept, which aims to imitate the concentration of relevant molecules and energy substrates found in the female reproductive system [2]. Several sequential systems were proposed, such as Quinn's series in the USA, the MediCult/Origio in Europe, and Cook in Australia. This type of media requires a culture media change on day 3 of development, when the embryo changes its metabolism (Fig. 2). On the contrary, Lawitts and Biggers in the early 1990s designed a new media, based on the principle of 'simplex optimization'. The approach, defined as 'let-the-embryo-choose', was based on testing different possible concentrations of a set of chemical compounds, and then culture mouse embryos in each possible combination and choose the particular 'mixture' that resulted in the highest blastocyst rate [26]. The result was the formulation of a simplex optimization medium (SOM), which for the first time allowed one single medium to be used to culture human embryos from fertilization to the blastocyst stage [8, 27]. The reputed advantage of this type of media is that there is no need to replace culture media on day 3, therefore limiting an extra step that could be stressful to embryos [26–28].

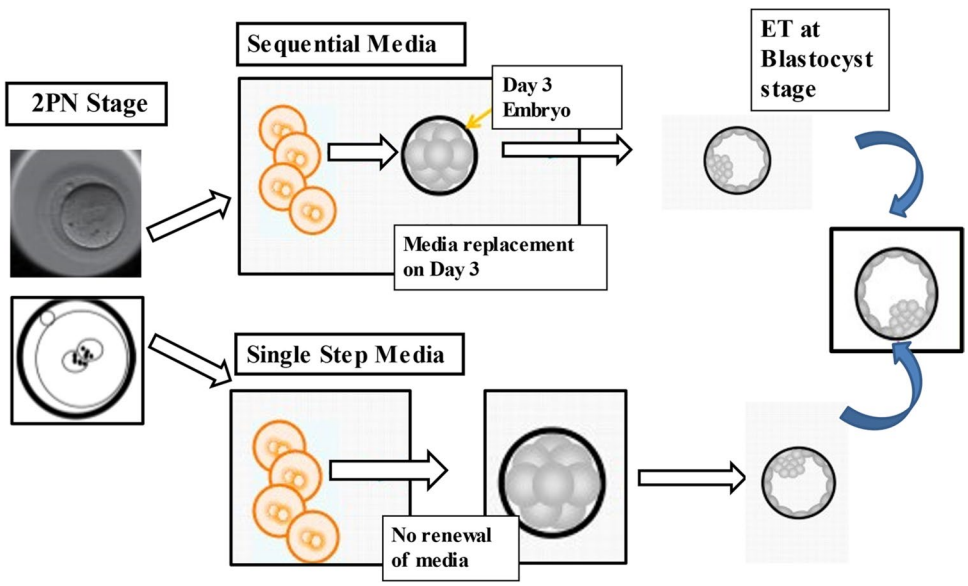

Fig. 2 Depicts the comparison between the use of sequential media and single step approach typically used to culture human embryos

Culture environment and potential effect on embryo development

Multiple factors may play a role in embryo development. Here, we will analyse how each particular condition may affect human embryo development.

Different culture media

Different culture media are currently available. In addition, embryos can be cultured under different culture conditions, with different protein sources and macromolecules. All these parameters can affect embryo development. A large set of culture media are commercially available for purchase. The exact concentration of the media components for either single-step or sequential media is not disclosed by the manufacturers [29], since media cannot be patented, and the company maintains the composition as a trade secret. However, all culture media contain pyruvate, lactate and glucose as carbohydrate sources, at different concentrations (Table 1). Glucose is an essential molecule needed not only for glycolysis but also as a precursor for the synthesis of numerous biomolecules such as lipids, phospholipids, complex carbohydrates, glycoproteins, and nucleic acids [30]. One critical question is to understand if any of these culture media is superior to the others, in terms of embryo development, implantation or live birth rate. The answer is not straightforward [31–34] since many comparative studies were underpowered, designed with an inappropriate experimental design or failed to control for all possible variables. For example, some studies included blastocyst rate independently if it occurred on days 5, 6 or 7 [35]. A review of randomized control trials conducted between January 1985 and July 2012 was published by Mantikou. The primary outcome studied included live birth rate, new-born health, clinical pregnancy rate, miscarriages, multiple pregnancies, implantation rate, cryopreservation rate, embryo quality and fertilization rate [32]. The authors concluded that a conventional meta-analysis comparing culture media was not feasible because of suboptimal study designs. The live birth rate was only disclosed in four studies, with one study reporting a significant difference between different culture media [36]. Nine trials reported the on-going and/or clinical pregnancy rates, and four studies found a significant difference [37]. Collectively, this analysis did not show the superiority of any culture media. A follow-up Cochrane review analysis [38] found similar results. In conclusion, while it appears that there is not a clearly superior culture media, additional and better designed studies are needed to answer this question.

Table 1 The main components of human culture media

Components of embryo culture media	
Salts	Sodium chloride
	Potassium chloride
	Calcium chloride
	Potassium phosphate
	Magnesium sulphate
Buffer	Sodium bicarbonate
Energy substrates	Glucose
	Sodium lactate
	Sodium pyruvate
Amino acids	Non-essential amino acids
	Essential amino acids
Chelator	EDTA
Antibiotic	Gentamicin
pH indicator	Phenol Red

Addition of amino acids (AAs)

Culture media have other important functions beyond providing developing embryos with energy. One aspect that warrants special attention is the composition of AAs. It has been proposed that the existing twenty AAs are regulators of several processes that occur during mammalian embryo development [39]. Specific AAs are known to support many cellular processes by acting as metabolites, antioxidants, osmolality regulators, internal pH buffers and chelators for heavy metals. For example, glutamine and non-essential AAs trigger the growth of the early cleavage embryo. Both essential and non-essential AAs favour the growth of the inner cell mass, while non-essential AAs promote stimulation of the trophectoderm and hatching from the zona pellucida [40]. Mouse studies have demonstrated that limiting AAs even briefly can impair embryo development [39, 41]. Therefore, culture media should comprise always AAs, and different combinations have been investigated by several authors [39–42]. There is also evidence indicating that a shortage of certain AAs, such as methionine, is associated with monozygotic twinning in humans [42]. Menezo summarized how methionine is involved in the glutathione, hypotaurine and taurine pathways and might play an important role in imprinting and DNA methylation; these processes are of paramount importance for chromosomal stability [43]. A recent study by Clare and collaborators investigated the effect of reduced methionine (from 50 to 10 μM) in bovine embryos and demonstrate that low levels of methionine might lead to alterations in DNA methylation in > 1600 genes, including a group of imprinted genes associated with an abnormal foetal-overgrowth phenotype [44]. An important concern during the in vitro culture is that the AA supplementation might raise the production of ammonium. When culture media is incubated at 37°C, AAs spontaneously undergo breakdown and produce ammonium. Unfortunately, ammonium is toxic to embryos, reduces implantation

and might negatively affect foetal development. The impact of ammonium is intensified when culture is performed at 20% oxygen tension [41]. This potential concern may be resolved by performing a media change in a sequential system. The amino acid glutamine is considered the greatest contributor to the ammonium production in vitro, as one of the most unstable amino acids. However, a newer strategy is to include a more stable dipeptide form alanyl-glutamine or glycyl-l-glutamine to significantly reduce the accumulation of ammonium in modern embryo culture media and avoid building a toxic embryo environment [28, 39, 41, 45, 46].

Addition of protein source and macromolecules

Common sources for macromolecules in culture media are the addition of human serum albumin (HSA), as well as complex protein supplements containing HSA and a combination of alpha and beta globulins (at a concentration between 5% and 20%) [47, 48]. It has been shown that macromolecule supplementation is beneficial, improves embryo development and positively affects the live birth rate [49]. Albumin is largely present in the oviduct [50]. The addition of albumin results in several advantages: it minimizes gametes and embryos sticking to consumables and stabilizes membranes. It provides a nitrogen source, pH buffer and chelator to bind trace elements and toxins [51, 52]. It also helps to stabilize the cytoskeleton of cells after cryopreservation during warming. Unfortunately, protein supplements might contain unwanted preservatives, stabilizers, growth factors and hormones; an example is octanoic acid, which contains high levels of pro-oxidant metals, which may be toxic to embryos [53]. A molecule highly investigated is hyaluronic acid, which is an adherence compound, and it is often added to the culture media. Hyaluronic acid is found in the female reproductive system, specifically in the endometrium at concentrations that vary during the menstrual cycle [54]. Furthermore, hyaluronan receptors have been identified on the embryo surface [55, 56]. A prospective randomized controlled study by Urman and colleagues [57] investigated the effect of the use of a hyaluronan-based transfer medium on implantation rate (IR) and clinical pregnancy rate (CPR) in a cohort of 1282 fresh embryo transfer cycles. The authors found an increase in both IR and CPR with the use of hyaluronan; in particular, hyaluronan was beneficial, especially for women who were > 35 years of age and those who had poor-quality embryos. A recent Cochrane review that includes seventeen studies and a total of 3898 participants concluded that the addition of hyaluronic acid to embryo transfer medium resulted in an increase in LBR. However, only six trials reported LBR, and the quality of the evidence was only moderate. Thus, large and randomized studies are needed [58]. Additional molecules, such as growth factors, have been suggested to be beneficial to embryo development. Some studies on the use of media containing granulocyte macrophage colony-stimulating factor (GM-CSF), in human and animal models, have found contradictory results [59–65]. Adanacıoglu and colleagues [60] in a retrospective trial including 131 unexplained infertility patients undergoing fresh embryo transfer compared the use of media containing or not GM-CSF in terms of embryo development, pregnancy outcomes and live birth rate. Results showed no statistical differences between the groups although a higher pregnancy of 39.13% and on-going pregnancy rates of 36.23% were seen in patients whose embryos were cultured in GM-CSF (compared to the control 30.65% and 29.03%, respectively). A Cochrane review published in 2020 reported that due to the very low quality of the evidence, the authors cannot be certain whether GM-CSF is any more or less effective than culture media not supplemented with GM-CSF for clinical outcomes in ART treatments. The claims from marketing information that GM-CSF has a positive effect on pregnancy rates are not supported by the scientific available evidence [61]. In contrast, in a trial performed on 430 women undergoing to frozen-warmed blastocyst transfer, Okabe-Kinoshita and co-authors [62] reported a beneficial effect when embryos were cultured in a GM-CSF containing medium. The authors found the percentage of clinical pregnancies, on-going pregnancies and live birth rates was significantly higher in the GM-CSF group (52.6%, 42.9% and 40.9%, respectively), as compared with the control group 41.8%, 31.1%, and 30.5%, respectively. The incidence of pregnancy loss (22.3% vs. 27.0%) did not significantly differ between the groups. The authors concluded that the addition of GM-CSF in culture media following blastocyst warming could increase the pregnancy rate. Collectively, additional well-designed, properly powered randomized controlled trials are urgently needed to provide certainty to the evidence.

Oxygen concentrations and ROS

The role of oxygen in embryonic metabolism and development is critical, as a delicate balance exists between therapeutic benefit and iatrogenic harm. Oxygen is consumed during mitochondrial oxidative phosphorylation, while reactive oxygen species (ROS) may be generated by the leakage of high-energy electrons as they pass down the electron transport chain. ROS may impair cell metabolism, genomic integrity and embryo viability. Culturing embryos in atmospheric oxygen (20%) might result in an increase in ROS production. Extensive research in mammals has determined that the oxygen concentration in the female reproductive tract varies between 2 and 8% [66–68]. Thus in vivo, embryos are never exposed to atmospheric oxygen concentration (i.e. 20% oxygen), and current best practices for culture systems recommend incubating embryos at 5% oxygen. Published evidence

from animal models, including mice, rats, cats, sheep and even pigs has observed the benefit of using reduced oxygen concentrations for embryo culture. Human studies have demonstrated that culturing embryos at low oxygen tension might be associated with improved embryo development, pregnancy and live birth rates [68–71]. Further reports have illustrated the overall adverse effect of atmospheric oxygen on the embryo, with alteration to the transcriptome, proteome, gene expression and changes in both carbohydrate and amino acid metabolism [72, 73]. A prospective randomized controlled trial by Meintjes and co-authors [71] demonstrated that culture human embryos at low oxygen concentration significantly improved pregnancy, implantation and live birth rate. These findings have been confirmed by other investigators (Table 2) [68–71]. Although the exact mechanism of the benefits of low oxygen has not been fully elucidated, it has hypothesized to be related to a reduction of ROS, improved air quality by reduced volatile organic compounds with the net results of an improvement of embryo gene expression, epigenetic profile or other cellular processes [73, 76]. ROS can induce serious damage to cell organelles, including DNA fragmentation and impairment of proteins functions and signalling [77, 78]. Damage to lipids secondary to high oxygen concentration has been reported in several species [79, 80]. Mitochondria are also affected by oxidative stress [81]. A recent study using mouse embryos found that IVF-generated embryos, more significantly if cultured under 20% O_2, had a reduced number of total mitochondria and an increase in abnormal mitochondria (containing vacuoles) compared to embryos generated after spontaneous mating [73, 82]. Studies have suggested that oxidative stress can also modify embryonic epigenomes. Li and collaborators examined the effects of high oxygen tension (20%) on global DNA methylation using immunofluorescence in bovine and found that high oxygen tension significantly increased global DNA methylation in 4-cell embryos and blastocysts [78]. Gaspar and collaborators found that high oxygen tension had an important effect on the histone marks on H3K9me2 and H3K4me2 in bovine blastocysts [83]. Culture of mouse embryos at high oxygen can change the embryo proteome. For example, the culture of embryo in 20% O_2 was associated with the downregulation of 10 proteins/biomarkers compared to in vivo developed mouse embryos [84]. Several authors have studied the effect of oxygen concentration on embryo gene expression [73, 85], and overall culturing of embryos with high oxygen was associated with a 10-fold increase of abnormal genes expression [86].

In summary, culturing embryos at low oxygen concentration is correlated with faster embryo development and lower perturbation in the global pattern of gene expression, and it is currently the favoured method adopted in clinical practice [84–86]. It is believed that the majority of IVF laboratories culture embryos under 5% oxygen, although as late as 2014, Christianson noted that only 25% of IVF laboratories worldwide used exclusively 5% oxygen tension [87].

The benefit of antioxidant supplementation

The female reproductive tract not only is exposed to a low oxygen tension [88] but is equipped with antioxidant systems to reduce the oxidative harm and assure protection to the embryo [89]. Accordingly, recent studies have explored the benefit of antioxidant supplementation in the culture media. Some of the antioxidants investigated include *N*-acetyl-L-cysteine (NAC), Acetyl-L-Carnitine (ALC), and α-Lipoic Acid (ALA) [90, 91]. These antioxidants appear to benefit both mouse and cow embryo development by protecting against oxidative stress through scavenging free radicals [92]. For example, Abdelrazik found that in the presence of oxidative stress, the supplementation of ALC to mouse embryo culture diminishes DNA damage and improves blastocyst development [93]. ALA is a robust free radical scavenger and metal chelator involved in recycling other cellular antioxidants including glutathione (GSH). Its addition to mouse embryos cultured in 20% oxygen resulted in improved embryo development [90, 91]. Since GSH production is dependent on the availability of cysteine, the addition of cysteine supported bovine embryo development and correlated with an increase in GSH levels in cleavage

Table 2 Some human reports showing the effect of oxygen tension on pregnancy rate, implantation and live birth rates

		Pregnancy rate (%)			(%) Implantation (IR) or live birth rate (LBR)		
Study [references]	Cycles (n)	20% O_2	5% O_2	*p* value	20% O_2	5% O_2	*p* value
Catt and Henman [69]	261	19	32	$P < 0.02$	IR: 10	IR: 14	$P < 0.02$
Kovacic and Vlaisavljević [74]	100	40	60	$P = 0.082$	IR: 38.3	IR: 50.5	$P = 0.217$
Waldenström et al. [70]	600	35.2	45.7	$P = 0.03$	LBR: 32.2	LBR: 42.1	$P < 0.05$
Meintjes et al. [71]	230	48.7	64.3	$P < 0.05$	IR: 35.6	IR: 49.4	$P < 0.05$
Kasterstein et al. [75]	258	18.4	38.2	$P = 0.025$	IR:10.3 LBR: 15.8	IR: 22.1 % LBR: 34.2	$P = 0.04$ $P = 0.047$

and blastocyst stages embryo [94, 95]. Several studies by Gardner and his group [90, 91] showed that supplementation of antioxidants in human embryo culture might induce an increase in implantation and pregnancy rate. Most importantly, a prospective randomized trial (including 1,563 metaphase II oocytes from 133 patients) found an increased on-going pregnancy rate (from 37.8% to 47.1%) when using media supplemented with antioxidants. Antioxidant use benefited particularly female patients aged 35–40 years, and in this age group, the on-going pregnancy was significantly higher in the antioxidant group (50%) compared to their absence (25.8%) ($P < 0.05$) [90].

Variables influencing culture environment

As important as the formulation of commercially produced culture media, it is equally important that different variables within the culture system are strictly controlled by the embryology laboratory. Here, some of the key environmental variables that might impact embryo development and outcomes will be investigated [96].

Temperature

It is well established that temperature can influence several features correlated with gamete and embryo physiology, including metabolism and the meiotic spindle (MS) stability [97, 98]. The MS is a cytoskeletal structure that is actively involved in the segregation of homologous chromosomes during meiosis I and sister chromatids during meiosis II to produce haploid gametes and has been considered an indicator of oocyte health [99]. Its stability is linked with fertilization and embryo division, as well as with errors in chromatin division, accountable for aneuploidies, implantation failure and miscarriage rate [97, 98]. Oocyte MS becomes unstable outside of the physiologic pH and temperature; it has been shown that MS begins to depolymerize at a temperature of 33°C [98], and depolarization continues as temperatures drop. Only 10 min of exposure to non-physiologic pH or temperature is sufficient to induce the spindle to disassemble [100]. Both animal and human studies have demonstrated a negative association between the variation of in vitro environmental pH, temperature, and osmolality on normal microtubule disassembly and spindle alterations [98, 100–102]. It has been observed that keeping the media temperature between 35 and 37°C compared with 25 to 30°C during oocyte recovery is beneficial for bovine and mouse embryo development. Similarly, a stable temperature of 37°C during oocyte manipulation has been associated with improved fertilization rate [103]. Overall, a temperature of 37°C is widely used to culture embryos, since this temperature approximates the natural body temperature in vivo. However, human body temperature is not a constant 37°C, as this value might change due to individual conditions such as metabolism, diet, sex, time of the day and area of the body where the measurement is obtained [104]. Indeed, the female body temperature rises in the luteal phase, and variations of 1°C or 2°C have been reported along the reproductive tract. The oviduct temperature may be 1.5°C cooler, and the follicle may be 2.3°C cooler than the body [67]. Few studies have investigated if different temperatures in vitro could alter and improve embryo development. De Munck and colleagues compared a stable temperature of 37.0 ± 0.3°C to a gradual variation from 36.6 to 37.5°C throughout the day. The authors found no difference in fertilization rate, embryo quality and live birth rate [105]. A prospective, double-blind, randomized controlled trial by Fawzy divided 412 women into two groups: the control group with embryo culture at 37°C and the intervention group with culture at 36.5°C [106]. The authors found no significant difference in terms of pregnancy or implantation rates. However, embryo culture at 36.5°C was associated with a significantly higher cleavage rate, but reduced fertilization rate, fewer high-quality embryos on day 3 and a significant reduction in blastocyst formation rate on day-5 when compared with culture at 37°C. An additional study by Hong and collaborators [88] analysed whether the culture at a cooler temperature of 36°C would increase the blastocyst formation and pregnancy rates. Controlling for temperature variations, incubator type and pH, the trial demonstrated that human embryos cultured at 37°C obtained a higher rate of blastocyst formation, while fertilization, aneuploidy and implantation rates were similar. Thus, although embryos have remarkable plasticity and are able to develop and tolerate modest variations of temperature, it is recommended to perform in vitro culture in the ART programmes at the temperature of 37°C (Table 3).

pH in embryo culture

Culture media pH is of paramount importance for embryo culture. Media pH is directly correlated with the bicarbonate concentration of the media and the CO_2 concentration of the incubator. Several other factors may also influence media pH such as laboratory location (elevation compared to sea level), protein supplementation, macromolecules and media formulation. Therefore, the pH value reported in every laboratory is unique, and it must be carefully calibrated by each laboratory. Although embryos seem to tolerate a wide range of pH, variations outside of physiological ranges negatively affect embryo metabolism and development and viability. Studies conducted in the mouse model have shown that abhorrent culture pH might be correlated with impaired foetal growth and development [79]. Interestingly, it appears that embryos might change their internal pH and secreted pH according to the metabolic stress they are exposed to [107]. Compared to embryos, oocytes are more

Table 3 Effect of different culture temperatures on human embryo development. Embryo cultured at 37°C results in an improved embryo development compared with 36°C. Adapted from Hong et al. (2012)

Number of mature oocyte (MII)	Temperature 36 °C 399	Temperature 37°C 406	*p*-value
Fertilization rate	86.2%	83.0%	$P = 0.23$
Number of cells on day 3 (mean)	7 ± 0.1	7.7 ± 0.1	$P = 0.001$
Blastocyst formation rate/2PN	51.6%	60.1%	$P = 0.03$
Usable blastocysts rate/2PN	41.2%	48.4%	$P = 0.03$
Aneuploidy rate	42.5%	46.1%	$P = 0.31$
Implantation rate	67.4%	73.3%	$P = 0.28$

fragile as they lack robust internal pH regulation and thus are more vulnerable to pH fluctuation. Indeed, at the time of oocyte retrieval, and during the entire period of embryo culture, it is mandatory to keep pH in the physiological range with minimal fluctuations, to ensure optimal embryo development [108]. For this reason, additives such as synthetic zwitterionic buffers MOPS and HEPES are recommended, since these compounds stabilize pH anytime gametes are outside the incubators. These buffers appear to be safe [96, 109–111]. A particular note regards the use of phenol red, a compound that changes colour based on the pH of the solution. It was commonly added to the media in the 1980s, to provide a visual representation of pH changes. However, phenol red has been linked with ROS generation following light exposure; also, possible estrogenic effects have been linked to its use [112]. As a result, new generation media have removed phenol red entirely. In summary, optimal pH management is extremely important to ensure consistency. On this regard, the newer and smaller bench-top incubators are considered better than large incubators commonly utilized in the past [16, 17].

Culture media osmolality

Media osmolality can impact embryo development. Different studies have reported that high osmolality negatively influences cell volume, surface and membrane stability. Mouse studies indicate that variation in osmolality might induce cell stress and inhibits embryo development [113]. The osmolality of the culture media depends on the chemical composition of the media itself and the presence of proteins or AAs. Early stages of embryo development are very sensitive to changes in osmolality, and cell volume homeostasis is essential for embryo development and further development. It has been demonstrated that mammal embryos develop optimally at an osmolality of 255–295 mOsm/kg, whereas osmolality greater than 300 mOsm/kg can induce stress and negatively influence embryo development [114]. Today, osmolality is set by the commercial media manufacturers; however, laboratory conditions such as evaporation during dish preparation, length of preparation, droplet volume, incubator humidity levels, airflow and temperature can lead to its variation (Table 4) [109–111]. Thus, consistent enforcement of laboratory protocols is needed to ensure that media osmolality is constant to ensure optimal embryo development.

Oil overlay

The oil overlay was first proposed by Brinster in the early 1960s [115]. Oil serves as a direct buffer between the media and the surrounding air, which normally is between 22 and 24°C, and reduces media evaporation, which can alter the media osmolality. Additionally, the oil overlay maintains a stable pH, controls the temperature and separates the culture media from potentially toxic or detrimental compounds and microorganisms present in the air. With the current widespread utilization of bench-top incubators with no humidification, the oil overlay is an important component of successful embryo culture. Two types of oil are utilized: paraffin oil and light mineral oil. The two oils offer similar advantages, although the polycarbonic lipid tail in mineral oil contains more unsaturated bonds than paraffin oil, making it more sensitive to photooxidation and peroxidation [116] Studies have investigated the possible negative effect overlaying oil on embryo development and pregnancy. Toxicants present in

Table 4 Variables influencing media evaporation and osmolality, which might affect spindle stability and impair further embryo development

Laboratory Procedure	Variables affecting media evaporation and increasing pH/osmolality	References
Dish preparation	Preparation time, surface temperature, humidity, air flow and temperature, size of culture media drop, microdrop preparation method, oil overlay	Swain [96] Swain et al. [111]
In vitro culture duration	Humidity of incubator, air humidity, media drop size, amount of oil overlay, type of oil utilized, length of the culture period (days 5, 6 and 7), media changes	Swain and Pool [109] Swain et al. [110] Swain et al. [111]

crude oil such as unsaturated/aromatics hydrocarbons, volatile organ compounds, peroxides, zinc and other compounds may contaminate oil [117, 118]. Of note, oil products can differ significantly in contaminant composition and are a large source of variation within the culture system. Morbeck and collaborators proposed a potential solution of washing oil with either water or culture media to remove some of these toxins [119]. A recent study published by Mestres and colleagues compared the performance of thirteen different commercial oils and found differences in osmolality, equilibration and stability of pH between different oils [120]. Oils with greater viscosity (heavy oil) provided additional protection against fluctuations in the culture conditions; however, the differences in temperature recorded were minimal (Fig. 3). Differences were also observed in the total number of cells in the inner cell mass in embryos cultured with different oils [120]. In addition, improper oil storage, particularly UV light exposure, could lead to oil peroxidation with increased toxicity to embryos. Currently, there is no consensus on which oil is superior, and choices of a specific laboratory might be based on oil viscosity, the impact of dish preparation techniques, or embryologist preference [119–121]. Of note, the potential synergistic interactions between oil and the type of culture media used have not been fully explored [122].

Plastic consumables used in the embryology laboratory

IVF laboratories must have excellent quality control systems to test not only reagents and culture media but also consumables like Petri dishes. This is done to prevent a possible negative influence of these factors on gamete and embryo viability and function [123]. These products, especially plastic materials, could release specific molecules or chemical compounds that could be toxic to the embryo. If not correctly and promptly identified, these toxic compounds can induce a detrimental impact on fertilization and ultimately on embryo development, resulting in reduced implantation and pregnancy rates [124]. Many plastic consumables used in IVF laboratory contain bisphenols. These chemicals are added to plastic products to make them more resistant to breakage and elevated temperature (used during the sterilization process). However, bisphenols are known endocrine disruptors that can have harmful estrogenic effects. It has been proposed that reprotoxic effects could arise from any of the following features: the composition of materials, the production process, the packaging, the storage, the transport and the sterilization of laboratory equipment. Several studies have reported how bisphenols and other chemicals might impair embryo development: by inducing apoptosis of gametes and embryos, interfering with steroid secretion in human granulosa cells and subsequent oocyte maturation, as well as reducing the live birth rates of women undergoing ART [123, 124]. Nijs and colleagues, investigating commercially available consumables used in ART, found that 13 out of 36 products were found to be toxic to embryos [125]. These toxic products included surgical gloves, hysterometers, ovum pickup procedure needles, oocyte retrieval tubing, sterile Pasteur pipettes, Petri dishes and even a type of embryo transfer catheter (Table 5). Many producers of IVF consumables report that their products are embryo tested, having passed the mouse embryo assay (MEA) or human sperm survival assay (HSSA). However, these tests are heterogeneous and not uniformly defined [126]. Therefore, it is essential that each laboratory performs a quality assurance and monitoring program for the products used in their clinic.

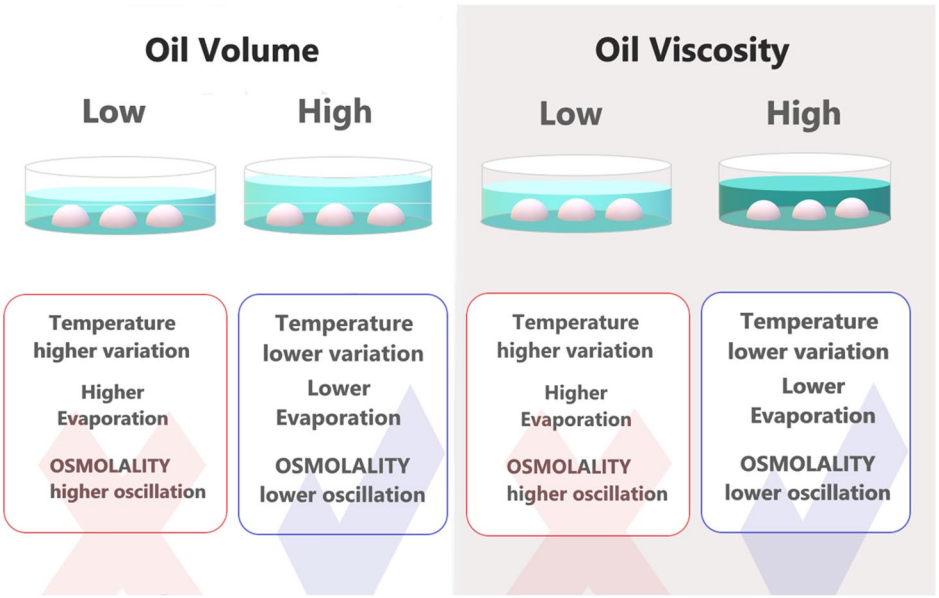

Fig. 3 Oil overlay and osmolality in human embryo culture. Strategies to decrease the risk of media evaporation, with the formation of endogenous contaminants that may impair further embryo development and implantation potential

Table 5 Human sperm survival assay (HSSA) of products and disposables generally used in daily procedures in the assisted reproductive technology (ART) laboratory. Adapted from Nijs et al. (2009)

Product	Related ART procedure	Exposure method	Exposure time
Condom Surgical glove	IUI, Oocyte collection Embryo transfer Surgical sperm retrieval Semen sperm preparation Echo-guided	0.5 cm × 0.5 cm sample in sperm suspension	30 min
Needles, needle guide Oocyte collection needles Latex tubing	Oocyte collection	Contact with semen sperm suspension	30 min
Pipette	Oocytes collection Semen sperm preparation	10 × flushing with semen sperm suspension	30 min
Freezing straws/devices Filling tips	Semen sperm cryopreservation Oocytes and embryos freezing	Filled with semen sperm suspension	24 h
ICSI injection pipette Holding pipette Denudation pipette Pipette to move embryos	Embryo washing/moving, denudation procedure before and After ICSI	Filled with semen sperm suspension	24 h
Tubes	Oocyte collection, IUI, embryo transfer, surgical sperm retrieval, semen sperm preparation	Fill with 0.5 mL of semen sperm suspension	24–96 h
Petri dishes	Gamete and embryo culture, Surgical sperm retrieval, ICSI procedure, gamete/embryo biopsy for genetic testing	Surface contact with semen Sperm suspension	1 h
Flasks Specimen container and lid	Oil and media storage Semen storage/production	Contact with semen sperm suspension	1 h

The same precaution should be extended to consumables used by physicians and nurses.

Stiffness of environment

The current standard environment for culture mammalian preimplantation embryos is the polystyrene Petri dish (PD). Particularly striking is the fact that a PD has an elastic modulus, E (expressed in Pascals: Pa) close to 10^9 Pa (1GPa), roughly six orders of magnitude stiffer than epithelia [127, 128]. Although no studies have formally measured the stiffness of the genital tract, a number of reports provide indirect evidence that the fallopian tube and uterine epithelium are all soft, on the order of 100–1000 Pa [128–130]. Culturing embryos on the proper substrate are important since every living structure (from cells to tissues and up to the level of the entire organism) has the capability to sense and respond to physical forces; this property is defined as mechanotransduction [131–133] mark principle is conveyed by mesenchymal stem cells (MSC), which sense the stiffness of their environment and initiate alternative differentiation patterns based on the overall biophysical cues. If cultured on soft matrices that mimic brain microenvironments, MSCs will differentiate into neurons; when cultured on stiffer matrices akin to muscle, MSCs generate myocytes; rigid matrices that imitate collagenous bone are instead osteogenic [134–136]. While multiple groups are studying the molecular mechanisms mediating the mechanotransduction effects, relatively few studies have evaluated the role of mechanotransduction during early embryo development, which is surprising given that the first forms of cell fate specification occur during these stages [137]. Few studies on mouse and bovine zygotes cultured on a substrate with a stiffness approximating that of the fallopian tube and uterus found a more rapid preimplantation development [138, 139]. More specifically in mice, the frequency of development from zygote to the 2-cell stage, 2-cell to blastocyst and the percentage of embryos hatching after 4 days of culture was significantly higher for embryos grown on soft collagen gels with a stiffness of 1k Pascal (Col-1k) versus stiffer substrates (collagen-coated Petri dish with a stiffness of 1 Giga Pascal = Col-1G or PD) [138, 139]. In addition, embryos grown on Col-1k had a higher number of TE cells, but a similar number of inner cell mass (ICM) cells.

New technologies in IVF laboratory

The rapid pace of improvement is continuing in the IVF laboratory. The utilization of new technologies, including microfluidic, time-lapse and artificial intelligence (AI), has an increasing role to play in modern ART, not only to support and increase pregnancy but also to assist embryologists to standardize routine procedures. The introduction of

time-lapse technologies, which allows continuous embryo assessment in a non-invasive fashion (since there is no need to remove embryos from the culture), allowed observation of embryonic developmental events that could not be detailed with such precision before [13]. Since microfluidic and artificial intelligence (AI) play a direct role in embryo development, we will delve into this technology. Microfluidic indicates a system that manipulates a liquid flowing in a microchannel of micrometre sizes. It is a multidisciplinary field that involves molecular and biological analysis and microelectronics. Its application started in the 1980s and is used in the development of inkjet printers, DNA chips, and recent advances in these technologies have found an obvious application in the ART laboratory [140, 141]. Processes that would benefit from microengineering include oocyte collection, micromanipulation, identification of follicular aspirates and isolation of individual oocyte cumulus masses and removal of the cumulus mass fertilization (conventional or by ICSI). Novel investigations have been performed to realize an IVF laboratory on a chip, incorporating all the current laboratory procedures operated during an IVF cycle, including non-invasive technology of embryo selection [141–146]. The endorsement of automated IVF systems offers potential benefits: standardization of workflows, error elimination, reduction in contamination and the potential combination with AI technologies. Furthermore, space reduction, miniature and automation IVF laboratories can easily increase accessibility to IVF cycles, especially for economically disadvantaged countries. Particularly useful would appear the use of 'perfusion culture', which involves a gradient of nutrients and the removal of waste products in real time [142]. Microfluidic devices have been used in the porcine and mouse models. Clark et al. showed that culture with a microfluidic device resulted in significantly more monospermic fertilization compared with standard insemination and culture [143], while Mancini and collaborators in the mouse model showed that zygotes had similar, although slightly slower development to the blastocyst stage [144]. Another relevant aspect of microfluid is that it could provide a dynamic environment to the embryo culture. In vivo, the human embryo develops in a moving environment and travels from the oviduct to the uterus, in a dynamic and constantly changing setting. This contrasts with what is generally performed in vitro worldwide in ART programmes, where embryos are cultured in a static manner. Indeed, there is evidence that continuous 'tilting' during culture provides a uniform shear stress and improves the development of mouse and human pre-implantation embryos [145]. However, a more recent study found no benefit. Microfluidic would be particularly advantageous to create dynamic culture conditions with the removal of toxic products released by the embryo [140–147]. Another area with rapid, 'logarithmic' development is the utilization of AI. Currently, AI is utilized for sperm selection or embryo selection prior to transfer, but also as a tool to analyse the performance of IVF personnel, and by physicians to optimize and personalize the stimulation protocols [148–151]. AI usage will expand in IVF laboratories, hopefully leading to increasing implantation potential [148]. It is likely that in the future, there will be automated systems for embryo culture and embryo selection, based on non-invasive measures of embryo competency (such as morphological, morphokinetic, morphometrics or molecular analysis of culture media-metabolomics or nucleic acid sequencing for example). These advantages, together with the possibility to standardize embryology procedures and remove human errors, will offer great benefits. Additionally, the integration of new generations of microscopies and evolution in the field of genetics will be valuable to assess not only the metabolic state of the embryo but also indirectly to describe the embryo epigenetic status [152]. Examples include polarized microscopy and Raman microspectroscopy (RM). Montag and collaborators have largely revisited the use of polarized optics to assess human oocytes [153]. Polarized light is a contrast-enhancing method that yields quality images obtained by birefringent materials. The main advantage is that it is non-invasive imaging, and it can be performed in real time and on living cells. It can detect the intracellular organelles of gametes and embryos, which might be important for selecting embryo viability. In oocytes, polarized optics can be applied to visualize the spindle [154] or the organization of ZP around the oocyte [155, 156]. RM is a combination of Raman spectroscopy and confocal microscopy and can be used to identify interactions between light and live matter. The photon scattering generates unique spectra that can be used to detect molecules and their molecular bonds in living cells [157–160]. RM is also used in association with other technologies such as hyperspectral and fluorescence lifetime imaging, or multiphoton excitation fluorescence microscopy [157, 158]. These methods are in continuous evolution and have distinguished themselves for having very low phototoxicity and high spatial and temporal resolution, making them ideally suited for studies of development and cellular dynamics [159]. RM has been used to evaluate mouse and Xenopus oocytes [160–162]. Studies, using RM, were able to identify patterns of intracellular lipids and areas of high protein content, including mitochondria [160, 161], as well as describe significant differences in lipid and protein components [162]. Such reports, however, require further investigations. Future experiments will examine the application of those novel microscopy technologies of viewing the intrastructure, function and organization of human embryos, to obtain biomarker data on oocyte competencies and advice on the selection of embryos to transfer in ART.

Why culture environment matters: possible effect on offspring health

The first 1000 days of development are believed to be highly sensitive to suboptimal nutrient or toxicant exposure. Exposure to abnormal stimuli during this time window can affect the future health of the individual [163, 164]. For example, a maternal isocaloric but low-protein diet limited to the preimplantation period in rats results in offspring being hypertensive [165]. It is well described that foetuses exposed in utero to famine during the Dutch winter in 1944 resulted in offspring with significant metabolic alterations [166]. It is believed that epigenetic changes are at the basis of these alterations. Importantly for the IVF practitioner, the preimplantation embryos undergo important epigenetic changes [167], which can be modified by culture in vitro [168, 169]. Currently, it is still unclear whether in vitro culture disturbance, as well as ovarian stimulation protocols, might perturb the homeostasis and influence long-term growth and metabolism. Overall current evidence does not show significant adverse effects on offspring health, but possible consequences in later life are still unknown. Several studies are encouraging and show no differences between ART-conceived children and the general population regarding mental health, growth and neurological outcome [170–172]. However, few studies with a limited number of patients show an increase in glucose intolerance and hypertension in ART-generated offspring [173–176]. These data together with the slight increase in obstetrical and perinatal complications, as well as congenital defects observed in ART-generated offspring [176–180], warrant caution. It is of paramount importance to continue follow-up of the health of IVF-conceived offspring to clarify possible adverse outcomes not only at the delivery but also later in life, during childhood or adulthood.

Concluding remarks

There have been remarkable progresses in the IVF laboratories that have resulted in improving pregnancy rates while reducing multiple pregnancies. It is believed that these progresses will continue in the future thanks to the use of novel engineering (microfluidic, AI) and molecular (non-invasive metabolic or transcriptomic testing of embryos) solutions. However, it is also clear that each factor (type of media, oxygen concentration, plastic dish, oil, etc.) involved in a gamete or embryo manipulation has a profound impact on embryo development. Cell stressors like pH, ROS, osmolality and temperature may play a pivotal role in permitting optimal embryo development and, if not well-controlled, may have a negative impact, ultimately lowering the implantation potential. Meticulous quality control in the laboratory and novel research to improve embryo development will be always an indispensable aspect of the IVF laboratory.

Author contribution RS and PR conceptualized and wrote this manuscript.

Data availability No data are available.

Declarations

Human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments. For this type of study, formal consent is not required.

Informed consent Not applicable.

Competing interests The authors declare no competing interests.

References

1. Bigger JD. Thoughts on embryo culture conditions. Reprod Biomed Online. 2001;4:30–8.
2. Gardner DK, Lane M. Culture and selection of viable blastocysts: a feasible proposition for human IVF? Hum Repod Update. 1997;3:367–82.
3. Fouks Y, Yogev Y. Twinning in ART: single embryo transfer policy. Best Pract Res Clin Obstet Gynaecol. 2022;84:88–95. https://doi.org/10.1016/j.bpobgyn.2022.03.010.
4. De Neubourg D, Dancet EAF, Pinborg A. Single-embryo transfer implies quality of care in reproductive medicine. Reprod Biomed Online. 2022;45(5):899–905. https://doi.org/10.1016/j.rbmo.2022.04.001.
5. Abe K, Schauer T, Torres-Padilla ME. Distinct patterns of RNA polymerase II and transcriptional elongation characterize mammalian genome activation. Cell Rep. 2022;41(13):111865. https://doi.org/10.1016/j.celrep.2022.111865.
6. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature. 1988;332:459–61.
7. Leese HJ. Formation and function of oviduct fluid. J Reprod Fertil. 1988;82:843–56.
8. Leese HJ, Barton AM. Production of pyruvate by isolated mouse cumulus cells. J Exp Zool. 1985;234:231–6.
9. Baltz JM, Smith SS, Biggers JD, Lechene C. Intracellular ion concentrations and their maintenance by Na+/K(+)-ATPase in preimplantation mouse embryos. Zygote. 1997;5(1):1–9.
10. Troncoso C, Bosch E, Rubio C, Remohí J, Simón C, Pellicer A. The origin of biochemical pregnancies: lessons learned from preimplantation genetic diagnosis. Fertil Steril. 2003;79:449–50.
11. Quinn P, Kerin J, Warnes G. 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;44:493–8.
12. 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;65:349–53.
13. Sciorio R, Meseguer M. Focus on time-lapse analysis: blastocyst collapse and morphometric assessment as new features of embryo viability. Reprod Biomed Online. 2021;43(5):821–32.
14. Sciorio R, Miranian D, Smith GD. Non-invasive oocyte quality assessment. Biol Reprod. 2022;106(2):274–90.
15. Sciorio R, Campos G, Palini S, Baldini D, Janssens R. Real-time image and time-lapse technology to select the single blastocyst to transfer in assisted reproductive cycles. Zygote. 2023:1–10. https://doi.org/10.1017/S0967199423000151.

16. Sciorio R. Use of time-lapse monitoring in medically assisted reproduction treatments: a mini-review. Zygote. 2021;29(2):93–101.
17. Sciorio R, Thong JK, Pickering SJ. Comparison of the development of human embryos cultured in either an EmbryoScope or benchtop incubator. J Assist Reprod Genet. 2018;35(3):515–22.
18. Rock J, Menkin MF. In vitro fertilization and cleavage of human ovarian eggs. Science. 1944;100:105–7.
19. Ruffenach SC. Landrum Brewer Shettles (1909-2003). In: Embryo Project Encyclopedia (2009-07-22). http://embryo.asu.edu/handle/10776/1958.
20. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet (London, England). 1978;2:366.
21. Steptoe PC, Edwards RG. Laparoscopic recovery of preovulatory human oocytes after priming of ovaries with gonadotrophins. Lancet (London, England). 1970;1:683–9.
22. Edwards RG, Bavister BD, Steptoe PC. Early stages of fertilization in vitro of human oocytes matured in vitro. Nature. 1969;221:632–5.
23. Edwards RG. Test-tube babies. Nature. 1981;293:253–6.
24. Edwards RG, Purdy JM, Steptoe PC, et al. The growth of human preimplantation embryos in vitro. Am J Obstet Gynecol. 1981;141:408–16.
25. Menezo Y, Testart J, Perone D. Serum is not necessary in human in vitro fertilization and embryo development. Fertil Steril. 1984;42:750–5.
26. Lawitt JA, Biggers JD. Joint effects of sodium chloride, glutamine, and glucose in mouse preimplantation embryo culture media. Mol Reprod Dev. 1992;31:189–94.
27. Summers MC, Biggers JD. Chemically defined media and the culture of mammalian preimplantation embryos: historical perspective and current issues. Hum Reprod Update. 2003;9:557–82.
28. Biggers JD, Summers MC. Choosing a culture medium: making informed choices. Fertil Steril. 2008;90:473–83.
29. Morbeck DE, Krisher RL, Herrick JR, Baumann NA, Matern D, Moyer T. Composition of commercial media used for human embryo culture. Fertil Steril. 2014;102:759–66.e9.
30. Gardner DK, Harvey AJ. Blastocyst metabolism. Reprod Fertil Dev. 2015;27(4):638–54. https://doi.org/10.1071/RD14421.
31. Balaban B, Urman B. Comparison of two sequential media for culturing cleavage-stage embryos and blastocysts: embryo characteristics and clinical outcome. Reprod Biomed Online. 2005;10:485–91.
32. Mantikou E, Youssef MA, van Wely M, van der Veen F, Al-Inany HG, Repping S, et al. Embryo culture media and IVF/ICSI success rates: a systematic review. Hum Reprod Update. 2013;19:210–20.
33. Reed ML, Hamic A, Thompson DJ, Caperton CL. Continuous uninterrupted single medium culture without medium renewal versus sequential media culture: a sibling embryo study. Fertil Steril. 2009;92:1783–6.
34. Summers MC, Bird S, Mirzai FM, Thornhill A, Biggers JD. Human preimplantation embryo development in vitro: a morphological assessment of sibling zygotes cultured in a single medium or in sequential media. Hum Fertil (Camb). 2013;16:278–85.
35. Pool TB, Schoolfield J, Han D. Human embryo culture media comparisons. Methods Mol Biol. 2012;912:367–86.
36. Campo R, Binda MM, Van Kerkhoven G, Frederickx V, Serneels A, Roziers P, et al. Critical reappraisal of embryo quality as a predictive parameter for pregnancy outcome: a pilot study. Facts Views Vision ObGyn. 2010;2:289–95.
37. Nelissen EC, Van Montfoort AP, Coonen E, Derhaag JG, Geraedts JP, Smits LJ, et al. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. Hum Reprod. 2012;27:1966–76.
38. Youssef MA, Mantikou E, van Wely M, Van der Veen F, Al-Inany HG, Repping S, et al. Culture media for human pre-implantation embryos in assisted reproductive technology cycles. Cochrane Database Syst Rev. 2015;(11):CD007876. https://doi.org/10.1002/14651858.CD007876.pub2.
39. Biggers JD, McGinnis LK, Raffin M. Amino acids and preimplantation development of the mouse in protein-free potassium simplex optimized medium. Biol Reprod. 2000;63:281–93.
40. Krisher RL, Schlenker T. Culture of human preimplantation embryos in a clinical ART setting. Methods Mol Biol. 2019;2006:355–71. https://doi.org/10.1007/978-1-4939-9566-0_24.
41. Wale PL, Gardner DK. Oxygen affects the ability of mouse blastocysts to regulate ammonium. Biol Reprod. 2013;89:75.
42. Cassuto G, Chavrier M, Menezo Y. Culture conditions and not prolonged culture time are responsible for monozygotic twinning in human in vitro fertilization. Fertil Steril. 2003;80:462–3.
43. Menezo Y, Lichtblau I, Elder K. Newinsights into human preimplantation metabolism in vivo and in vitro. J Assist Reprod Genet. 2013;30:293–303.
44. Clare CE, Pestinger V, Kwong WY, Tutt DAR, Xu J, Byrne HM, Barrett DA, Emes RA, Sinclair KD. Interspecific variation in one-carbon metabolism within the ovarian follicle, oocyte, and preimplantation embryo: consequences for epigenetic programming of DNA methylation. Int J Mol Sci. 2021;22(4):1838. https://doi.org/10.3390/ijms22041838.
45. Summers MC, McGinnis LK, Lawitts JA, Biggers JD. Mouse embryo development following IVF in media containing either L-glutamine or glycyl-Lglutamine. Hum Reprod. 2005;20:1364–71.
46. Lane M, Gardner DK. Ammonium induces aberrant blastocyst differentiation, metabolism, pH regulation, gene expression and subsequently alters fetal development in the mouse. Biol Reprod. 2003;69:1109–17.
47. Weathersbee PS, Pool TB, Ord T. Synthetic serum substitute (SSS): a globulin-enriched protein supplement for human embryo culture. J Assist Reprod Genet. 1995;12:354–60.
48. Pool TB, Martin JE. High continuing pregnancy rates after in vitro fertilization– embryo transfer using medium supplemented with a plasma protein fraction containing alpha- and beta-globulins. Fertil Steril. 1994;61:714–9.
49. Meintjes M, Chantilis SJ, Ward DC, Douglas JD, Rodriguez AJ, Guerami AR. A randomized controlled study of human serum albumin and serum substitute supplement as protein supplements for IVF culture and the effect on live birth rates. Hum Reprod. 2009;24:782–9.
50. Tay JI, Rutherford AJ, Killick SR, Maguiness SD, Partridge RJ, Leese HJ. Human tubal fluid: production, nutrient composition and response to adrenergic agents. Hum Reprod. 1997;12(11):2451–6. https://doi.org/10.1093/humrep/12.11.2451.
51. Gardner DK. Dissection of culture media for embryos: the most important and less important components and characteristics. Reprod Fertil Dev. 2008;20:9–18.
52. Morbeck DE, Baumann NA, Oglesbee D. Composition of single-step media used for human embryo culture. Fertil Steril. 2017;107:1055–60.
53. Fredrickson J, Krisher R, Morbeck DE. The impact of the protein stabilizer octanoic acid on embryonic development and fetal growth in a murine model. J Assist Reprod Genet. 2015;32:1517–24.
54. 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;306:335–40.
55. Knudson CB, Knudson W. Hyaluronan-binding proteins in development, tissue homeostasis, and disease. FASEB J. 1993;7:1233–41.

56. Campbell S, Swann HR, Aplin JD, Seif MW, Kimber SJ, Elstein M. CD44 is expressed throughout pre-implantation human embryo development. Hum Reprod. 1995;10:425–30.
57. 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(3):604–12. https://doi.org/10.1016/j.fertnstert.2007.07.1294.
58. Bontekoe S, Heineman MJ, Johnson N, Blake D. Adherence compounds in embryo transfer media for assisted reproductive technologies. Cochrane Database Syst Rev. 2014;(2):CD007421. https://doi.org/10.1002/14651858.CD007421.pub3.
59. Pock T, Schulte K, Schlatt S, Boiani M, Nordhoff V. GM-CSF perturbs cell identity in mouse pre-implantation embryos. PloS One. 2022;17(2):e0263793.
60. Adanacıoglu F, Çetin C, Tokat G, Adanacıoglu D, Karasu AFG, Çetin MT. Comparison of the effects of GMCSF-containing and traditional culture media on embryo development and pregnancy success rates. Rev Bras Ginecol Obstet. 2022;44(11):1047–51. https://doi.org/10.1055/s-0042-1759630.
61. Armstrong S, MacKenzie J, Woodward B, Pacey A, Farquhar C. GM-CSF (granulocyte macrophage colony-stimulating factor) supplementation in culture media for women undergoing assisted reproduction. Cochrane Database Syst Rev. 2020;7(7):CD013497.
62. Okabe-Kinoshita M, Kobayashi T, Shioya M, Sugiura T, Fujita M, Takahashi K. Granulocyte-macrophage colony-stimulating factor-containing medium treatment after thawing improves blastocyst-transfer outcomes in the frozen- thawed blastocyst-transfer cycle. J Assist Reprod Genet. 2022;39(6):1373–81. https://doi.org/10.1007/s10815-022-02493-1.
63. Siristatidis C, Vogiatzi P, Salamalekis G, Creatsa M, Vrachnis N, Glujovsky D, 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. https://doi.org/10.1155/2013/704967.
64. Ziebe S, Loft A, Povlsen BB, Erb K, Agerholm I, Aasted M, et al. A randomized clinical trial to evaluate the effect of granulocyte-macrophage colonystimulating factor (GM-CSF) in embryo culture medium for in vitro fertilization. Fertil Steril. 2013;99:1600–9.
65. Chen P, Huang C, Sun Q, Zhong H, et al. Granulocyte-macrophage colony stimulating factor in single blastocyst conditioned medium as a biomarker for predicting implantation outcome of embryo. Front Immunol. 2021 Jun;30(12):679839. https://doi.org/10.3389/fimmu.2021.679839.
66. Fischer B, Bavister BD. Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. J Reprod Fertil Nov. 1993;99(2):673–9.
67. Ng KYB, Mingels R, Morgan H, Macklon N, Cheong Y. In vivo oxygen, temperature and pH dynamics in the female reproductive tract and their importance in human conception: a systematic review. Hum Reprod Update. 2018;24(1):15–34. https://doi.org/10.1093/humupd/dmx028.
68. Sciorio R, Smith GD. Embryo culture at a reduced oxygen concentration of 5%: a mini review. Zygote. 2019;27(6):355–61.
69. Catt JW, Henman M. Toxic effects of oxygen on human embryo development. Hum Reprod. 2000;15(Suppl 2):199–206.
70. Waldenström U, Engström AB, Hellberg D, Nilsson S. Low-oxygen compared with high-oxygen atmosphere in blastocyst culture, a prospective randomized study. Fertil Steril. 2009;91(6):2461–5.
71. Meintjes M, Chantilis SJ, Douglas JD, et al. A controlled randomized trial evaluating the effect of lowered incubator oxygen tension on live births in a predominantly blastocyst transfer program. Hum Reprod. 2009;24(2):300–7.
72. Gardner DK, Lane M. Ex vivo early embryo development and effects on gene expression and imprinting. Reprod Fertil Dev. 2005;17:361–70.
73. Rinaudo PF, Giritharan G, Talbi S, Dobson AT, Schultz RM. Effects of oxygen tension on gene expression in preimplantation mouse embryos. Fertil Steril. 2006;86(4 Suppl):1252–65.
74. Kovacic B, Vlaisavljević V. Influence of atmospheric versus reduced oxygen concentration on development of human blastocysts in vitro: a prospective study on sibling oocytes. Reprod Biomed Online. 2008;17(2):229–36. https://doi.org/10.1016/s1472-6483(10)60199-x.
75. Kasterstein E, Strassburger D, Komarovsky D, Bern O, Komsky A, Raziel A, et al. The effect of two distinct levels of oxygen concentration on embryo development in a sibling oocyte study. J Assist Reprod Genet. 2013;30(8):1073–9. https://doi.org/10.1007/s10815-013-0032-z.
76. Sciorio R, El Hajj N. Epigenetic risks of medically assisted reproduction. J Clin Med. 2022;11(8):2151. https://doi.org/10.3390/jcm11082151.
77. Cajas YN, Cañón-Beltrán K, Ladrón de Guevara M, Millán de la Blanca MG, Ramos-Ibeas P, Gutiérrez-Adán A, Rizos D, González EM. Antioxidant nobiletin enhances oocyte maturation and subsequent embryo development and quality. Int J Mol Sci. 2020;21(15):–5340. https://doi.org/10.3390/ijms21155340.
78. Li W, Goossens K, Van Poucke M, Foreir K, Braeckmans K, Van Soom A, Peelman LJ. High oxygen tension increases global methylation in bovine 4-cell embryos and blastocysts but does not affect general retrotransposon expression. Reprod Fertil Dev. 2014;28(7):948–59.
79. Bradley J, Swann K. Mitochondria and lipid metabolism in mammalian oocytes and early embryos. Int J Dev Biol. 2019;63(3-5):93–103. https://doi.org/10.1387/ijdb.180355ks.
80. Dunning KR, Russell DL, Robker RL. Lipids and oocyte developmental competence: the role of fatty acids and β-oxidation. Reproduction. 2014;148(1):R15–27. https://doi.org/10.1530/REP-13-0251.
81. van der Reest J, Nardini Cecchino G, Haigis MC, Kordowitzki P. Mitochondria: their relevance during oocyte ageing. Ageing Res Rev. 2021;70:101378. https://doi.org/10.1016/j.arr.2021.101378.
82. Belli M, Zhang L, Liu X, Donjacour A, Ruggeri E, Palmerini MG, Nottola SA, Macchiarelli G, Rinaudo P. Oxygen concentration alters mitochondrial structure and function in in vitro fertilized preimplantation mouse embryos. Hum Reprod. 2019;34(4):601–11. https://doi.org/10.1093/humrep/dez011.
83. Gaspar RB, Arnold DR, Corrêa CAP, da Rocha CV Jr, et al. Oxygen tension affects histone remodeling of in vitro-produced embryos in a bovine model. Theriogenology. 2015;83(9):1408–15. https://doi.org/10.1016/j.theriogenology.2015.01.002.
84. Katz-Jaffe MG, Linck DW, Schoolcraft WB, Gardner DK. A proteomic analysis of mammalian preimplantation embryonic development. Reproduction. 2005;130(6):899–905.
85. Rinaudo P, Schultz RM. Effects of embryo culture on global pattern of gene expression in preimplantation mouse embryos. Reproduction. 2004;128(3):301–11. https://doi.org/10.1530/rep.1.00297.
86. Feuer S, Liu X, Donjacour A, Simbulan R, Maltepe E, Rinaudo P. Common and specific transcriptional signatures in mouse embryos and adult tissues induced by in vitro procedures. Reproduction. 2016;31:REP-16-0473. https://doi.org/10.1530/REP-16-0473.
87. Christianson MS, Zhao Y, Shoham G, Granot I, Safran A, Khafagy A, Leong M, Shoham Z. Embryo catheter loading and embryo culture techniques: results of a worldwide Web-based survey. J Assist Reprod Genet. 2014;31:1029–36.
88. Hong K, Forman E, Lee H, et al. Optimizing the temperature for embryo culture in IVF: a randomized controlled trial (RCT)

comparing standard culture temperature of 37C to the reduced more physiologic temperature of 36C. Fertil Steril. 2012;98(3):s167.
89. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. Reprod Biol Endocrinol. 2012;10:49.
90. Gardner DK, Kuramoto T, Tanaka M, Mitzumoto S, Montag M, Yoshida A. Prospective randomized multicentre comparison on sibling oocytes comparing G-Series media system with antioxidants versus standard G-Series media system. Reprod Biomed Online. 2020;40(5):637–44. https://doi.org/10.1016/j.rbmo.2020.01.026.
91. Truong T, Harvey AJ, Gardner DK. Antioxidant supplementation of mouse embryo culture or vitrification media support more in-vivo-like gene expression post-transfer. Reprod Biomed Online. 2022;44(3):393–410. https://doi.org/10.1016/j.rbmo.2021.11.013.
92. Guerin P, El Mouatassim S, MenezoY. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Hum Reprod Update. 2001;7:175–89.
93. Abdelrazik H, Sharma R, Mahfouz R, Agarwal A. L-carnitine decreases DNA damage and improves the in vitro blastocyst development rate in mouse embryos. Fertil Steril. 2009;91:589–96.
94. Furnus CC, de Matos DG, Picco S, Garcia PP, Inda AM, Mattioli G, Errecalde AL. Metabolic requirements associated with GSH synthesis during in vitro maturation of cattle oocytes. J Anim Sci. 2008;109:88–99.
95. Hammond CL, Lee TK, Ballatori N. Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. J Hepatol. 2001;34:946–54.
96. Swain JE. Optimal human embryo culture. Semin Reprod Med. 2015;33(2):103–117. https://doi.org/10.1055/s-0035-1546423.
97. Sun XF, Wang WH, Keefe DL. Overheating is detrimental to meiotic spindles within in vitro matured human oocytes. Zygote. 2004;12(1):65–70.
98. Wang WH, Meng L, Hackett RJ, Oldenbourg R, Keefe DL. Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates. Fertil Steril. 2002;77(6):1274–7.
99. Gorbsky GJ. The spindle checkpoint and chromosome segregation in meiosis. FEBS J. 2015;282(13):2471–87.
100. Montag M, van der Ven H. Symposium: innovative techniques in human embryo viability assessment. Oocyte assessment and embryo viability prediction: birefringence imaging. Reprod Biomed Online. 2008;17(4):454–60.
101. Swearman H, Koustas G, Knight E, Liperis G, Grupen C, Sjoblom C. pH: the silent variable significantly impacting meiotic spindle assembly in mouse oocytes. Reprod Biomed Online. 2018 Sep;37(3):279–90.
102. Pickering SJ, Braude PR, Johnson MH, Cant A, Currie J. Transient cooling to room temperature can cause irreversible disruption of the meiotic spindle in the human oocyte **Supported by program grant PG 8302273 to P.R.B, and M.H.J, from the Medical Research Council of Great Britain, Cambridge, England. Fertil Steril. 1990;54:102–8.
103. Pollard JW, Martino A, Rumph ND, Songsasen N, Plante C, Leibo SP. Effect of ambient temperatures during oocyte recovery on in vitro production of bovine embryos. Theriogenology. 1996;46(5):849–58.
104. Vinales KL, Begaye B, Thearle MS, Krakoff J, Piaggi P. Core body temperature, energy expenditure, and epinephrine during fasting, eucaloric feeding, and overfeeding in healthy adult men: evidence for a ceiling effect for human thermogenic response to diet. Metabolism. 2019;94:59–68. https://doi.org/10.1016/j.metabol.2019.01.016.
105. De Munck N, Janssens R, Santos-Ribeiro S, Tournaye H, Velde H, Verheyen G. The effect of different temperature conditions on human embryos in vitro: two sibling studies. Reprod Biomed Online. 2019;38(4):508–15.
106. Fawzy M, Emad M, Gad MA, Sabry M, Kasem H, Mahmoud M, Bedaiwy MA. Comparing 36.5°C with 37°C for human embryo culture: a prospective randomized controlled trial. Reprod Biomed Online. 2018;36(6):620–6. https://doi.org/10.1016/j.rbmo.2018.03.011.
107. Lee SH, Liu X, Jimenez-Morales D, Rinaudo PF. Murine blastocysts generated by in vitro fertilization show increased Warburg metabolism and altered lactate production. Elife. 2022;15(11):e79153. https://doi.org/10.7554/eLife.79153.
108. Hentemann M, Mousavi K, Bertheussen K. Differential pH in embryo culture. Fertil Steril. 2011;95(4):1291–4.
109. Swain JE, Pool TB. New pH-buffering system for media utilized during gamete and embryo manipulations for assisted reproduction. Reprod Biomed Online. 2009;18(6):799–810. https://doi.org/10.1016/s1472-6483(10)60029-6.
110. Swain JE, Carrell D, Cobo A, Meseguer M, Rubio C, Smith GD. Optimizing the culture environment and embryo manipulation to help maintain embryo developmental potential. Fertil Steril. 2016;105(3):571–87. https://doi.org/10.1016/j.fertnstert.2016.01.035.
111. Swain JE, Cabrera L, Xu X, Smith GD. Microdrop preparation factors influence culture-media osmolality, which can impair mouse embryo preimplantation development. Reprod Biomed Online. 2012;24(2):142–7. https://doi.org/10.1016/j.rbmo.2011.10.008.
112. Nakayama T, Noda Y, Goto Y, Mori T. Effects of visible light and other environmental factors on the production of oxygen radicals by hamster embryos. Theriogenology. 1994;41(2):499–510.
113. Baltz J, Shou C. Cell volume regulation in mammalian oocytes and preimplantation embryos. Mol Reprod Dev. 2012;79:821–31.
114. Brinster RL. Studies on the development of mouse embryos in vitro. The effect of osmolarity and hydrogen ion concentration. J Exp Zool. 1965;158:49–57.
115. Brinster RL. A Method for in vitro cultivation of mouse ova from two-cell to blastocyst. Exp Cell Res. 1963;32(1):205–8.
116. Elder K, Van den Bergh M, Woodward B. Troubleshooting and problem solving in the IVF laboratory. Cambridge: Press CU; 2015. 35–7
117. Morbeck DE, Leonard PH. Culture systems: mineral oil overlay. Methods Mol Biol. 2012;912:325–31.
118. Erbach GT, Bhatnagar P, Baltz JM, Biggers JD. Zinc is a possible toxic contaminant of silicone oil in microdrop cultures of preimplantation mouse embryos. Hum Reprod. 1995;10:3248–54.
119. Morbeck DE, Khan Z, Barnidge DR, Walker DL. Washing mineral oil reduces contaminants and embryotoxicity. Fertil Steril. 2010;94(7):2747–52.
120. Mestres E, Matia-Algué Q, Villamar A, Casals A, Acacio M, García-Jiménez M, Martínez-Casado A, Castelló C, Calderón G, Costa-Borges N. Characterization and comparison of commercial oils used for human embryo culture. Hum Reprod. 2022;37(2):212–25.
121. Ainsworth AJ, Fredrickson JR, Morbeck DE. Improved detection of mineral oil toxicity using an extended mouse embryo assay. J Assist Reprod Genet. 2017;34(3):391–7.
122. Hughes PM, Morbeck DE, Hudson SB, Fredrickson JR, Walker DL, Coddington CC. Peroxides in mineral oil used for in vitro fertilization: defining limits of standard quality control assays. J Assist Reprod Genet. 2010;27(2-3):87–92.

123. Mortimer D, Mortimer ST, editors. Quality and risk management in the IVF Laboratory. Cambridge: Cambridge University Press; 2005.
124. Ray BD, Mcdermott A, Wardle PG, Corrigan E, Mitchell EA, McLaughlin EA, et al. In vitro fertilization: fertilization failure due to toxic catheters. J In Vitro Fert Embryo Transf. 1984;4:58–61.
125. Nijs M, Franssen K, Cox A, Wissmann D, Ruis H, Ombelet W. Reprotoxicity of intrauterine insemination and in vitro fertilization-embryo transfer disposables and products: a 4-year survey. Fertil Steril. 2009;92(2):527–35.
126. Delaroche L, Oger P, Genauzeau E, Meicler P, Lamazou F, Dupont C, Humaidan P. Embryotoxicity testing of IVF disposables: how do manufacturers test? Hum Reprod. 2020;35(2):283–92.
127. Discher DE, Janmey P, Wang Y-L. Tissue cells feel and respond to the stiffness of their substrate. Science (New York, NY). 2005;310:1139–43.
128. Thie M, Röspel R, Dettmann W, Benoit M, Ludwig M, Gaub HE, Denker HW. Interactions between trophoblast and uterine epithelium: monitoring of adhesive forces. Hum Reprod. 1998;13:3211–9.
129. Manoogian SJ, Bisplinghoff JA, McNally C, Kemper AR, Santago AC, Duma SM. Dynamic tensile properties of human placenta. J Biomech. 2008;41:3436–40.
130. Filas BA, Bayly PV, Taber LA. Mechanical stress as a regulator of cytoskeletal contractility and nuclear shape in embryonic epithelia. Ann Biomed Eng. 2011;39:443–54.
131. DuFort CC, Paszek MJ, Weaver VM. Balancing forces: architectural control of mechanotransduction. Nat Rev Mol Cell Biol. 2011;12:308–19.
132. Parsons JT, Horwitz AR, Schwartz MA. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. Nat Rev Mol Cell Biol. 2010;11:633–43.
133. Vogel V, Sheetz MP. Cell fate regulation by coupling mechanical cycles to biochemical signaling pathways. Curr Opin Cell Biol. 2009;21:38–46.
134. Sun Y, Villa-Diaz LG, Lam RH, Chen W, Krebsbach PH, Fu J. Mechanics regulates fate decisions of human embryonic stem cells. PloS One. 2012;7:e37178.
135. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006;126:677–89.
136. Titushkin IA, Shin J, Cho M. A new perspective for stem-cell mechanobiology: biomechanical control of stem-cell behavior and fate. Crit Rev Biomed Eng. 2010;38:393–433.
137. Wozniak MA, Chen CS. Mechanotransduction in development: a growing role for contractility. Nat Rev Mol Cell Biol. 2009;10:34–43.
138. Rivera RM, Rinaudo P. Bovine preimplantation embryo development is affected by the stiffness of the culture substrate. Mol Reprod Dev. 2013;80:184.
139. Kolahi KS, Donjacour A, Liu X, Lin W, Simbulan RK, Bloise E, Maltepe E, Rinaudo P. Effect of substrate stiffness on early mouse embryo development. PloS One. 2012;7:e41717.
140. Wheeler MB, Walters EM, Beebe DJ. Toward culture of single gametes: the development of microfluidic platforms for assisted reproduction. Theriogenology. 2007 Sep;1(68 Suppl 1):S178–89. https://doi.org/10.1016/j.theriogenology.2007.04.042.
141. Gu W, Zhu X, Futai N, Cho B, Takayama S. Computerized microfluidic cell culture using elastomeric channels and Braille displays. PNAS. 2004;101:15861–6.
142. Volpatti LR, Yetisen AK. Commercialization of microfluidic devices. Trends Biotechnol. 2014;32:347–50.
143. Clark SG, Haubert K, Beebe DJ, Ferguson CE, Wheeler MB. Reduction of polyspermic penetration using biomimetic microfluidic technology during in vitro fertilization. Lab Chip. 2005;5:1229–32.
144. Mancini V, McKeegan PJ, Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA, Picton HM, Pensabene V. Probing morphological, genetic and metabolomic changes of in vitro embryo development in a microfluidic device. Biotechnol Prog. 2021;37:e3194.
145. Matsuura K, Hayashi N, Kuroda Y, Takiue C, Hirata R, Takenami M, Aoi Y, Yoshioka N, Habara T, Mukaida T, Naruse K. Improved development of mouse and human embryos using a tilting embryo culture system. Reprod Biomed Online. 2010;20:358–64.
146. Kushnir VA, Smith GD, Adashi EY. The future of IVF: the new normal in human reproduction. Reprod Sci. 2022;29(3):849–56.
147. Yanez LZ, Camarillo DB. Microfluidic analysis of oocyte and embryo biomechanical properties to improve outcomes in assisted reproductive technologies. Mol Hum Reprod. 2017;23(4):235–47. https://doi.org/10.1093/molehr/gaw071.
148. Bormann CL, Curchoe CL, Thirumalaraju P, Kanakasabapathy MK, Gupta R, Pooniwala R, Kandula H, Souter I, Dimitriadis I, Shafiee H. Deep learning early warning system for embryo culture conditions and embryologist performance in the ART laboratory. J Assist Reprod Genet. 2021;38:1641–6.
149. Blank C, Wildeboer RR, DeCroo I, Tilleman K,Weyers B, Petra de Sutter, Massimo Mischi, Benedictus Christiaan Schoot Prediction of implantation after blastocyst transfer in in vitro fertilization: a machine-learning perspective. Fertil Steril 2019; 111:318-326.
150. Curchoe CL, Bormann CL. Artificial intelligence and machine learning for human reproduction and embryology presented at ASRM and ESHRE. J Assist Reprod Genet. 2018;2019:1–10.
151. Gardner DK, Reineck P, Gibson BC, Thompson JG. Microfluidics and microanalytics to facilitate quantitative assessment of human embryo physiology. In: Agarwal A, Varghese A, Nagy ZP, editors. Practical Manual of In Vitro Fertilization: Advanced Methods and Novel Devices. 2nd ed. New Jersey: Humana Press; 2019. p. 557–66.
152. Nagaoka SI, Hassold TJ, Hunt PA. Human aneuploidy: mechanisms and new insights into an age-old problem. Nat Rev Genet. 2012;13(7):493–504.
153. Montag M, Koster M, van der Ven K, van der Ven H. Gamete competence assessment by polarizing optics in assisted reproduction. Hum Reprod Update. 2011;17(5):654–66.
154. Swann MM, Mitchison JM. Refinements in polarized light microscopy. J Exp Biol. 1950;27(2):226–37.
155. Keefe D, Tran P, Pellegrini C, Oldenbourg R. Polarized light microscopy and digital image processing identify a multilaminar structure of the hamster zona pellucida. Hum Reprod. 1997;12(6):1250–2.
156. Silva CP, Kommineni K, Oldenbourg R, Keefe DL. The first polar body does not predict accurately the location of the metaphase II meiotic spindle in mammalian oocytes. Fertil Steril. 1999;71(4):719–21.
157. Squirrell JM, Wokosin DL, White JG, Bavister BD. Long-term two-photon fluorescence imaging of mammalian embryos without compromising viability. Nat Biotechnol. 1999;17(8):763–7.
158. Wan Y, McDole K, Keller PJ. Light-sheet microscopy and its potential for understanding developmental processes. Annu Rev Cell Dev Biol. 2019;35:655–81.
159. Albert-Smet I, Marcos-Vidal A, Vaquero JJ, Desco M, Munoz-Barrutia A, Ripoll J. Applications of light-sheet microscopy in microdevices. Front Neuroanat. 2019;13:1.
160. Wood BR, Chernenko T, Matthaus C, Diem M, Chong C, Bernhard U, Jene C, Brandli AA, McNaughton D, Tobin MJ, Trounson A, Lacham-Kaplan O. Shedding new light on the molecular architecture of oocytes using a combination of synchrotron

Fourier transform-infrared and Raman spectroscopic mapping. Anal Chem. 2008;80(23):9065–72.
161. Rusciano G, Pesce G, Salemme M, Selvaggi L, Vaccaro C, Sasso A, Carotenuto R. Raman spectroscopy of Xenopus laevis oocytes. Methods. 2010;51(1):27–36.
162. Bogliolo L, Murrone O, Di Emidio G, Piccinini M, Ariu F, Ledda S, Tatone C. Raman spectroscopy-based approach to detect aging-related oxidative damage in the mouse oocyte. J Assist Reprod Genet. 2013;30(7):877–82.
163. Fleming TP, Watkins AJ, Velazquez MA, Mathers JC, Prentice AM, Stephenson J, Barker M, Saffery R, Yajnik CS, Eckert JJ, Hanson MA, Forrester T, Gluckman PD, Godfrey KM. Origins of lifetime health around the time of conception: causes and consequences. Lancet. 2018;391:1842–52.
164. Rinaudo P, Wang E. Fetal programming and metabolic syndrome. Annu Rev Physiol. 2012;74:107–30.
165. Kwong W, Wild A, Roberts P, Willis A, Fleming T. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. Development. 2000;127:4195–420.
166. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Mol Cell Endocrinol. 2001;185:93–8.
167. Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. Nature. 2007;447:425–32.
168. 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;62:1526–35.
169. 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;79:618–23.
170. Basatemur E, Shevlin M, Sutcliffe A. Growth of children conceived by IVF and ICSI up to 12 years of age. Reprod Biomed Online. 2010;20:144–9.
171. Schendelaar P, Middelburg KJ, Bos AF, Heineman MJ, Jongbloed Pereboom M, Hadders AM. The Groningen ART cohort study: the effects of ovarian hyperstimulation and the IVF laboratory procedures on neurological condition at 2 years. Hum Reprod. 2011;26:703–12.
172. Wagenaar K, vanWeissenbruch 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;95:611–6.
173. Ceelen M, van Weissenbruch MM, Prein J, Smit JJ, Vermeiden JP, Spreeuwenberg M, van Leeuwen FE, de Waal HA D-v. 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;24:2788–95.
174. Ceelen M, van Weissenbruch MM, Roos JC, Vermeiden JPW, van Leeuwen FE, van de Waal HAD. Body composition in children and adolescents born after in vitro fertilization or spontaneous conception. J Clin Endocrinol Metab. 2007;92:3417–23.
175. Meister TA, Rimoldi SF, Soria R, von Arx R, Messerli FH, Sartori C, Scherrer U, Rexhaj E. Association of assisted reproductive technologies with arterial hypertension during adolescence. J Am Coll Cardiol. 2018;72:1267–74.
176. Scherrer U, Rimoldi SF, Rexhaj E, Stuber T, Duplain H, Garcin S, de Marchi SF, Nicod P, Germond M, Allemann Y, Sartori C. Systemic and pulmonary vascular dysfunction in children conceived by assisted reproductive technologies. Circulation. 2012;125:1890–6.
177. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, Haan EA, Chan A. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012;366:1803–13.
178. Tararbit K, Lelong N, Thieulin AC, Houyel L, Bonnet D, Goffinet F, Khoshnood B, Group ES. The risk for four specific congenital heart defects associated with assisted reproductive techniques: a population based evaluation. Hum Reprod. 2013;28:367–74.
179. Luke B, Brown MB, Wantman E, Forestieri NE, Browne ML, Fisher SC, Yazdy MM, Ethen MK, Canfield MA, Watkins S, Nichols HB, Farland LV, Oehninger S, Doody KJ, Eisenberg ML, Baker VL. The risk of birth defects with conception by ART. Hum Reprod. 2021;36(1):116–29. https://doi.org/10.1093/humrep/deaa272.
180. Luke B, Brown MB, Wantman E, Schymura MJ, Browne ML, Fisher SC, Forestieri NE, Rao C, Nichols HB, Yazdy MM, Gershman ST, Sacha CR, Williams M, Ethen MK, Canfield MA, Doody KJ, Eisenberg ML, Baker VL, Williams C, et al. The risks of birth defects and childhood cancer with conception by assisted reproductive technology. Hum Reprod. 2022;37(11):2672–89. https://doi.org/10.1093/humrep/deac196.