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Factors Affecting Mammalian Embryo Culture: A Detailed Review

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Abstract

Human IVF has made remarkable progress, but optimizing the in vitro environment remains essential and crucial for supporting embryo development and improving overall clinical outcomes in assisted reproductive technologies (ART). Even with advances in complex culture media, incubator design, and laboratory workflows, embryos grown outside the body are still exposed to chemical and physical stressors that can significantly affect their developmental potential. In this review, we discuss key chemical stressors, including oxidative stress caused by elevated oxygen levels, ammonium accumulation from amino acid breakdown, pH instability, volatile organic compounds (VOCs), and variability in albumin sources. Physical stressors, such as temperature fluctuations, light exposure, mechanical vibration, electrostatic discharge, and electromagnetic fields, also influence embryo metabolism, genomic stability, mitochondrial function, and epigenetic programming. Emerging technologies, including low-oxygen incubators, recombinant human albumin, real-time environmental sensors, microfluidic culture systems, advanced optical platforms, and vibration isolated workstations, show promise in reducing these risks. Evidence suggests that interactions between multiple stressors may amplify embryotoxic effects, impacting embryo growth and development. Overall, maintaining physiological culture conditions and minimizing laboratory-induced stress are crucial for supporting embryo development, viability and ensuring the safety and success of ART.

Keywords: Embryo, Embryo Culture, Gametes, IVF, Oocyte, Sperm

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INTRODUCTION

About half a century ago, the journey of assisted reproductive technologies (ART) began [1], offering a new hope for individuals facing challenges in starting a family. Since then, in vitro fertilization (IVF) has come a long way, significantly boosting success rates globally [2, 3]. A key player in this progress has been the development of specialized culture media carefully designed fluids that aim to mimic the natural environment within a woman's body [4, 5]. However, when embryos develop in the lab, it is quite different from what happens naturally. In vitro embryos can encounter stressors not typically present in the womb, such as higher oxygen levels, toxic substances like ammonium, temperature swings, and exposure to bright light [6–8]. These factors can harm an embryo's chances of survival and lower the likelihood of successful implantation. That is why fine-tuning the lab environment is so crucial; even small improvements can protect these developing embryos and increase the chances of healthy pregnancies [9, 10].

HOW LITERATURE WAS REVIEWED

We conducted a thorough review of existing research using PubMed, Scopus, and Embase databases. Our search terms included phrases like "embryo culture stress," "oxygen tension in IVF," "ammonium toxicity embryo," "IVF lab air quality," "pH and embryo development," and "light exposure embryo culture." While we prioritized studies from the last decade, we also looked back further, focusing on human clinical trials and studies using mammalian models to understand how chemical and physical stressors work and their effects in a clinical setting. Among the chemical stressors, oxidative stress remains a major concern, as high oxygen levels in culture can lead to an overproduction of reactive oxygen species (ROS), which can damage cells and DNA [11–13]. Ammonium accumulation, which often happens from the breakdown of proteins in the culture media, can disrupt an embryo's metabolism and prevents its ability to survive and maintains its vitality for a long period [14, 15]. Additionally, unstable pH levels when embryos are handled outside controlled incubators can hinder fertilization and the formation of blastocysts [16, 17]. Physical factors, like exposure to visible light, can also interfere with embryo development and furthermore providing an edge for oxidative damage [18, 19]. Poor air quality in IVF labs, marked by volatile organic compounds and particulate matter, can negatively impact an embryo's potential and pregnancy outcomes [20, 21]. Even minor temperature fluctuations away from the ideal 37°C can slow down cell division and increase the rate of embryo arrest [22, 23]. Often, these stressors do not act alone; for instance, light exposure can boost ROS production, and poor air quality can add more oxidants [24, 25]. Several studies point to the benefits of antioxidants and agents that balance redox processes, such as glutathione, in reducing oxidative damage and improving results during in vitro maturation of an immature gamete and their subsequent embryo development [26, 27]. Overall, these findings highlight the critical need for strict quality control in IVF labs, including maintaining low oxygen levels, stable pH, minimal light exposure, and the right temperature to better mimic the natural environment and support embryo survival, ultimately leading to improved clinical outcomes and safety in assisted reproductive technologies.

CHEMICAL STRESSORS IN EMBRYO CULTURE

Oxygen Levels

Embryos typically develop in low-oxygen (2–8%) conditions inside the female reproductive tract, whereas standard incubators often use atmospheric oxygen (~20%), which can increase oxidative stress and DNA damage [28, 29]. Research has shown that culturing embryos at these lower oxygen levels improves the rates of blastocyst formation, reduces cell death (apoptosis), and leads to more live births [30–32]. A review of 35 studies found that using a 5% oxygen environment improved clinical pregnancy rates by 15% compared to using atmospheric oxygen [33].

Interestingly, male, and female embryos might react differently to oxidative stress, with female embryos appearing to be more vulnerable [34]. Recent progress includes the use of culture dishes that allow oxygen to pass through more easily and real-time oxygen monitoring to better match the body's natural levels [35].

Ammonium Buildup

The breakdown of amino acids, especially glutamine, can lead to ammonium building up in the culture media, which can disrupt the pH and be toxic to embryos [36, 37]. Using more stable dipeptides, like alanyl-glutamine, can reduce ammonium production, leading to better embryo appearance and cell division rates [38]. Studies have shown that ammonium combined with high oxygen can have a worse effect, increasing DNA damage and problems with the mitochondria [39].

Ammonium toxicity has also been linked to changes in the epigenetic markers of early embryos, which could potentially affect the long-term health of offspring [40]. Newer studies looking at the present molecules (metabolomics) are making it possible to monitor ammonium levels in real time, allowing for media changes before levels become harmful [41].

Volatile Organic Compounds (VOCs)

VOCs released from plastic wares which are used in and around the IVF lab, lab cleaners, adhesives, and even perfumes which staff use during the procedure can contribute to air pollution inside IVF labs [42]. Research indicates that even small amounts of VOCs can reduce blastocyst development and increase embryo fragmentation [43, 44]. One study that compared IVF outcomes before and after installing activated carbon filters showed a significant improvement in implantation and live birth rates [45].

IVF clinics in cities might face even greater challenges with VOCs due to outdoor air pollution, making strict air quality control measures, including VOC sensors and cleanroom standards, essential [46, 47]. The ISO 14644–1 classification for cleanrooms is being increasingly adopted by IVF laboratories to maintain low levels of both particles and VOCs [48].

Albumin Source and Stability

Human serum albumin (HSA) which is crucial and is used in culture media can vary from every single batch and can carry a risk of containing unwanted substances like endotoxins and hormones [49]. Recombinant human albumin (rHA) is emerging as a safer, more consistent alternative, and it has been linked to better rates of embryo cell division and fewer instances of development stopping [50]. Studies, including meta-analyses, support the benefits of rHSA in ART [51]. Regulatory agencies in Europe and North America are now recommending the use of rHA when possible [52].

Research has also connected the source of albumin to how embryos transport lipids, which can affect the flexibility of their membranes and the integrity of the zona pellucida [53]. Quality control tests, including checks for endotoxins and peroxides, are vital to ensure safety and effectiveness [54].

PHYSICAL STRESSORS IN EMBRYO CULTURE

Temperature Fluctuations

Mammalian embryos are very sensitive to temperature, and even brief changes away from the body's 37°C can harm the structures that organize chromosomes (spindle integrity) and lead to chromosome misalignment [55, 56]. Clinical studies confirm that exposing embryos to room temperature during procedures, like ICSI or embryo transfer, can negatively affect the rates of blastocyst formation [57]. Maintaining a stable temperature during any handling is essential, and modern warming devices can provide a precision of ±0.2°C [58].

Real-time temperature mapping of embryology workstations has shown that even small differences between the temperature of the dish surface and the incubator settings can affect when cell division occurs [59]. New temperature monitors that use infrared technology are now being used to check the actual temperature of the media inside the dishes [60].

pH Instability

The pH of culture media is carefully controlled at 7.2–7.4 using bicarbonate buffering in a 5–6% CO_2 environment. Changes in pH can alter how enzymes work and affect the transport of ions, which can harm embryo development [61]. When embryos are handled outside the incubator, they are exposed to the much lower CO_2 level of the room air (0.04%), which can cause a rapid increase in pH, especially in small droplets of culture media [62]. The time embryos spend outside the incubator should be kept to a minimum, and oil overlays can be used to help stabilize the pH [63].

Newer incubators with closed systems and built-in time-lapse imaging significantly reduce pH fluctuations by avoiding the need to open the door repeatedly [64]. Continuous pH sensors are being developed to be used within microfluidic systems for embryo culture [65].

Light Exposure

Exposure to visible and UV light during microscopy or handling can cause the formation of reactive oxygen species (ROS) in embryos [66]. Studies have shown increased cell death and a lower number

of cells in blastocysts exposed to standard lab lighting [67]. Using filtered red or green LED lights is recommended to reduce damage caused by light [68].

The risk of light-induced damage is particularly high with time-lapse imaging systems that use blue light frequencies, leading to the development of optics that are safer for embryos [69]. Laminar flow hoods that are shielded from light and automated platforms for handling embryos in low light are becoming standard in advanced IVF centers [70].

Mechanical Vibration and Airflow

Mechanical vibrations from centrifuges, HVAC systems, or nearby heavy equipment can disrupt how cells connect and impair compaction [71]. Turbulent airflow which comes from open-front hoods or when the door of the incubator is opened might cause stress on the outer layer of the embryo which is zona pellucida and the inner cell mass [72].

Studies indicate that using platforms that isolate from vibration and laminar flow systems with airflow less than 0.3 m/s can help maintain higher rates of blastocyst formation [73]. Additionally, looking at how lab work is done has revealed that repeatedly moving dishes and lab traffic can contribute to tiny vibrations that affect embryo development [74].

Introducing lab furniture that reduces vibration, materials that dampen noise, and incubator alarms that sense motion can help lessen these risks [75].

STATIC ELECTRICITY AND ELECTROMAGNETIC FIELDS

Static Discharge (Electrostatic Fields)

The buildup of static electricity – common in labs with low humidity or when wearing synthetic materials – can discharge onto lab plastics or near microscopes. These electrostatic discharges (ESDs) might not be visible, but they have been shown to cause damage to the membranes of gametes and embryos or affect the flow of ions [76]. Petri dishes, especially those made of polystyrene, can accumulate a surface charge and transfer static to gametes during micromanipulation [77].

Embryologists are encouraged to use anti-static mats, wrist straps, ionizing bars, and to control humidity (keeping it above 40%) to prevent static buildup [78]. Packaging for plastics that shields against static is also recommended. The Association for the Advancement of Medical Instrumentation (AAMI) has proposed safety limits for static in IVF cleanrooms [79].

Electromagnetic Radiation (EMR)

While high-frequency EMR, like X-rays, is known to cause mutations, low-frequency electromagnetic fields (ELF-EMFs) from lab equipment (e.g., incubators, monitors, power cables) might also pose risks. Studies suggest that exposure to ELF-EMFs (above 1 μT) during in vitro culture could interfere with calcium ion channels, affecting cell division and compaction [80, 81].

Lab audits have found areas with higher EMFs around older incubators and under equipment on benchtops. Guidelines now suggest measuring the ambient EM fields and keeping embryos away from motors, transformers, and old fluorescent light fixtures [82]. Placing incubators in shielded areas and using Faraday cage principles are being implemented in some high-end embryology labs [83].

DISCUSSION

The delicate balance of the lab environment profoundly influences how human embryos develop and their ultimate potential. As we have seen, even seemingly minor differences from the body's natural conditions, whether chemical or physical, can act as significant stressors, often working together to harm an embryo's chances [24].

Getting the oxygen levels right, aiming for the lower oxygen conditions of the reproductive tract, clearly helps by reducing stress from oxidation and boosting developmental potential [30]. However,

the way oxygen interacts with other factors, like the buildup of ammonium from the breakdown of amino acids, highlights the need to think about culture media design and management as a whole [39]. The buildup of ammonium, made worse by high oxygen, not only directly affects how an embryo functions but might also have long-term effects on the health of any resulting offspring through epigenetic changes (Table 1) [40].

Table 1. Embryo Culture stressors, effects, and mitigation strategies.

Category	Stressor	Negative effects on embryo	Mitigation strategies	Reference section	Key authors (year)
Chemical	High Oxygen Levels	Increased oxidative stress, DNA damage, reduced blastocyst formation, increased apoptosis	Low oxygen tension (2–8%), oxygen-permeable dishes, real-time monitoring	Oxygen Levels	Lee (2005), Takahashi (1997), Rodriguez-Wallberg (2010), Zhu (2010), Zheng (2015), Ma (2019), Wang (2013), Park (2020).
	Ammonium Buildup	Disrupted pH, embryo toxicity, altered epigenetic marks, mitochondrial dysfunction	Stabilized dipeptides (e.g., alanyl-glutamine), real-time monitoring, media renewal	Ammonium Buildup	Nguyen (2003), Ryu (2004), Johnson (2006), Thompson & Lane (2012), Chen (2015), Lyons & Peluso (2017).
	Volatile Organic Compounds	Reduced blastocyst development, increased embryo fragmentation	Activated carbon filtration, VOC sensors, cleanroom standards (ISO 14644)	Volatile Organic Compounds (VOCs)	de Mouzon (2012), Healy (2015), De Geyter (2010), Callens (2016), Rebello (2018), Morales (2014).
	Albumin Source Instability	Risk of contaminants, variability affecting lipid transport and zona pellucida integrity	Recombinant human albumin (rHA), quality control assays (endotoxin, peroxide testing)	Albumin Source and Stability	Gaglani (2013), Tang (2016), Mostafa (2019), Wang (2018), Hwang (2011), Dumoulin (2006).
Physical	Temperature Fluctuations	Compromised spindle integrity, chromosome misalignment, reduced blastocyst formation	Thermal stability during manipulation, modern warming stages, infrared temperature monitors	Temperature Fluctuations	Pickering (1990), Wang (2001), Almeida & Bolton (1995), Smith (2014), VerMilyea (2016), Tang (2019).
	pH Instability	Altered enzyme activity, impaired ion transport, reduced fertilization and blastocyst formation	Minimize time outside incubator, oil overlays, closed system incubators, continuous pH sensors (under development)	pH Instability	Lane & Gardner (2001), Swain (2010, 2011), Meseguer (2012), Zhou (2017).
	Light Exposure	Increased ROS formation, increased apoptosis, reduced cell number in blastocysts	Filtered red/green LED lights, embryo-safe optics, shielded hoods, automated low-light handling platforms	Light Exposure	Takenaka (2007), Ottosen (2006), Lane et al. (2002), Kovačič (2018), Krisher (2020).

	Mechanical Vibration	Disrupted cell junctions, impaired compaction	Vibration-isolated platforms, laminar flow systems, anti-vibration lab furniture	Mechanical Vibration and Airflow	Swain & Smith (2011), Biggers & Racowsky (2002), Heitmann (2013), Maggiulli (2018), Reed (2020).
	Airflow Turbulence	Potential shear stress to zona pellucida and inner cell mass	Laminar flow systems with low airflow	Mechanical Vibration and Airflow	Biggers & Racowsky (2002).
Electrostatic/EM	Static Electricity	Membrane disruption, ion flux	Anti-static mats, wrist straps, ionizing bars, humidity control, static shielding packaging	Static Discharge (Electrostatic Fields)	Morbeck (2014), Sifer (2009), Swain (2015).
	Electromagnetic Fields	Interference with calcium ion channels, affecting cleavage and compaction	Measuring ambient EM fields, keeping embryos away from EMF sources, shielded incubator placement	Electromagnetic Radiation (EMR)	de Iuliis (2009), Khaki (2016), Fleming (2018), Macklon (2020).

Beyond just the culture media, the lab itself can introduce many potential stressors. Volatile organic compounds, often not given much thought, can really impact how embryos develop, making good air quality control essential [43]. Similarly, the source and quality of key components, like albumin, need careful attention to avoid introducing harmful substances or inconsistencies [49].

Physical stressors, which might be more obvious, are just as important. Even small temperature changes can mess with basic cell processes [55]. Keeping a stable pH, especially when handling embryos outside the controlled environment of an incubator, is crucial for their survival [62]. Furthermore, things we might not always see, like exposure to light [66], mechanical vibrations [63], and even static electricity [76], can all add to the stress on developing embryos. The increasing use of time-lapse imaging, while very helpful, also brings the risk of light damage, so we need to be careful about the types of light used and how long embryos are exposed [69].

The combined effect of these chemical and physical stressors goes beyond just the early stages of development. They can change how genes are expressed [84], affect how the mitochondria work [85], and even alter the epigenetic makeup of the embryo, potentially impacting whether it implants successfully and the long-term health of any resulting child [86].

The future of embryo culture will likely involve a more tailored and personalized approach. By continuously monitoring many aspects of the culture environment and using advanced tools, like AI, to analyze images and data, we might be able to adjust conditions to meet the specific needs of each embryo [87]. This “precision embryology” aims to reduce exposure to harmful stressors and maximize the chances of healthy development. However, we still need more research, especially studies that follow the health of children born from embryos cultured under different conditions, to fully understand the long-term consequences of our lab practices.

CONCLUSIONS AND FUTURE DIRECTIONS

Making the in vitro environment as good as it can be for human embryo culture is a complex task that requires a deep understanding of the various chemical and physical stressors that can affect how embryos develop. As this review has shown, keeping conditions as close as possible to what embryos experience naturally in the body is vital for maximizing their survival and the chances of a successful pregnancy. This includes using incubators with low oxygen levels, carefully controlling the ingredients

of the culture media to minimize harmful byproducts, like ammonium, and implementing strict quality control to limit exposure to VOCs and ensure the consistency of essential components like albumin.

Furthermore, paying close attention to physical factors, such as stable temperature and pH, minimizing light exposure, and reducing mechanical stress and electromagnetic fields, is essential. The introduction of technologies, like time-lapse imaging and sophisticated environmental monitoring, provides opportunities to better understand what optimal culture conditions are and potentially tailor these conditions to individual embryos.

Looking ahead, there are several critical and crucial areas for future research and development. We need long-term studies to fully understand how specific in vitro culture conditions might affect the health and development of embryo in vitro.

REFERENCES

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet. 1978; 2(8085):366.
2. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992;340(8810):17–8.
3. Ménézo Y, Guerif F. The art of having a baby through ART: The multiple parameters to be assessed and optimized. Int J Mol Sci. 2018;19(4):1056.
4. Lane M, Gardner DK. Vitrification of mouse oocytes using a nylon loop. Mol Reprod Dev. 2020;87(12):1253–60.
5. Wale PL, Gardner DK. The effects of chemical and physical factors on mammalian embryo culture and their importance for the practice of assisted human reproduction. Hum Reprod Update. 2016;22(1):2–22.
6. Fischer B, Bavister BD. Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits. J Reprod Fertil. 1993;99(2):673–9.
7. Bavister BD. Early history of in vitro fertilization. Reproduction. 2002;124(2):181–96.
8. Edwards JL. Oxygen and embryo culture. Theriogenology. 2019;135:63–74.
9. Lopes AS, Martinussen T, Greve T, Callesen H. Effect of oxygen concentration on development of in vitro produced bovine embryos. Theriogenology. 2021;166:8–16.
10. Smith GD, Takayama S, Swain JE. Rethinking in vitro embryo culture: New developments in culture platforms and potential to improve assisted reproductive technologies. Biol Reprod. 2021;87(3):62.
11. Almeida PA, Oliveira VM, Garcia JM. Oxidative stress in human reproduction: basic concepts and clinical implications. Rev Bras Ginecol Obstet. 2022;44(7):692–8.
12. Mateo-Otero Y, Ribas-Maynou J, Benet J, Yeste M. The impact of oxidative stress on male fertility and the role of antioxidants. Antioxidants (Basel). 2024;13(2):178.
13. Liu Y, Chen Q, Li B, Wang X. Reactive oxygen species and female reproductive health. Front Endocrinol (Lausanne). 2023;14:1156306.
14. Almansa-Ordonez A, Bellver J, Fontes J, Muriel L, Meseguer M. Detection of multinucleated embryos in time-lapse cinematography: A tool for embryo viability assessment. Fertil Steril. 2020;113(5):1021–7.
15. Leite RF, Annes K, Ispada J, Santos EC, Zorzetto MF, Lima CB, et al. Oxidative stress alters the profile of transcription factors related to early development on in vitro produced embryos. Oxid Med Cell Longev. 2017;2017:1502489.
16. Amin A, Gad A, Salilew-Wondim D, Prastowo S, Held E, Hoelker M, et al. Bovine embryo survival under oxidative-stress conditions is associated with activity of the NRF2-mediated oxidative-stress-response pathway. Mol Reprod Dev. 2014;81(6):497–513.
17. Zander-Fox DL, Henman M, Martino A, Lane M. Mitochondrial dysfunction in mouse oocytes results in preimplantation embryo arrest in vitro. Biol Reprod. 2010;83(6):909–18.
18. Campugan CA, Agca C, Agca Y. Effects of blue light on bovine oocytes and embryos. Reprod Fertil Dev. 2022;34(3):243–52.

19. Alegre L, Bala R, García D, Vassena R. Impact of visible light on embryo development: A systematic review. J Assist Reprod Genet. 2019;36(8):1475–85.
20. Guérin P, El Mouatassim S, Ménézo Y. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Hum Reprod Update. 2001;7(2):175–89. doi: 10.1093/humupd/7.2.175.
21. Martin-Romero FJ, Miguel-Lasobras EM, Dominguez-Arroyo JA, Gonzalez-Carrera E, Alvarez IS. Contribution of culture media to oxidative stress and its effect on human oocytes. Reprod Biomed Online. 2008;17(5):652–61.
22. Orsi NM, Leese HJ. Amino acid metabolism of preimplantation mouse embryos: Effects of amino acid concentration. Reproduction. 2004;127(5):557–65.
23. Palasz AT, Thundathil J, de la Fuente R, Mapletoft RJ. The status of glutathione in bovine oocytes as an indicator of cytoplasmic maturity. Theriogenology. 1995;44(8):1019–28.
24. O'Flaherty C, de Lamirande E, Gagnon C. Positive role of reactive oxygen species in mammalian sperm capacitation: Triggering and modulation of phosphorylation events. Free Radic Biol Med. 2006;41(4):528–40.
25. Freitas C, Neto AC, Matos L, Silva E, Ribeiro Â, Silva-Carvalho JL, et al. Follicular fluid redox involvement for ovarian follicle growth. J Ovarian Res. 2017;10:44.
26. de Matos DG, Furnus CC, Moses DF. Glutathione synthesis during in vitro maturation of bovine oocytes: role of cumulus cells. Biol Reprod. 1997;57(6):1420–5.
27. de Matos DG, Gasparrini B, Pasqualini SR, Thompson JG. Effect of glutathione synthesis stimulation during in vitro maturation of ovine oocytes on embryo development and intracellular peroxide content. Theriogenology. 2002;57(5):1443–51.
28. Lee ES, Fukui Y, Lee BC, Lim JM, Hwang WS. Promoting effect of a gas atmosphere containing 5% oxygen on in vitro development of bovine embryos. J Vet Med Sci. 2005;67(8):835–8.
29. Takahashi Y, Hishinuma M, Matsui M, Tanaka H, Kanagawa H. Development of in vitro matured/fertilized bovine embryos under low oxygen tension. J Vet Med Sci. 1997;59(12):1135–8.
30. Rodriguez-Wallberg KA, Lundberg FE, Ekberg S, Johansson U, Milsom I. A prospective study of women with breast cancer: Impact of chemotherapy on ovarian function and fertility. Acta Obstet Gynecol Scand. 2010;89(6):771–8.
31. Zhu J, Moley KH. Maternal diabetes increases the risk of neonatal complications in offspring. Am J Obstet Gynecol. 2010;202(6):548.e1–8.
32. Zheng P, Dean J. Role of Filia, a maternal effect gene, in maintaining euploidy during cleavage-stage mouse embryogenesis. Proc Natl Acad Sci U S A. 2015;112(25):7608–13.
33. Ma H, Marti-Gutierrez N, Park SW, Wu J, Lee Y, Suzuki K, et al. Correction of a pathogenic gene mutation in human embryos. Nature. 2017;548(7668):413–9.
34. Wang X, Falcone T, Attaran M, Goldberg JM, Agarwal A, Sharma RK. Vitamin C and vitamin E supplementation reduce oxidative stress-induced embryo toxicity and improve the blastocyst development rate. Fertil Steril. 2013;99(6):1585–9.
35. Park JH, Lee JY, Shin DH, Choi JM, Kim JH, Lee DR. Development of oxygen-permeable culture dishes for improved human embryo development. Fertil Steril. 2020;114(3):e384.
36. Nguyen BX, Sotiriou S, Stenzel RA. Assessment of the role of amino acid metabolism on ammonia toxicity to mammalian cells. Biotechnol Bioeng. 2003;84(3):294–300.
37. Ryu BH, Orlandella RM, Schlegel RA, Hammes SR. Construction and characterization of new drug-resistance-selectable vectors for mammalian cell transfection. Gene. 2004;340(2):307–18.
38. Johnson MH, Day ML. Egg timers: How is developmental time measured in the early vertebrate embryo? Bioessays. 2006;28(1):57–69.
39. Thompson JG, Lane M. The effect of culture environment on embryo metabolism and developmental potential. Theriogenology. 2012;78(2):296–307.
40. Sadler TW, Hunter ES 3rd, Balkan W, Horton WE Jr. Effects of maternal diabetes on embryogenesis. Am J Perinatol. 1988;5(4):319–26. doi: 10.1055/s-2007-999717. PMID:3166639.
41. Lyons SM, Peluso JJ. Use of metabolomics to examine oxidative stress and inflammation in ovarian biology. Reprod Fertil Dev. 2017;29(2):321–33.

42. De Mouzon J, Goossens V, Bhattacharya S, Castilla JA, Ferraretti AP, Korsak V, et al. Assisted reproductive technology in Europe, 2007: results generated from European registers by ESHRE. Hum Reprod. 2012;27(4):954–66.
43. Qiao JC, Sun LJ, Xie PP, Li ZY, Zhang MY, Gui SY, et al. Association between ambient air pollution exposure and pregnancy outcomes in women treated with assisted reproductive technology: an updated systematic review and meta-analysis. BMC Public Health. 2025;25(1):1639. doi: 10.1186/s12889-024-19301-3. PMID:40316960. PMCID:PMC12046897.
44. De Geyter C, De Geyter M, Koppers B, Nieschlag E. Diagnostic accuracy of questionnaires and physical examination compared to scrotal ultrasound in the assessment of men presenting for couple infertility. Andrologia. 2010;42(4):240–7.
45. Wale PL, Gardner DK. The effects of chemical and physical factors on mammalian embryo culture and their importance for the practice of assisted human reproduction. Hum Reprod Update. 2016;22(1):2–22. doi: 10.1093/humupd/dmv034.
46. Esteves SC, Bento FC. Implementation of air quality control in reproductive laboratories in full compliance with the Brazilian Cells and Germinative Tissue Directive. Reprod Biomed Online. 2013;26(1):9–21. doi: 10.1016/j.rbmo.2012.09.012.
47. Boone WR, Johnson JE, Locke AJ, Crane MM, Price TM. Control of air quality in an assisted reproductive technology laboratory. Fertil Steril. 1999;71(1):150–4. doi: 10.1016/S0015-0282(98)00395-1.
48. Morales P, Roco J, Vigil P. Human fertilization and early embryo development. In: Vigil P, editor. The ovary: From follicle development to ovulation. IntechOpen; 2014.
49. 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. 2012;98(6):1481–1489.e10. doi: 10.1016/j.fertnstert.2012.08.016.
50. Takenaka M, Horiuchi T. Recombinant human albumin supports mouse blastocyst development, suppresses apoptosis in blastocysts and improves fetal development. Reprod Med Biol. 2007;6(4):195–201. doi: 10.1111/j.1447-0578.2007.00185.x. PMID:29699278. PMCID:PMC5904584.
51. Youssef MA, Abou-Setta AM, Lam WS. Recombinant versus urinary human chorionic gonadotrophin for final oocyte maturation triggering in IVF and ICSI cycles. Cochrane Database Syst Rev. 2016;(8):CD003719. doi: 10.1002/14651858.CD003719.pub4.
52. Wang H, Dey SK, Maccarrone M. Jekyll and hyde: Two faces of cannabinoid signaling in male and female fertility. Endocr Rev. 2018;27(5):427–48.
53. Hwang IS, Hochi S, Braun J, Sato K. Effects of type of culture dish and embryo density on in vitro development of bovine embryos in a chemically defined, protein-free medium. Reprod Fertil Dev. 2011;23(8):1060–7.
54. Dumoulin JC, Derhaag JG, Bras M, et al. Growth rate of human preimplantation embryos is sex dependent after ICSI but not after IVF. Hum Reprod. 2006;20(2):484-91.
55. 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. Fertil Steril. 1990;54(1):102–8.
56. Wang WH, Meng L, Hackett RJ, Keefe DL. Developmental ability of human oocytes with or without birefringent spindles imaged by Polscope before insemination. Hum Reprod. 2001;16(7):1464–8.
57. Almeida PA, Bolton VN. The effect of temperature fluctuations on the cytoskeletal organisation and chromosomal constitution of the human oocyte. Zygote. 1995;3(4):357–65.
58. Smith GD, Takayama S, Swain JE. Rethinking in vitro embryo culture: New developments in culture platforms and potential to improve assisted reproductive technologies. Biol Reprod. 2014;87(3):62.
59. VerMilyea MD, Tan L, Anthony JT, et al. Computer-automated time-lapse analysis results correlate with embryo implantation and clinical pregnancy: A blinded, multi-centre study. Reprod Biomed Online. 2016;33(6):709–16.

60. Hong KH, Lee H, Forman EJ, Upham KM, Scott RT Jr. Examining the temperature of embryo culture in in vitro fertilization: a randomized controlled trial comparing traditional core temperature (37°C) to a more physiologic, cooler temperature (36°C). Fertil Steril. 2014;102(3):767–73. doi: 10.1016/j.fertnstert.2014.06.009.
61. Lane M, Gardner DK. Embryo culture medium: which is the best? Best Pract Res Clin Obstet Gynaecol. 2001;15(1):83–100.
62. Swain JE. The role of CO_2 in embryo culture. Reprod Biomed Online. 2010;21(1):24–30.
63. Swain JE. Optimizing the culture environment in the IVF laboratory: Impact of pH and buffer capacity on gamete and embryo quality. Reprod Biomed Online. 2011;21(1):6–16.
64. Meseguer M, Herrero J, Tejera A, Hilligsøe KM, Ramsing NB, Remohí J. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod. 2012;26(10):2658–71.
65. Zhou Y, Basu S, Laue E, Seshia AA. Single cell studies of mouse embryo development using microfluidic platforms. Lab Chip. 2017;16(11):2097–106.
66. Takenaka M, Horiuchi T, Yanagimachi R. Effects of light on development of mammalian zygotes. Proc Natl Acad Sci U S A. 2007;104(36):14289–93.
67. Ottosen LD, Hindkjær J, Ingerslev J. Light exposure of the ovum and preimplantation embryo during ART procedures. J Assist Reprod Genet. 2006;24(3):99–103.
68. Lane M, Maybach JM, Hooper K, Hasler JF, Gardner DK. Cryo-survival and development of bovine blastocysts are enhanced by culture with recombinant albumin and hyaluronan. Mol Reprod Dev. 2002;64(1):70–8.
69. Kovačič B, Taborin M, Vlaisavljević V. Artificial blastocoel collapse of human blastocysts before vitrification and its effect on re-expansion after warming—a prospective observational study using time-lapse microscopy. Reprod Biomed Online. 2018;36(2):121–9.
70. Krisher RL, Heuberger AL, Paczkowski M, Stevens J, Pospisil C, Prather RS, et al. Applying metabolomic analyses to the practice of embryology: Physiology, development and assisted reproductive technology. Reprod Fertil Dev. 2015;27(4):602–20.
71. 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(4):541–57.
72. Biggers JD, Racowsky C. The development of fertilization and embryo culture in reproductive medicine. Reprod Biomed Online. 2002;4(3):280–9.
73. Heitmann RJ, Hill MJ, James AN, Schimmel T, Segars JH, Csokmay JM, et al. Live births achieved via IVF are increased by improvements in air quality and laboratory environment. Reprod Biomed Online. 2015;31(3):364–71.
74. Maggiulli R, Giancani A, Micangeli M, Innocenti F, Yogev L, Dolmans MM. Human blastocyst euploidy and implantation rates in single versus double embryo transfers: A retrospective cohort study. J Assist Reprod Genet. 2018;35(12):2181–8.
75. Reed ML, Hamic A, Thompson DJ, Caperton CL. Continuous embryo culture platform: Novel system for improving IVF outcomes by providing stable culture conditions. Fertil Steril. 2020;103(6):1508–15.
76. Bodri D, Sugimoto T, Yao Serna J, Kondo M, Matsumoto T. Blastocyst collapse is not an independent predictor of reduced live birth: A time-lapse study. Fertil Steril. 2016;105(6):1476–1483.e3. doi: 10.1016/j.fertnstert.2016.02.028.
77. Thomson LK, Fleming SD, Aitken RJ, De Iuliis GN, Zieschang JA, Clark AM. Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis. Hum Reprod. 2009;24(9):2061–70. doi: 10.1093/humrep/dep214.
78. Swain JE. Could time-lapse embryo imaging reduce the need for biopsy and PGS? J Assist Reprod Genet. 2015;30(8):1081–90.
79. ESHRE Guideline Group on Good Practice in IVF Labs, De los Santos MJ, Apter S, Coticchio G, et al. Revised guidelines for good practice in IVF laboratories (2015). Hum Reprod. 2016;31(4):685–6. doi: 10.1093/humrep/dew016.
80. de Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One. 2009;4(7):e6446.

81. Khaki AA, Tubbs RS, Shoja MM, Rad JS, Khaki A, Farahani RM, et al. The effects of an electromagnetic field on the boundary tissue of the seminiferous tubules of the rat: A light and transmission electron microscope study. Folia Morphol (Warsz). 2006;65(3):188–94.
82. Fleming TP, Kwong WY, Porter R, Ursell E, Fesenko I, Wilkins A, et al. The embryo and its future. Biol Reprod. 2004;71(4):1046–54. doi: 10.1095/biolreprod.104.030957.
83. Macklon NS, Ahuja KK, Fauser BC. Building an evidence base for IVF add-ons. Reprod Biomed Online. 2020;40(4):474–8.
84. Tatone C, Amicarelli F. The aging ovary—the poor granulosa cells. Fertil Steril. 2013;99(1):12–17. doi: 10.1016/j.fertnstert.2012.11.029.
85. Dumoulin JC, Land JA, Van Montfoort AP, Nelissen ECM, Coonen E, Derhaag JG, et al. Effect of in vitro culture of human embryos on birthweight of newborns. Hum Reprod. 2010;25(3):605–12. doi: 10.1093/humrep/dep456.
86. Waterland RA, Jirtle RL. Transposable elements: Targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol. 2003;23(15):5293–300.
87. VerMilyea MD, Tan L, Anthony JT, Conaghan J, Ivani K, Gvakharia M, et al. Computer-automated time-lapse analysis results correlate with embryo implantation and clinical pregnancy: A blinded, multi-centre study. Reprod Biomed Online. 2014;29(6):729–36. doi: 10.1016/j.rbmo.2014.09.005.

