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Air quality in the clinical embryology laboratory: a mini-review

Romualdo Sciorio, Erika Rapalini and Sandro C. Esteves

***Abstract*:** The scope of the clinical embryology laboratory has expanded over recent years. It now includes conventional in vitro fertilization (IVF) techniques and complex and time-demanding procedures like blastocyst culture, processing of surgically retrieved sperm, and trophectoderm biopsy for preimplantation genetic testing. These procedures require a stable culture environment in which ambient air quality might play a critical role. The existing data indicate that both particulate matter and chemical pollution adversely affect IVF results, with low levels for better outcomes. As a result, IVF clinics have invested in air cleaning technologies with variable efficiency to remove particulates and volatile organic compounds. However, specific regulatory frameworks mandating air quality control are limited, as are evidence-based guidelines for the best air quality control practices in the embryology laboratory. In this review, we describe the principles and existing solutions for improving air quality and summarize the clinical evidence concerning air quality control in the embryology laboratory. In addition, we discuss the gaps in knowledge that could guide future research to improve clinical outcomes.

Keywords: air quality (AQ), assisted reproductive technology (ART), embryo culture, in vitro fertilization (IVF), volatile organic compounds (VOCs)

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Background

The number of couples facing infertility has increased steadily, many of whom will ultimately need medically assisted reproductive (MAR) treatment. Global data report over 8 million in vitro fertilization (IVF) babies born in the last 40 years, and in the UK, IVF babies account for about 3% of all babies born in 2016.[1,2] Furthermore, in the last decades due to the social and legal equality for same-sex couples, MAR treatment are increasingly applied for those couples as well as single women/men and transgender couples. Consequently, increasing numbers of homosexual are seeking help to assisted reproductive technology (ART) to achieve parenthood in countries where it is allowed.[3] IVF is a high-complexity multi-step procedure, which has markedly evolved over the last decades.[4] Human embryogenesis involves a coordinated cascade of biochemical and molecular intracellular signaling events between gametes that results in the development of viable embryos capable of implantation and establishment of viable pregnancies to term. Indeed, the extended *in vitro* culture of human embryos constitutes one of the most challenging applications of cell culture. This process demands a more critical growth environment as gametes and embryos are especially sensitive cells types, largely unprotected as they lack epithelial surfaces, immunological defenses, detoxifying mechanisms, thus being vulnerable to environmental influences. Studies that have examined environmental and airborne pathogens have indicated that both ambient air pollution as well as laboratory air quality (AQ) may play a significant role on embryogenesis, implantation, and conception of MAR treatments.[5,6] Thus, efforts have been devoted to optimizing the embryology laboratory environment to mitigate the possible adverse effect of ambient air on IVF outcomes. Our review aims to (1) describe the principles and existing solutions for improving laboratory air quality, (2) summarize the existing evidence concerning AQ control in the embryology laboratory, and (3) highlight

Correspondence to:
Romualdo Sciorio
Edinburgh Assisted Conception Programme, EFREC, Royal Infirmary of Edinburgh, 51 Little France Crescent, Old Dalkeith Road, Edinburgh EH16 4SA, UK.
sciorioromualdo@hotmail.com

Erika Rapalini
Ospedale Versilia, Centro di Procreazione medicalmente assistita, Lido di Camaiore, Italy

Sandro C. Esteves
ANDROFERT, Andrology and Human Reproduction Clinic, Campinas, Brazil; Division of Urology, Department of Surgery, University of Campinas (UNICAMP), Campinas, Brazil

Faculty of Health, Aarhus University, Aarhus, Denmark

the main gaps in this area of knowledge that could guide future research and improve ART clinical outcomes.

What are the threats?

Particulate matter (PM) is a mixture of microscopic solids and liquid droplets measuring from 1 to 100 microns, in temporary suspension in air. The embryology laboratory may be served by outdoor air, whose quality is influenced by many factors, including construction, vehicle traffic and exhaust, industrial and commercial emissions, waste management, and seasonal pollutants, to cite a few. Microorganisms, like viruses, spores, and bacteria, measure from <1 to up 8 microns, and are present on all inanimate surfaces and in air suspension, creating potentially sources of contamination in the embryology laboratory. They can adhere to PM and contaminate surfaces when the particles settle. Volatile organic compounds (VOCs) are any organic (carbon-containing) solid or liquid compound that evaporates at room temperature. They react with indoor ozone and create submicronic particles and harmful by-products, some of which have toxic properties and are potentially mutagenic.[7] VOCs are generated from a variety of materials,[8] the list is long and includes construction materials, such as wood furniture,[9] polyvinyl chloride flooring materials,[10] adhesives, and paints,[11] all of which release formaldehyde or aldehydes. Cosmetics like perfumes and aftershaves also release VOCs due to evaporation of their solvent, which is typically alcohol-based. Autoclaved materials release VOCs when packs are opened for use. Also, laboratory plasticware, which is made from a variety of plastics like polyethylene, polystyrene, polycarbonate, polypropylene, and acrylic, release VOCs. These compounds can also be found in CO_2 gas cylinders, insulation used in air handling systems, and refrigerant gases.[5,8,10] Similarly, cleaning products can be a source of VOCs (e.g. vinyl floor liquid wax and ammonia-based products, glass cleaners, and aerosols that contain butane or isobutane as propellants).[12] Although low-VOCs disinfectant, specifically designed to use in IVF laboratories, have been introduced, many laboratories still use ethanol-based products, which despite its effect against viruses and bacteria, release VOCs. Finally, mold growth produce carbon dioxide, water and VOC. VOCs and other small inorganic gaseous molecules, including nitrous oxide, sulfur dioxide, and heavy metals, seem to be detrimental to embryo development.[13,14] Furthermore, VOCs can harm sperm quality by attaching to the DNA, causing fragmentation and altering cell replication.[15]

Detecting VOCs in the embryology laboratory

In the clinical embryology laboratory, VOC levels are generally measured using portable direct reading instruments using VOC probes. Short- and long-term sampling (hours or days) can be utilized to provide a snapshot or average exposure over time, respectively. These instruments determine the total VOC concentration (TVOC), that is, the total concentration of all VOCs in a defined measuring range, using the photo-ionization detection (PID) method. The measurements are expressed in parts per million (ppm) or parts per billion (ppb), depending on the instrument's type and detection limits. Although the PID method has the advantage of obtaining results immediately, it does not identify the VOC type or quantify them separately. The latter will require active sampling on TenaxTA® Sorbent, thermal desorption, and gas chromatography using mass spectrometry, which provides detailed information about non-polar and weakly polar VOCs. Aldehydes will need different measurement methods (eg., 2.4-dinitrophenylhydrazine impregnated silica tubes or cartridges with subsequent solvent desorption, clean-up and liquid chromatographic analysis). Therefore, the VOC assessment in indoor air is highly dependent on how this evaluation is performed. All available methods are selective in what they can measure, and there is no device capable of measuring all VOCs. Also, the type of instrument chosen will determine the sensitivity of the measurements.[14] Importantly, the portable devices that measure TVOCs in ppm might not be able to detect potentially genotoxic, or mutagenic VOCs due to their low detection limits.[16] In the IVF laboratory, it would be more appropriate to perform VOC measurement with instruments providing readings in ppb. The results might reflect better the indoor air quality concerning the potential genotoxicity of the biochemical interaction between gametes/embryos and contaminants. In one of the author's (S.C.E.) facility, a portable VOC meter in ppb is turned on during operational hours; 100 ppb is set as the alarm level above which critical activities such as incubator openings and gamete/embryo manipulation should be avoided. Threshold values above

which an adverse effect of VOCs is to be expected in the context of human cell culture are yet to be established. Studies have reported that increased levels of VOCs were related with a statistically significant decrease in clinical pregnancy rates, but it remains mostly unknown the specific thresholds according to the type of VOC, except for aldehydes, which should be kept below the detection limit of 80–100 ppb.[8,17–19] Currently, it has been generally recommended that IVF laboratories maintain total VOC levels below 400 to 800 ppb.[20] However, a study by Worrilow and colleagues,[17] including 8 years of clinical outcomes, and evaluating the dynamic levels of VOCs and viable and nonviable particulates within the ambient air of the IVF laboratory, indicated that VOC levels far below 100 ppb affected preimplantation embryogenesis negatively.

Solutions to improve air quality

The principles of AQ control in the embryology laboratory involves four main aspects, namely: (1) air pressure differential, (2) turbulent air, (3) high-efficiency particulate air (HEPA) filtration, and (4) VOCs filtration.[21] Positive pressure creates an air pressure differential between adjacent rooms, thus minimizing retention as both particulate matter in air suspension and VOCs are carried away, whereas the newly filtered air dilutes the remaining particles and VOCs. Air pressurization also creates turbulent air, which washes out "dead" air in critical spots like those under workstations, microscopes, and other equipment. Similarly, the introduction of particles and VOCs from the outside air is avoided by the forced air movement, which uses positive air pressurization through filters. As mentioned above, microorganisms can adhere to PM; thus, a decrease in the number of particles in air suspension equates to a decrease of contamination by pathogens. Removal of air particles is commonly achieved with the use of HEPA filters. These filters are designed to remove 99.97% of particles greater than or equal to 0.3 microns. However, due to their physical characteristics, HEPA filters remove not only particles measuring 0.3 microns or higher (by sieving or impaction) but also smaller particles the size of viruses (eg., SARS-CoV-2; ~0.1 microns) by diffusion and interception methods.[22] VOCs removal is also critical for improving the AQ in the embryology laboratory. Typically, VOCs are removed by sorption filtration (mass transfer from air to adsorbent) using chemical filters, typically manufactured as a mesh embedded with activated carbon, potassium permanganate, activated alumina, and silica gels.[23] The space between the carbon particles contains delocalized electrons that act as electronic glue, thus inducing the chemical contaminants to attach to the carbon.[24] Alcohols and ketones, which cannot be removed by carbons filters, are detoxified with the use of potassium permanganate.[14] Alternatively, VOCs can be removed using ultraviolet photocatalytic oxidation (UVPCO), generally combined with the use of carbon filters.[25] UVPCO uses the energy of UV lights absorbed by a semiconductor metal oxide (eg., titanium oxide) to produce reactive species which adsorb VOCs. The photo-oxidation of VOCs causes the production of CO_2, water, and partially oxidized by-products. Volatile by-products can be released as secondary pollutants, whereas the filters adsorb non-volatile by-products. Thus, the real effectiveness of UVPCO filters is still debatable and not wholly accepted by the scientific community.[26,27] Many air cleaning solutions are commercially available with variable efficiency to control air contamination. One option consists of portable filtration systems, first proposed by Cohen and colleagues.[12] This system employs stand-alone VOCs filtration units equipped with VOCs and HEPA filters; some units may also have UVPCO (Figure 1). Data on their efficacy are minimal, and it is still premature to make a firm conclusion on the actual effectiveness of these portable units.[24,25] Another option is the use of centralized air filtration systems, in which a mix of outside and recirculated indoor air is pressurized and filtered through a series of dust, VOCs, and terminal HEPA filters (Figure 2). Typically, the amount of outside air and recirculated air in IVF laboratory cleanrooms is 20%–25% and 75%–80%, respectively. This balance is important as recirculated air is stable and has already been filtered, thus securing the optimal life for the expensive HEPA filters and reducing the running costs and energy waste. On the other hand, VOC can accumulate in the indoor environment, and fresh outside air provides a way of both diluting VOCs and reducing the risk of cross-contamination between spaces. The filtered air is distributed to the embryology laboratory and adjacent rooms via ducts. The choice of the optimal filtration system should be based on a risk management analysis. This analysis should include the design,

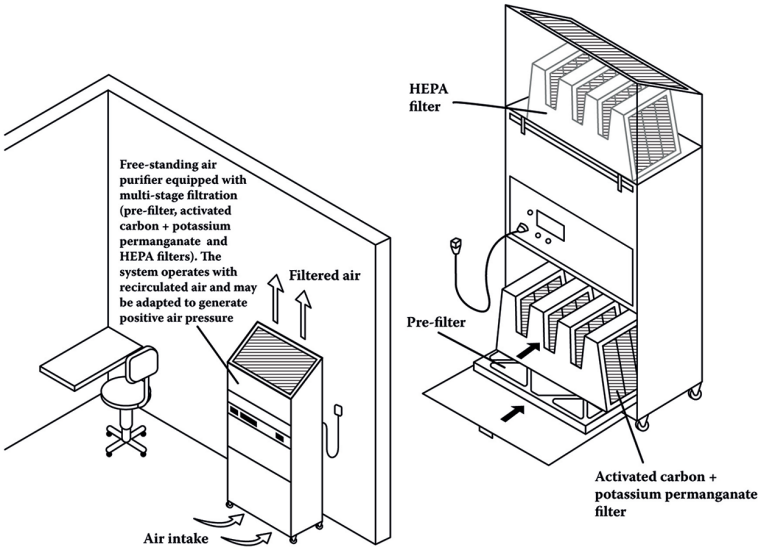

Figure 1. Illustration depicting a portable four-stage free-standing air filtration system. Reprinted from: Sadir and colleagues,[22] with permission from Taylor & Francis.

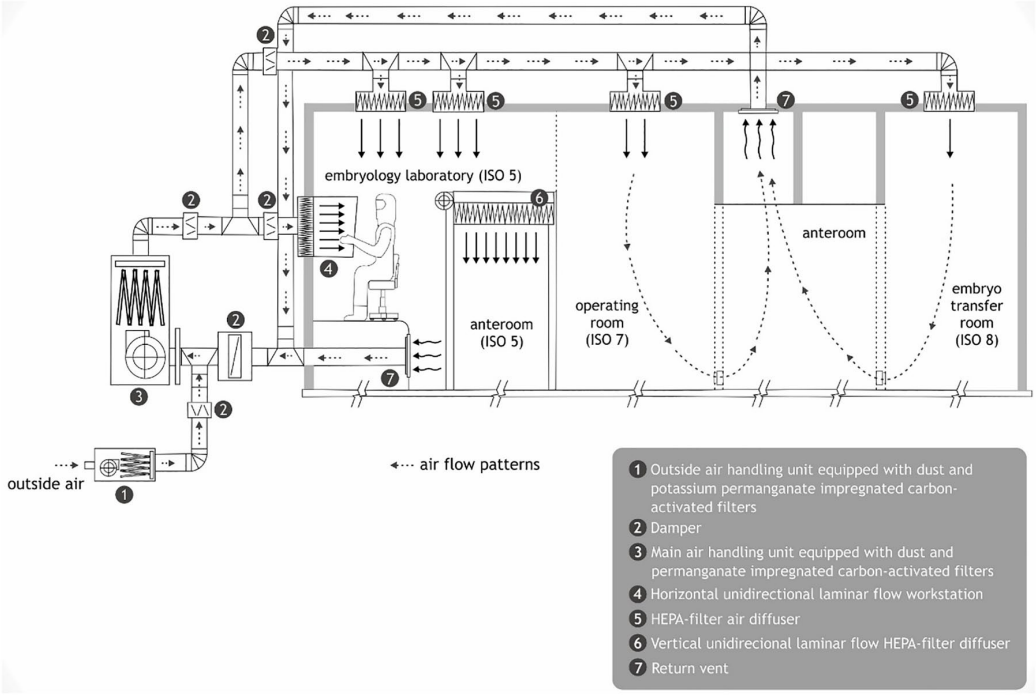

Figure 2. Schematic representation of cleanrooms (embryology suite, operating theater, and embryo transfer suite). Airflow patterns and filtration units are also depicted. The air-handling ventilation unit is located in a separate room. An external rooftop subunit draws outside air that goes through coarse and activated carbon prefilters before entering into the main unit. The main ventilation unit pulls prefiltered outside air and the cleanrooms' return air through coarse filters, past a 16-unit potassium permanganate-impregnated pelletized coconut shell-based activated carbon filters and then through fine dust filters. Finally, filtered air enters the cleanrooms through a set of high-efficiency particulate air (HEPA) filters. Floor- and ceiling-level vents in the cleanrooms return air to the main ventilation unit to be remixed with the existing air. Differential positive pressure is maintained between rooms. The embryology laboratory/anteroom is positive to the operating room, which is positive to both the embryo-transfer room and the dressing room/hallways. *Reprinted with permission from* Esteves and Bento.[7]

qualification, and operation of the embryology laboratory.[26] Critical aspects to be taken into account include age and size of the laboratory, outdoor ambient pollution, whether the facility is old or new, which affects the VOCs levels, and the existence of regulatory directives dictating the air cleanliness to be achieved. While compact cleaning solutions are less expensive and easy to implement, they provide less control of air contamination than centralized filtration systems due to inherent technical limitations concerning their capability to address the four principles of AQ control discussed above. The available clinical data—albeit limited—seem to favor the use of cleanroom laboratories for AQ.[27,28] However, it remains to be determined if a cleanroom is necessary as no randomized controlled trial has examined this issue. Also, it is not known if the practice of using a cleanroom would be more useful in specific situations, such as laboratories installed in highly polluted areas. Attention to construction materials, equipment, furniture, and human activity is also essential to reduce contamination. The use of low off-gas materials is preferable, and laboratory personnel should be trained on the principles of air quality control, including the function of airflows and airlocks, hygiene, dress code, and the use of cleaning agents.[5] Equally important is the use of inline gas filtration systems as compressed gases (eg., CO_2) are known sources of contaminants, including n-butane, acetaldehyde, isopropanol, freon, and benzene.[29–33] The use of modern incubators, properly designed for the embryology laboratory, with built-in VOC and HEPA filters (some with UV light) and thus providing clean air in the embryo culture chambers, may offer additional benefit. These incubators keep culture conditions, including pH, temperature, and air quality steady through the entire period of culture, and therefore, may offer a valid option for AQC for laboratories that adopt uninterrupted single step culture media.[34]

Monitoring

A risk management analysis is paramount to determine what measures should be implemented to mitigate the risks associated with chemical and particulate contamination, as discussed above, as many activities involving gametes and embryos are performed in the laboratory environment. Irrespective of the chosen filtration system, monitoring the AQ is also essential. The critical air cleanliness elements to control periodically includes (1) no. particles in air suspension, (2) air pressure differential, (3) air exchanges per hour, (4) total VOC in air, and (5) microbiological control. Microbiological contamination is a critical element to be monitored and must be a part of the routine quality control process, which is usually carried out periodically (every 3-6 months) using air samples, sedimentation plates (e.g. 90 mm plates), and swabbing methods. The number of colony forming units is measured and the type of microbial can be determined. Besides, contamination control measures are of utmost importance to mitigate the risk of microbial contamination (e.g. use of gloves, masks, and coveralls). The heating, ventilation, and air conditioning (HVAC) is an integral element of the air quality system. It has to be properly installed, maintained, and cleaned. Air ducts, coils, drain pan, grills, blower motor, air plenum, and air filters are some of the parts that need to be decontaminated. During HVAC cleaning, air pressurization should be placed under negative pressure or vacuum to prevent the spread of contaminants. The negative pressure also helps to get rid of dust and fine particles, as well as loosened contaminants. After mechanical cleaning, sanitizers are applied to nonporous surfaces of HVAC systems to control for microbial contamination. As for filter replacement, it is essential to consider that filter saturation depends on outside air quality and pollution levels and on the strategies adopted by the embryology laboratory to mitigate the introduction, generation, and retention of particles and VOCs. Thus, filter replacement should be guided by the concurrent analysis of AQ rather than the manufacturer's specifications. A detailed description of how to perform AQ control in the embryology laboratory can be found elsewhere.[35–37]

Summary evidence

In 1997, Cohen and colleagues[12] investigated the effect of chemical contamination on IVF outcomes in different settings. In this study, a new IVF laboratory was built above one of the busiest streets of southern Italy, known for its high industrial emission and stagnant air. At the beginning of the clinical activities, the authors reported a significant drop in pregnancy rate. After the installation of a pump to push air through a water-filtered gas bottle before entering the incubator, the pregnancy results were returned to average values. In another

setting, the authors installed solid carbon and potassium permanganate filters in the IVF laboratory to remove adhesive and paint smells and other pollutants. Sixteen months after the installation, they found an overall increased implantation rate from 22% to 36% in a cohort of 1400 patients. This study suggested that chemical air contamination might adversely affect *in vitro* embryo culture and pregnancy outcomes, albeit the VOC levels were not precisely evaluated before and after the introduction of air filtration. The same group in a subsequent trial investigated the effect of acrolein on mouse embryo development.[14] Acrolein belongs to the group of aldehydes, and is found in the air, probably as a result of industrial activity. The compound has also been detected in tobacco smoke.[38] The authors found that the mouse embryos growth was significantly affected after acrolein was added at different concentrations to the culture environment.[14] The physiological effect was noted at concentrations in the low ppm range. Although this is not a typical condition in ART, it could be, however, speculated that acrolein and other aldehydes might be associated with reduced growth of mammalian embryos.[39] Other studies in the late 1990s provided insights into the compounds and factors that impact AQ. Schimmel and colleagues[40] showed that both incubators and compressed medical gasses are sources of VOCs, as well as sterile Petri dishes, incubators, cleaning supplies, monitors, microscopes, and even furniture, all of which contribute to the emission of VOCs.[13] A study published by Nijs and colleagues used the human sperm survival test to identify potential reprotoxic products and consumables used in ART procedures. They analyzed several products customarily used in the IVF laboratory, including surgical gloves, ovum pickup needle, type of embryo transfer catheter, and also sterile Pasteur pipette and culture Petri dish, and found that 13 of 36 products analyzed were potentially toxic. The authors speculated that these products might release chemical compounds toxic to sperm.[41] Another critical aspect to consider concerning chemical contamination to human embryo culture is the use of mineral oil as a protective overlay during human embryo culture. On the one hand, an oil overlay could be a barrier to water-soluble VOCs such as ethanol, acetone, and formaldehyde. On the other hand, benzene, isobutylene, and styrene are oil-soluble, highlighting the importance of controlling VOC sources in the IVF lab. Added to this, mineral oil is a petroleum-derived product that can vary widely in quality. Commercially available oil might contain aromatic and unsaturated hydrocarbons that are susceptible to peroxidation and free radical formation,[42] particularly during storage or culture conditions.[43] Lately, with the widespread use of the single-step medium in human *in vitro* embryo culture, the mineral oil might be exposed to a longer incubation time at 37°C up to 7 days, thus increasing the risk of peroxidation and generation of toxic compounds that could be directly transferred to the embryo.[44] In a 1997 retrospective cohort study involving infertile couples undergoing IVF, Boone and colleagues[31] observed that the construction of class 100 cleanroom for air particulates improved air quality and increased the number of high-quality embryos available for transfer, ultimately increasing clinical pregnancy rates. Later in 2013, Esteves and Bento[5] also reported their results after the construction of a new IVF facility that used a centralized air ventilation system to supply filtered air in terms of particles and VOCs to the IVF laboratory, operating room and embryo transfer room. The new filtration system used forced air movement through a series of pre-filters, 16 beds of activated carbon mixed with potassium permanganate and silica, and HEPA filters before the air supplied the critical areas. The authors reported that during the study period, few changes other than the environmental conditions were made. They measured air particles and VOC levels before and after the installation of the new system and noticed a sharp decrease in both contaminants. In parallel, they showed an increased live birth rate (35.6% vs 25.8%, $P=.02$) and decreased miscarriage rate (20.0% vs 28.7%; $P=.04$) compared to their results before the implementation of AQ.[5] In 2015, Munch and colleagues[45] assessed the fertilization, cleavage, blastocyst formation, clinical pregnancy, and live birth rates in the presence and absence of carbon filtration. This retrospective cohort included a total of 524 fresh cycles. The authors found that fertilization, cleavage, and blastocyst formation rates were significantly reduced in fresh IVF when, by mistake, the carbon filters were not replaced. They also noticed that these metrics returned to normal ranges when new carbon filters were installed, thus suggesting that the lack of carbon air filtration adversely affected embryo development, in particular, around the time of fertilization, when the oocytes are very sensitive to air quality changes.[45] However, VOC levels were not reported before and after filters' replacement, and it remains speculative if indeed VOC levels were high in the absence of carbon filtration. In

another study, Heitmann and colleagues[32] assessed IVF pregnancy outcomes before and after renovating their IVF facility. The new facility included a dedicated centralized air filtration system for particles and VOCs. The authors emphasized that no changes occurred in the personnel, laboratory equipment, or protocols during this period. Overall, the total VOC (819.4 g/m^3 vs 32.0 ug/m^3) and aldehyde (13.7 μg/m^3 vs 5.2 μg/m^3) concentrations in the IVF laboratory decreased after the installation of the new AQ system. The decrease in VOC levels were associated with increased rates of implantation (24.3% vs 32.4%, $P < .01$), as well as clinical pregnancy (40.8% vs 50.2%, $P = .01$) and live birth (31.8% to 39.3%, $P = .03$). Table 1 summarizes the relevant clinical studies on AQ and IVF outcomes published to date. These studies collectively suggest that laboratory air filtration might improve both embryonic and pregnancy outcomes of infertile couples undergoing ART. However, it is worth noting that the existing studies are mainly retrospective in nature and lack proper control groups. Moreover, the implementation of other relevant changes rather than air filtration alone could have positively impacted IVF outcomes. Thus, a cause-effect relationship remains to be demonstrated in prospective controlled trials.

Gaps in knowledge and future directions

Due to the increasing evidence suggesting an association between laboratory air quality and ART outcomes, regulatory bodies issued cleanroom specifications.[48,49] While these directives are intended to safeguard public health in line with the precautionary principle, they differ in the specifications on how AQ control has to be handled.[5] For instance, the EU directive focuses on particulate air only, whereas the Brazilian directive tackles both particles and VOCs. Along the same lines, VOC thresholds for embryology laboratories are lacking, as are specific practice guidelines on good embryology laboratory practices concerning AQ control. Nevertheless, a recent document discussed the relevant aspects concerning AQ control in the embryology laboratory and provided practical recommendations based on expert judgment to guide laboratory design, qualification, and operation concerning AQ control.[20] Nevertheless, level 1 evidence is still lacking to support any recommendation concerning the minimum AQ requirements for optimal human gamete manipulation and embryo culture. Indeed, several gaps in knowledge exist in this area. Notably, it is challenging to perform well-designed studies on the effect of air quality and environmental pollutants on embryo development. First, there are many chemical compounds partitioned in the air. Second, the VOC mass transfer from air to water/culture phase of the cultured cells and embryos is difficult to model. Nowadays, culture systems are not biphasic due to the effect of the commonly used oil interphase. Thus, all gaseous interactions occur through mineral oil. Third, the relative solubility of a chemical compound in oil is described by its oil–water partition coefficient. Partitioning into mineral oil or water/culture medium is not the same for different classes of compounds. Compounds with a high partition coefficient are hydrophobic (e.g. benzene, styrene) and achieve a much higher concentration in the oil phase than in the aqueous phase at equilibrium. By contrast, hydrophilic compounds (e.g. ethanol, acrolein) accumulate preferentially into the aqueous phase. Future studies should focus on the objective determination of the most relevant chemical contaminants in the IVF lab, and their thresholds. In this sense, triphasic models would be essential to study the effects of chemical contamination on embryo development. Equally important would be to investigate (e.g. using well-designed pragmatic clinical trials) the effectiveness of different air filtration approaches (eg., cleanroom vs portable filtration systems), and whether modern "sealed" incubators equipped with VOC and HEPA filters could compensate the lack of stringent AQ control. The results of these studies will be invaluable to elaborate evidence-based practice guidelines for AQ control in embryology laboratories.

Conclusion

The association between the environment, preimplantation toxicology, and successful embryogenesis demands a comprehensive evaluation of the main variables that contribute to air contamination in the embryology laboratory. Particulate matter and VOCs are the primary pollutants to be controlled for in the embryology laboratory as they might adversely affect embryogenesis, implantation, and conception in ART cycles. Embryology laboratories that are willing to adopt strict air quality control should be constructed and used to minimize the introduction, retention, and generation of particles and VOCs, in which temperature, humidity, and pressure are continuously controlled and monitored. Attention should also be paid to

Table 1. Evidence summary[a] concerning the impact of air quality control in the embryology laboratory on the outcomes of assisted reproductive technology cycles.

Study	Study design	Study population	Air quality control	Outcome
Schimmel and colleagues[40]	Descriptive qualitative study	None	Air sampling and VOCs in human IVF laboratories	Higher levels of VOC found in CO_2 tanks and incubators compared to outside air. Air filtration using carbon activated and potassium permanganate reduced VOC levels
Hall and colleagues[14]	Descriptive qualitative and observational cohort studies	*In vitro* mouse embryos	Air sampling and VOCs in human IVF laboratories and Acrolein bioassay using mouse embryos	Increased levels of VOC observed in ambient air of human IVF laboratories. Reduction in aldehyde levels by air filtration using carbon-activated and permanganate. *In vitro* mouse embryo development, implantation, and post-implantation development inversely correlated with acrolein concentration
Boone and colleagues[31]	Observational study	275 infertile couples undergoing IVF; fresh ET	Centralized system (Class 100 cleanroom) for particle filtration.	Air particles decreased ($P < .001$) after implementation of centralized air filtration. Reduction in air particles was associated with an increase in the number of high-quality embryos (62% vs. 71, $P < .001$) and CPR per transfer (59% vs. 35%, $P = .003$).
Khoudja and colleagues[52]	Descriptive qualitative and observational study	1403 infertile couples undergoing IVF/ICSI cycles; fresh ET	Combination of centralized and portable system to filter particles and VOCs	VOC levels decreased and overall air quality improved after installation of centralized VOC air filtration system. Higher fertilization (83.7% vs. 70.1%, $P < .001$) blastocyst rate (51.1% vs. 41.7%, $P < .001$), IR (34.4% vs. 26.4%, $P = .001$), and CPR (54.6% vs. 40.6%, $P = .001$) were observed after the incorporation of strict air quality control.
Esteves and Bento[5]	Descriptive qualitative and observational study	2060 ICSI cycles; fresh ET	Centralized system for particles (ISO 5 cleanroom) and VOC filtration (new facility) compared with portable air filtration system (old facility)	Higher rates of high-quality embryos and live birth rates (35.6% vs. 25.8%, $P = .02$), and lower miscarriage rates (28.7% vs. 20.0%, $P = .04$) in the new facility than in the old facility.
Munch and colleagues[45]	Observational study	524 fresh and 156 cryopreserved IVF/ICSI cycles	VOC and HEPA air filtration (not specified if centralized or portable)	Embryonic (cleavage and blastocyst rates rates) and pregnancy (IR, CPR, LBR) outcomes decreased significantly ($P < .05$) in fresh cycles carried out in the absence of VOC air filtration and increased ($P < .05$) after installation of VOC filters.
Heitmann and colleagues[32]	Combination of descriptive qualitative and observational study	820 IVF/ICSI cycles; fresh ET	Centralized system for particle and VOC filtration (new facility) compared with portable air filtration system (old facility)	Lower VOC levels (Total VOC 819.4 μm^3 vs. 32 ug/m^3, and aldehyde 13.69 ug/m^3 vs. 5.2 ug/m^3), and higher IR (32.4% vs. 24.3%; $P < .01$) and LBR (39.3% vs. 31.8%, $P = .03$) in the new facility than in the old facility.
Agarwal and colleagues[53]	Combination of descriptive qualitative and observational study	1036 IVF/ICSI cycles; fresh ET	Portable air filtration system for particles and VOCs	Lower VOC levels ($P < .0001$), higher No. blastocysts (6.3 ± 0.8 vs. 4.5 ± 0.4; $P = .04$), and higher IR (42% vs. 31%, $P = .001$) as well as LBR (31% vs. 23%, $P = .009$) after incorporation of air filtration.

CPR: clinical pregnancy rate; ET: embryo transfer; HEPA: high-efficiency particulate air; IR: implantation rate; ISO: international organization for standardization; IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection; LBR: live birth rate; VOC: volatile organic compound.
[a]We conducted a PubMed search to identify relevant studies published in English until March 3rd, 2020 (start search date not specified). The search terms used were "air quality" AND "in vitro fertilisation" OR "assisted reproductive technology." We limited the search to full-text human studies that reported pregnancy outcomes, including cohort studies, case series, cross-sectional studies, and prospective studies.

other critical issues that might affect indoor air quality and VOCs emissions, such as laboratory furniture, wood, equipment, clothing and cosmetics, and sterile plasticware, gloves, incubators and numbers of embryologist working simultaneously in the same area. Ideally, a risk management analysis concerning laboratory air quality should take into consideration not only to reduce but also to avoid the risks associated with poor air quality conditions.[50] Fair evidence indicates that laboratory air quality is associated with IVF outcome, although the evidence is not unequivocal.[46,47,51] Solid evidence based on prospective trials on the optimal solution for AQ in ART is still missing. Most published studies cited earlier are retrospective and lack proper controls. Some studies were performed after laboratory renovation; therefore, additional features might be changed, not only the air filtration. However, evaluating the impact of AQ on human early embryo development using randomized controlled trials are not easy to perform due to technical and possible ethical implications. Furthermore, there are a multitude of factors affecting ART results, including the characteristics of treated patients, protocols and procedures, and environmental pollutants. Finally, we do believe that the optimal approach of AQ would involve an embryology laboratory cleanroom, in which a mixture of filtered fresh outside air and recirculated air for PM and VOC air is supplied, and that includes both the transfer and operating rooms. Such facility should be constructed and used in a way to minimize the introduction, generation, and retention of particles and VOCs.

Author contributions

R.S., E.R., and S.C.E. contributed to the conception and designed the manuscript. R.S. and E.R. wrote the first draft of the manuscript. R.S. and S.C.E. wrote sections of the manuscript and revised it for content. All authors contributed to manuscript revision, read, and approved the submitted version.

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ORCID iD

Romualdo Sciorio https://orcid.org/0000-0002-7698-8823

Data availability statement

All data are included in the study.

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