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Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting

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KEY MESSAGE

An international expert meeting on the technical and operational requirements for assisted reproduction tehnology laboratory air quality established 50 consensus points regarding site suitability, design criteria for new construction, laboratory commissioning and ongoing volatile organic compounds management that provide aspirational benchmarks for existing laboratories and guidelines for constructing new laboratories.

A B S T R A C T

This proceedings report presents the outcomes from an international Expert Meeting to establish a consensus on the recommended technical and operational requirements for air quality within modern assisted reproduction technology (ART) laboratories. Topics considered included design and construction of the facility, as well as its heating, ventilation and air conditioning system; control of particulates, micro-organisms (bacteria, fungi and viruses) and volatile organic compounds (VOCs) within critical areas; safe cleaning practices; operational practices to optimize air quality while

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minimizing physicochemical risks to gametes and embryos (temperature control versus air flow); and appropriate infection-control practices that minimize exposure to VOC. More than 50 consensus points were established under the general headings of assessing site suitability, basic design criteria for new construction, and laboratory commissioning and ongoing VOC management. These consensus points should be considered as aspirational benchmarks for existing ART laboratories, and as guidelines for the construction of new ART laboratories.

Background

Regulation and licensing of IVF laboratories is increasing, especially in relation to their associated procedure rooms ('non-hospital surgical facility'). Examples of this include European Union Member States enabling legislation for licensing and accreditation across the European Union in response to the European Union Tissues and Cells Directive; College of American Pathologists, Joint Commission on Accreditation of Healthcare Organizations; state licensing authorities in the USA; Provincial Colleges of Physicians and Surgeons in Canada; and Reproductive Technology Accreditation Committee in Australia and New Zealand (and elsewhere in south-east Asia). As a result, authorities have tended to develop air-quality requirements as if the entire IVF suite was a surgical facility and require the same level of operational infection control procedures as hospitals. Although this might sound a prudent approach, these requirements do not consider the unique needs of the assisted reproduction technology (ART) laboratory, where the environment must cause the lowest levels of physicochemical stress possible to the gametes and embryos.

The aim of this consensus workshop, held at the Upper Egypt Assisted Reproduction Symposium (UEARS) 2017 conference (Cairo, Egypt) and involving international experts (**Table 1**), was to establish the recommended technical and operational requirements for air quality to achieve the safe and effective operation of an IVF Centre's procedural suite regarding design and construction of the facility heating, ventilation and air conditioning (HVAC) system, controlling particulates, micro-organisms and volatile organic compounds (VOCs) within the critical areas; selection of construction materials and methods to minimize VOCs and contaminating agents, including particulates; safe cleaning practices to protect gametes and embryos from toxins; operational practices to optimize air quality while minimizing physicochemical risks (temperature control versus air flow); and infection-control practices minimizing exposure to VOCs, e.g. cold sterilizers, surface cleaners and hand sanitizers.

Invitations were based on individuals' experience in modern IVF laboratory design, qualifications in specific topics in the environmental field, expertise in consensus building, or both; all participants but one were invited speakers on the UEARS congress programme. The workshop was structured as a series of presentations, each followed by open discussion and the establishment of consensus points with a dual goal to establish safe and effective operational recommendations as well aspirational benchmarks for air quality in existing ART laboratories, and to provide guidelines for the construction of new ART laboratories.

Table 1 – List of consensus workshop participants.

Participant	Contribution	Affiliation
David Mortimer	Co-convenor, presenter, contributor, participant	Oozoa Biomedical, West Vancouver, Canada
Jacques Cohen	Co-convenor, presenter, contributor, participant	ART Institute of Washington, 3 Regent Street, Livingston NJ, USA
Sharon Mortimer	Writer/editor, participant	Oozoa Biomedical, West Vancouver, Canada
Mohamed Fawzy	Local organizer, presenter, participant, UEARS liaison	Ibnsina and Banon IVF Centers, Egypt
Antonia Gilligan	Presenter, contributor, participant	Alpha Environmental, Emerson, NJ USA
David McCulloh	Presenter, contributor, participant	NYU Fertility Center, New York University Langone Medical Center, New York, USA
Dean Morbeck	Contributor	Fertility Associates, Auckland, New Zealand
Xavier Pollet-Villard	Participant	Nataliance, IVF and Andrology Centre, Laboratoire Medibio, Saran, France
Ragaa Mansour	Participant	The Egyptian IVF-ET Center, Maadi, Cairo, Egypt
Daniel Brison	Participant	University of Manchester, Manchester, UK
Alpesh Doshi	Participant	The Embryology and PGD Academy Ltd, Saffron Walden, Essex, UK
Joyce Harper	Participant	Institute for Women's Health, University College London, UK
Jason Swain	Contributor	Colorado Center for Reproductive Medicine, Lone Tree, Colorado, USA

Participants were selected on the basis of their experience in designing modern IVF laboratories, qualifications in specific topics in the environmental field, expertise in consensus building, or both; all participants but one were invited speakers on the UEARS congress programme. All participants declare that no commercial bias or self-interest has influenced the recommendations presented in this report.
UEARS, Upper Egypt Assisted Reproductive Symposium.

Workshop presentations

How the laboratory environment affects gamete and embryo biology

The entire IVF process is governed by the biology of the gametes and embryos, to optimize their growth and development. The biochemical and biophysical requirements govern all IVF laboratory procedures, as well as the design of the laboratory, the HVAC system, the engineering of the laboratory equipment and the materials used. It would follow, then, that the best approach would be to protect the gametes and embryos against exposure to adverse external factors. Although embryos are highly adaptable to the environment to which they are exposed, any 'adaptation' represents a source of physiological stress (Wale and Gardner, 2016). Cellular stress can also result in alterations in embryonic gene expression, regulation, or both, including imprinting and epigenetic effects, which could be inherited.

A wide range of factors can affect the outcome of IVF. First, the patients themselves represent a source of influence, as their own biology will affect the potential of their gametes and resultant embryos. Clinical factors, such as the stimulation, oocyte retrieval and embryo transfer procedures and luteal phase support will also influence outcomes. Within the laboratory, the gametes and embryos can be affected by factors associated with the laboratory environment, equipment, contact materials, methodology and the staff themselves.

Environmental factors can be on the micro scale, such as the incubator temperature, oxygen or CO_2 control (which will affect the pH to which the gametes and embryos are exposed), or the macro scale, such as the laboratory design or air quality. Equipment factors are related to the selection of the appropriate piece of equipment for the purpose, regular maintenance and (verified) calibration, and the possibility of malfunction (Mortimer and Mortimer, 2015). Contact materials include the culture medium, plasticware, gases, handling devices, intracytoplasmic sperm injection microtools, oocyte retrieval needles and embryo transfer catheters. Methodological factors are related to choosing the correct technical approach and following the correct standard operating procedure for each laboratory process.

In addition to these general considerations, specific factors particularly affect the functional potential of oocytes and embryos. For example, attention should be paid to maintaining a stable temperature during follicular aspiration, transport of the aspirates to the 'egg search' workstation, the 'egg search' procedure and subsequent handling of cumulus–oocyte complexes (Mortimer and Mortimer, 2015). Oocytes and embryos also require stable pCO_2 for bicarbonate-buffered medium pH equilibration, and reduced pO_2 to help protect against oxidative stress. For example, the pH of bicarbonate-buffered media is above the expected range by 2-min exposure to air (Blake et al., 1999; discussed in Mortimer and Mortimer, 2015), so zwitterion-buffered media containing sufficient bicarbonate concentration for embryo metabolism should be used for any oocyte or embryo handling under air. Significant change in the microenvironment affects oocyte and embryo metabolism and homeostasis (Wale and Gardner, 2016). For example, even a 5-min exposure of fertilized mouse oocytes to a collection medium that did not contain amino acids resulted in fewer embryos reaching the blastocyst stage, and lower cell numbers in those that did (Gardner and Lane, 1996).

Gametes and embryos also require protection from exposure to toxic substances, such as VOCs and airborne chemically active compounds. This can be achieved in part by paying attention to the design of the laboratory and choice of building materials, the choice of the workstation and incubation system, and the choice of gas and design of the gas supply system (Mortimer and Mortimer, 2015). A range of other potential sources of challenges to IVF laboratory air quality include the materials used in the facility construction and finishing, e.g., paints, adhesives and sealants; HVAC system air quality; the cleaning products and air fresheners used within the facility; VOCs released by equipment and products used in the laboratory or facility; human-derived contaminants, such as cosmetics, hair and skin cells; fibres from clothing; laundry products; and sanitizing products used for infection control.

These factors will be considered in more detail in the following sections.

VOCs: a systematic review of impact data

Evidence in clinical medicine

Evidence-based medicine strives to rely on randomized controlled trials and uses systematic reviews and meta-analyses as major tools. As a result, evidence-based medicine is less effective in evaluating complex procedures as their success relies on the skill and experience of the person(s) performing them. If we agree that retrospective analysis is informative, but not decisive, and that the best available evidence is the randomized controlled trial, then we would need to conduct a blinded randomized controlled trial to test the clinical efficacy of VOC reduction.

The design and execution of this type of study is so complicated, however, that it seems unachievable within acceptable medical practice. The already established deleterious effects of some VOCs on embryo development would render such a study unethical with human embryos, as the test arm would involve exposing those embryos to harm. So, is there an alternative approach we could follow to assess evidence?

The fundamental prerequisites for conducting a randomized controlled trial in clinical embryology are as follows: removal of technological bias; randomization; allocation concealment; blinding; and analysis and reporting of the randomized controlled trial data. All of these embody substantial practical difficulties, especially when considering VOCs or VOC elimination technologies, e.g. needing two separate laboratories, but with otherwise identical culture systems, and with negligible cross-over of personnel between them. The 'Harper Model' (Harper et al., 2012) presents six steps for the investigation of whether a new technology could and should be introduced into the IVF laboratory, covering the range from 'concept' to 'safe routine implementation': hypothesis-driven research; testing in animal models; testing in donated embryo material; pre-clinical small-scale investigations; larger clinical trials; and assessment of clinical- and cost-effectiveness.

Careful application of this approach, in which evidence-based medicine is only one of the requirements, would seem to represent a practical way forward. The fourth and fifth stages could then be undertaken in existing laboratories using 'current technology' that apply the 'new technology' as the intervention. Power calculation and interim analyses would be prudent, allowing for acceptance of improved technology at the earliest possibility to avoid known harm.

An increasing number of studies of varying quality have been published on the effect of laboratory air quality on IVF outcomes (reviewed in Esteves and Bento, 2016) (**Table 2**). Unfortunately, many studies were poorly designed, owing to complicated circumstances, ethical concerns and cost. It is also difficult to attribute all the observed improvements to improved air quality, given that concomitant improvements have been made in laboratory design over the same time period.

Overall, although it seems likely that an effective air filtration system is essential for achieving optimal laboratory key performance indicators, and that it is feasible to effect a significant decrease in VOC and aldehyde concentrations, some properly designed, prospective trials would be beneficial. Readers are also referred to the review by Morbeck (2015).

The HVAC system and cleanroom standards

Historical features of ART laboratory design

Until around 2000, the design of most ART laboratories in a hospital followed the design of procedure rooms or surgical suites. Hospital-based laboratories were often squeezed into small spaces without regard to adjacent facilities for instrument sterilization. The design of laboratories outside of hospitals was largely based upon medical offices with little consideration for the needs of cell culture. Therefore, ART laboratories designed before 2000 typically failed to adequately consider a range of aspects of design and construction that could adversely affect the laboratory environment and outcomes. This range of aspects, partially reviewed by Cohen et al. (2012), includes vinyl flooring (either tiles or sheet); 'solid' hard ceiling but with lighting in the ceiling, allowing infiltration of dirty air from the plenum space; particle filtration, commonly including high efficiency particulate air (HEPA) filters; no dedicated gas supply room, cylinders often in the IVF laboratory; cryopreservation laboratory and cryostorage, but without floor protection; no dedicated air handling system, so the air supply into the laboratory was shared from multiple uncontrolled sources; older laboratories commonly used an open plenum design to return air to the HVAC system; casework commonly made from medium density fibreboard (MDF), a manufactured wood product composed of wood chips glued and bonded together; hot culture rooms were used in some facilities (37°C/98.6°F), which would have been detrimental to proper incubator function; entry vestibules (airlocks) that separate the IVF laboratories from the general office space were not common; procedure rooms and laboratories were often separated by hallways; and diagnostic andrology might occupy the same space as embryology.

Towards the end of this period, more stringent measures were suggested, including air showers to remove particulates on staff and ultra low particulate air filtration (American Society for Reproductive Medicine, 1998; Esteves and Agarwal, 2013). These measures are used in microprocessor clean rooms with high rates of air changes (40 + per hour), but they are not relevant in a biological clean room that has HEPA filtration and a laminar flow hood. Other suggested design improvements were the removal of the diagnostic andrology laboratory from the ART laboratory, and the use of positive pressure to reduce the risk of infiltration of contaminants from outside the laboratory.

The American Institute of Architects–Department of Health and Human Services Guidelines for Design and Construction of Hospital and Health Care Facilities issued in 1996–1997, and updated and revised in 2001, have no specific requirement for ART laboratories. The most stringent specifications are for operating or surgical cystoscopic rooms, based on the implied specification for IVF laboratories: air movement out of the room (positive pressure); a minimum of 15 total air changes per hour (ACH) or 'TACH'; a minimum of three fresh air changes per hour or 'FACH'; no use of recirculating room units, i.e. no window-mounted air conditioning units; room temperature of 20–23°C (68 to 73°F); and relative humidity between 30% and 60% (lower relative humidity levels risk eye irritation, evaporative loss from culture and increased static electricity, and higher relative humidity levels risk mould growth).

Current engineering guidelines from Europe and Asia stress particulate removal as being critical for ART laboratories, but particle removal alone is insufficient. As our understanding of the influence

Table 2 – Studies on the effect of air quality (adapted from Esteves and Bento, 2014).

Reference	Year of publication	Study design	Location/population	Method	Main outcome
Cohen *et al.*	1997	Descriptive VOC quantitative levels.	IVF laboratory.	Air sampling VOCs/aldehyde.	High VOC levels found in laboratory air and inside incubators.
Schimmel *et al.*	1997	Descriptive VOC quantitative levels.	IVF laboratory and gas cylinders.	Gas cylinders VOCs/aldehyde.	Varying levels of VOCs/reduction with activated carbon/ KMnO4.
Hall *et al.*	1998	Observational analytic cohort.	In-vitro cultured mouse embryos	Air sampling Acrolein bioassay.	Embryo development affected .
Mayer *et al.*	1999	Prospective randomized crossover.	Human treatment cycles (*n* = 110).	Incubators with and without filters.	Increased pregnancy rate with filters.
Boone *et al.*	1999	Observational analytic cohort.	Human couples (*n* = 275).	Centralized particle filtration.	Reduced particulates, improved embryo development.
Worrilow *et al.*	2001	Descriptive qualitative.	New IVF laboratory.	Central HVAC/ VOC filtration.	Significant reduction in particulates with the new HVAC system to achieve a US Fed Standard class 100 cleanroom (equivalent to ISO 14644-1 Class 5).
Worrilow *et al.*	2002	Observational analytic cross-sectional.	IVF cycles 2 year.	Outside/inside sampling.	Seasonal VOC variation affecting pregnancy rates.
Esteves *et al.*	2004	Observational analytic cohort.	Human ICSI cycles (*n* = 468).	Two laboratories: conventional versus HVAC/filter.	Improved embryo development, increased pregnancy/ decreased miscarriage rates.
von Wyl *et al.*	2004	Descriptive Qualitative.	IVF laboratory air sampling.	Old/new laboratory particle filter.	Reduced particulates and VOC.
Esteves *et al.*	2006	Observational analytic cohort.	Human male factor ICSI cycles (*n* = 399).	Two laboratories: conventional versus HVAC/filter.	Improved embryo development, increased pregnancy/ decreased miscarriage rates.
Knaggs *et al.*	2007	Observational analytic cohort.	IVF cohort.	Key performance indicators study/EU Tissues and Cells Directive.	Increased pregnancy and implantation rates.
Merton *et al.*	2007	Randomized controlled trial.	Bovine.	Incubator filter.	No effect on embryo development, slight increase in pregnancy rate.
Souza *et al.*	2009	Observational analytic cohort study.	Human ICSI cycles (*n* = 123).	Comparing class 8 and class 5 incubators.	No differences.
Khoudja *et al.*	2013	Descriptive qualitative observational analytic cohort.	Human IVF–ICSI cycles (*n* = 1403).	Standalone filtration versus novel Landson system.	Significant improvements in laboratory performance.
Esteves *et al.*	2013	Observational analytic cohort.	Human ICSI cycles in ISO 5 clean room laboratory (*n* = 2060), *cf* 255 ICSI cycles in older-style laboratory.	New ISO 5 clean room laboratory compared with older-style laboratory.	Increased proportion of high quality embryos on day 3.
Munch *et al.*	2015	Observational analytic cohort.	Human fresh IVF cycles (*n* = 524) and frozen embryo transfer cycles (*n* = 156).	Laboratory with and without carbon filter.	Decline in laboratory performance when filter removed.
Heitmann *et al.*	2015	Descriptive qualitative observational analytic cohort.	Human IVF–ICSI cycles (*n* = 820).	Old laboratory with standalone filter/new laboratory with HVAC and central filter .	Decreased VOC; Improvements in laboratory performance.

HVAC, heating, ventilation and air conditioning; ICSI, intracytoplasmic sperm injection; VOC, volatile organic compounds.

of laboratory conditions on outcomes has developed, concomitant changes have occurred in the engineering and design specifications (Mortimer, 2005). As a result, many contemporary ART laboratories have now segmented laboratory design, separating the diagnostic andrology and endocrinology services from the culture suite, as well as a separate gas room or closet and an entry vestibule. These design features each reduce the risk of introducing external contaminants into the culture area. Further control of air-borne contaminants is achieved using HEPA filtration, a dedicated air handling unit (AHU) or a dedicated supply duct, i.e. not via the plenum space, chemical filtration (either in the AHU or free standing) and positive pressure. Despite these improvements, most IVF laboratories worldwide still have some of the following design or operating issues: lack of complete isolation from surroundings; inadequate positive pressurization to prevent the inflow of 'dirty' air; and lack of robust chemical filtration or removal. Examples include incorrect filter media selection, using activated charcoal alone; incomplete oxidation of some oxygenated organics by photochemical oxidation systems; and a failure to monitor the removal absorption process or media; inappropriate materials used in construction (such as MDF, linoleum flooring, oil-coated ductwork and use of formaldehyde-urea insulation); low fresh air ventilation rates; the ability of air within the HVAC system to bypass particulate or chemical filters owing to poor pressure monitoring, lack of supporting frame or wire and inadequate maintenance; selection of a contaminated or compromised source of supply air, such as placing the air intake in the shipping dock; designs that render maintenance difficult or impossible (such as placing the AHU and chemical filter in the ceiling space); and inadequate control of humidity.

Design philosophy for a new ART laboratory suite

The first step in creating an optimized modern laboratory design is to define the functions of the proposed ART suite, i.e., the retrieval room, transfer room and IVF culture and biopsy laboratory. With the HVAC system, these rooms are contiguous, i.e., space for oocyte or embryo cryopreservation is typically attached. The use of double-door gasketed pass-through windows can reduce air flow from the IVF laboratory into clinical procedure rooms to achieve and maintain its positive pressure. This highly segmented arrangement requires a modification of the engineering of the surgical and clinical rooms used in a typical construction, such as ensuring that the rooms have a hard lid ceiling.

This is followed by separating 'dirty' rooms from the ART suite. These include, but are not limited to, gas cylinder rooms, storage rooms for bulk supplies, fixation and staining rooms, diagnostic andrology laboratories, endocrine assay laboratories, office space for laboratory staff, changing rooms, and janitorial closets. Indeed, the cryobank itself, owing to the requirement for continuous extraction ventilation, should also be separate from the ART suite.

Maintenance of the ART suite's clean environment is paramount and requires strategies to reduce outside infiltration and the loss of a high level of positive pressure. For example, requiring access via vestibules or staged interlocking doors, reducing the risk of air leakage by having lighting mounted on rather than in the ceiling, and ensuring that access panels are gasketed and joints sealed with silicone at the interfaces. It is critical that access panels are re-sealed every time they have been opened. The pressure across the IVF laboratory suite and the dedicated AHU should be monitored routinely to ensure that the system is functioning correctly. Larger, more complex ART laboratory designs will typically have supply and exhaust fans coupled with variable frequency drives. In all cases, all the ductwork and AHU components must be free of rust inhibitor, which is one of the major sources of VOCs in a newly constructed ART laboratory.

The gas rooms for compressed gasses and liquid nitrogen should be separate from, but ideally adjacent to, the ART laboratory suite. All gas lines into the IVF laboratory suite must be redundantly sealed at the piping–wall interface.

Local regulations aiming at fire protection often apply to gas and cryobank rooms as they contain significant volumes of volatile liquids. As a result, architects might request the presence of firebreak dampers in ventilation ducts. This must be avoided to prevent the risk of anoxia or CO_2 intoxication in case of accidental closure of ventilation dampers without an alternative fresh air supply.

The setting and range of temperature in the ART laboratory suite should be adjusted to the requirements of the staff. The current standards of the American Society of Heating, Refrigerating and Air-Conditioning Engineers are wide enough to accommodate the desired ranges, i.e., ANSI-ASHRAE Standard 62.1–2016 (see www.ashrae.org/technical-resources/bookstore/standards-62-1-62-2).

Physical isolation criteria

The ART laboratory suite is a controlled environment where microbial, particulate and chemical contaminants are removed, controlled to improve productivity, or both. Although the first design objective would logically be to ensure the environment around the laboratory is not a source of contaminants, the surrounding environment is not necessarily controllable. The other approach is that if the ART laboratory suite is fully isolated, the contaminants cannot enter and should not adversely affect outcomes. The use of secure sealed physical barriers (walls, doors, ceiling, floors), and significant levels of positive pressure, are both effective strategies towards achieving this goal, although positive pressure is not an absolute barrier, and sealing alone cannot remove every point of possible infiltration (leakage is a function of the square of the radius of all open holes, i.e. the total area of all the holes).

The effectiveness of the physical isolation measures can be measured by sulphur hexafluoride infiltration studies.

Retrofitting existing laboratory suites

Although the recommendations contained in this report are for new builds, common HVAC engineering strategies could improve the environment in existing ART facilities. These include installing a boost fan into the existing HVAC to increase the positive pressure; installing a double-door pass-through between the IVF laboratory and the procedures room (if the procedures room is not as clean as the IVF laboratory); reducing the number of new materials being brought directly into the laboratory; and using supplementary air cleaning systems to reduce the level of particulates.

Controlling VOCs: the fabric of the laboratory

The ART laboratory is a semi-enclosed compartment for the interaction of chemistry with biology. Although the physical factors of temperature and relative humidity are held to set limits, and the osmolality, pH and composition of culture media are controlled, the level of incidental chemical exposure is largely uncontrolled in many ART

facilities worldwide. This exposure can be from VOCs in the air (from outgassing of the laboratory fabric, consumables, or both), the gases supplying the incubators and workstations, or from contaminants that have dissolved in the culture medium or mineral oil. The VOCs are organic chemical compounds, and their composition makes it possible for them to evaporate under normal indoor atmospheric conditions of temperature and pressure.

All chemicals have different molecular and physical properties. In an environment with multiple materials, some will absorb selectively. By changing the concentration, temperature or atmospheric pressure, a chemical can be pushed from one medium, e.g. air, into a second medium, e.g. water. This is known as the 'sink' effect: in chemical terms, a sink is a reservoir that takes up a chemical element or compound from another phase. For instance, a water sink does not attract all VOCs away from culture medium, so it presumably competes by volume. Solubility and reactivity are crucial in reversing the transport of, for example, formaldehyde and higher aldehydes back into mineral oil or air with a sink such as water. Where the material ends up is competitive in the sense that one material may displace another in a matrix.

Not all VOCs are toxic to gametes, embryos, or both; examples of non-toxic VOCs include silicones, which are used in all incubator gaskets, tubing and high molecular weight alkanes, such as paraffinic oils that are stable and non-reactive. Obviously, exposure of gametes and embryos to unknown agents should be minimized as a rule, but pronounced effects require that the material be present in the environment, meaning it will have a significant vapour pressure at room temperature or be present in its containers, that it be soluble in the culture medium and possibly the mineral oil, and is reactive. Even then, the level of exposure can be modulated by the following: the ability of the contaminant to be changed or removed by pollution control technology; the route of contamination (air, culture media, gas supply and plastic ware); the ability of the environment and its components to absorb or release the contaminant (the 'sink' affect); the ability of the contaminant to react with the environment and its components (mineral oil, media, biologicals, embryos); the ability of material to penetrate the culture system; the ability to affect the gametes, zygotes, embryos and blastocysts; the ability of the contaminant to be changed by zygotes, embryos, and blastocysts; the biochemical capacity of gametes, zygotes, embryos and blastocysts to metabolize the contaminant; detoxify and excrete or transform into a more toxic form (cytochrome P450 enzymes); differential solubility and reactivity can reverse the transport of molecules between water and oil or air and water; and pollution control technology reducing the exposure in the laboratory, via the HVAC-AHU and gas supply filters.

A range of organic compounds is typically found in ART laboratories (**Table 3**), although their biological significance varies. d-limonene and α-pinene, common ingredients in colognes and cleaning products, are highly oil soluble and hence should not be used, but because they are unreactive under the conditions of culture, they do not seem to be biologically significant contaminants. Ethanol, however, which is one of the two most common contaminants in ART facilities, can be metabolized into acetaldehyde, and isopropyl alcohol (2-propanol) can be metabolized into formaldehyde, both of which are biologically damaging.

Measuring VOCs and aldehydes

No practical method will identify and quantify the levels of each VOC in the laboratory. The olfactory method, i.e. sense of smell, is not a reliable analytical method. The perception of odour is susceptible to inter-individual differences in sensitivity, is highly dependent on the substance and the amount in the environment, and the odour threshold for some substances exceeds the potential level of toxicity in an ART environment.

Table 3 – Odour thresholds of organic contaminants typically found in assisted reproduction technology laboratories (American Industrial Hygiene Association, 1989).

Organic compound	Geometric mean AIHA	Comment
Ethanol (ethyl alcohol)	18–100 ppm	Most common VOC in ART laboratories.
Isopropyl alcohol (2-propanol)	19–43 ppm	Second most commonly found VOC.
Acetone (2-propanone)	62–130 ppm	
Propene (propylene)	23–68 ppm	Plastic.
Hexamethylcyclotrisiloxane	No data	Silicone from gaskets.
Acetonitrile (methyl cyanide)	1160 ppm	Plastics.
Formaldehyde	0.03–9970 ppm	
Acetaldehyde	0.067 ppm	
d-Limonene	0.5 ppm	Scent of lemon.
α-Pinene	0.005 ppm	Scent of pine.

AIHA, American Industrial Hygiene Association, ART, assisted reproduction technology; VOC, volatile organic compound.

Photoionization detectors allow quantification of VOC levels in the ppm (mg/m^3) to ppb (μg/m^3) range, with the results expressed as the equivalent signal level of isobutylene. This means that they cannot report the identity of the VOCs in the environment. They are also poor detectors of aldehydes. They are useful, however, to measure the state of the chemical filter medium. When activated carbon is exhausted or in a closed, non-chemically filtered environment, the detectors will show a gradual to sudden increase. This technology has been added to many laboratories to monitor for any decline of environmental quality.

Gas chromatography or gas chromatography mass spectroscopy permits identification and quantification of 65–75 of the reference VOC listed for the TO15 methodology by the Environmental Protection Agency (Environmental Protection Agency, 1999) at low levels (μg/m^3 or ppb). It can also identify and semi-quantify materials not calibrated by the gas chromatography standards.

Sources of aldehydes in ART laboratory settings

Some evidence shows that the ability to culture human embryos in an ART facility to the blastocyst stage is reduced by a select group of contaminants. Data obtained from several laboratories (unpublished data provided by Alpha Environmental Inc., Emerson, NJ, USA) has correlated elevations of formaldehyde, acetaldehyde and higher molecular weight aldehydes with poor, delayed or no embryo development (**Table 4**), and subsequent reductions in aldehyde levels with improved embryo development.

In addition, according to the Hazardous Substance Data Bank (www.toxnet.nlm.nih.gov/newtoxnet/hsdb.htm), many of the common aldehydes found in the ART laboratory, e.g. formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, benzaldehyde, n-hexaldehyde, and acrolein (**Table 5**) are known carcinogens and mutagens. Another common compound, acetonitrile, has also been suggested as a possible source for the slow release of cyanide.

Table 4 – Aldehyde levels measured in four IVF laboratories that were experiencing significant decreases in outcomes (data from Alpha Environmental Inc., Emerson, NJ, USA).

Laboratory Number	Material	Concentration (μg/m³)		Observations
		IVF laboratory	Incubator	
I	Formaldehyde	29.0	N/A	Human embryos: unable to reach blastocyst stage.
	Acetaldehyde	13.0		
II	Formaldehyde	N/A	140	MEA: poor result
			81	Human embryos: unable to reach four-cell and blastocyst stages.
	Acetaldehyde		61	
	Hexaldehyde			
III	Formaldehyde	25.0	2.8	MEA: unable to pass
	Acetaldehyde	17.0	12.0	Human embryos: 'very
	Hexaldehyde	8.2	4.4	limited clinical success'
IV	Formaldehyde	21.0		Human embryos: unable to reach blastocyst stage; no pregnancies.
	Butyraldehyde	23.0		
	Benzaldehyde	8.8		
	Total with all aldehydes detected	74.1		

Air samples were taken at locations selected by the client and Alpha Environmental; aldehyde concentrations (generally ranging between 20 and 282 μg/m³) were determined using a US Environmental Protetction Agency (EPA) TO-11a method used by the EPA and the US Occupational Safety and Health Administration (OSHA); see www3.epa.gov/ttn/amtic/airtox.html.
MEA, mouse embryo assay.

Indeed, among modern ART laboratories, i.e. those built using cleanroom concepts, the mean and upper 95% confidence limit for total VOC levels were 339.5 μg/m³ and 1213.9 μg/m³, respectively, whereas those for older-style ART laboratories were 1323.2 μg/m³ and 3236.5 μg/m³, respectively (**Table 5**).

Formaldehyde is released from numerous sources in the external environment, as well as from inside the ART laboratory. Formaldehyde reacts with albumin and will denature it, so it is of concern in all biological systems.

Formaldehyde is photochemically produced in smog and is seen in areas with a high solar flux, e.g. California, Texas, Guatemala and Beijing. Incomplete combustion of fossil fuels, e.g. in gas water heaters and motor vehicles, can further increase environmental formaldehyde levels. Inside buildings, MDF is a significant source of formaldehyde owing to off-gassing and should not be used in ART laboratory cabinetry. If possible, MDF should not be used for cabinetry in adjacent office and clinical spaces in case poor or intermittent overpressure allows off-gassed formaldehyde to enter the ART laboratory. Off-gassing is a time-dependent reaction and can be accelerated by higher temperatures, e.g. 33°C. Therefore, at room temperature, this process will continue for quite some time, with significant levels of formaldehyde produced, in the mg/m³ range, but effects are seen in IVF laboratories in the μg/m³ range.

Formaldehyde can be added to paints, but the Material Safety Data Sheet may not list it as an ingredient (if its concentration is less than 0.1–1.0%) or as an impurity. The fixation process for pathology biopsy samples (10% formalin) can also be a source of formaldehyde. Formaldehyde partitions into air because of its very high vapor pressure.

Cold sterilization, e.g. Cidex, is a significant source of other molecular weight aldehydes, including glutaraldehyde. The cold sterilizer system is often located in the procedure room, in open containers, which allows the fumes to enter the IVF suite environment. The sterilizer solution is carcinogenic, mutagenic and water-soluble; therefore, it poses a significant risk to gametes and embryos.

Compressed gases, particularly CO_2, can also be a source of aldehydes. A range of aldehydes dissolved in the liquid CO_2 within the compressed gas tank are available, such as acetaldehyde, isovaleraldehyde, benzaldehyde and formaldehyde. If liquid CO_2 is present in the cylinder, they will remain dissolved and not enter the gas phase, but when the pressure in the gas tank becomes low, the aldehydes will enter the gas phase and pass into the incubator.

Styrene, the monomer used in the production of polystyrene, can also be found in ART laboratories because of incomplete polymerization during the manufacturing process, resulting in the release of styrene molecules when new packages of plasticware are opened. The octanol–water partition coefficient (log (K[O/W]) for styrene is 3.25, meaning that, for every 1000 molecules of styrene, well over 99% would be in the mineral oil phase with a very high affinity.

It is not inevitable that every IVF laboratory will have high levels of aldehydes; laboratories designed, built and operated on the basis of principles of reducing incidental chemical exposures can show sustained low aldehyde levels (**Table 6**).

Avoiding VOCs in culture

As a rule, the culture environment is optimized by minimizing the length of time gametes and embryos are outside of the incubators while performing required procedures, and the number of incubator openings, as this can lead to environmental fluctuations (Wale and Gardner, 2016). Nevertheless, even brief exposures to the external environment may be sufficient to introduce hazards such as bacteria, moulds and toxins that could affect gamete biology, embryo development, or both. Bacteria and moulds can be avoided by filtration of culture medium or air, and by using antibiotics in the culture medium and ultraviolet light photooxidation in air purification systems. Exposure to toxins, particularly VOCs, in the laboratory, however, is quite prevalent, and is suspected to affect embryonic development as early as the start of culture in the laboratory or as late as after implantation of embryos into the uterus. Culture environment can affect the appearance of embryos during culture and the subsequent performance of embryos extending well beyond the culture period for other mammals (Johnson and Gardner, 2011; Lane et al., 2008).

Table 5 – Measured volatile organic compounds concentrations in both older style IVF laboratories and modern assisted reproduction technology suites built using cleanroom concepts (data from Alpha Environmental, Inc, Emerson, NJ, USA).

Compound	Older-style ART laboratories (μg/m^3)		Modern ART laboratories (μg/m^3)	
	Mean ± SD	95% upper confidence limits	Mean ± SD	95% upper confidence limits
Ethanol	397.6 ± 338.6	1074.8	101.1 ± 158.7	418.5
Isopropyl alcohol (2-propanol)	570.8 ± 755.6	2082.0	101.1 ± 201.5	504.1
Acetone	86.0 ± 166.4	418.8	36.2 ± 48.0	132.1
Propene	22.4 ± 28.8	80.1	11.5 ± 11.9	35.3
Acetonitrile	9.1 ± 16.5	42.0	7.7 ± 13.2	34.2
Isobutane	4.0 ± 6.0	15.9	7.3 ± 29.9	67.0
Chlorodifluoromethane	57.7 ± 233.9	525.5	6.2 ± 31.3	68.8
Toluene	7.0 ± 10.1	27.3	5.8 ± 12.5	30.8
Hexamethylcyclotrisiloxane	14.7 ± 31.5	77.7	5.8 ± 14.8	35.3
1-Butanol	7.3 ± 18.0	43.3	4.6 ± 17.5	39.5
N-pentane	5.1 ± 11.7	28.5	4.5 ± 9.8	24.2
Sevoflurane	15.9 ± 49.1	114.0	4.2 ± 21.5	47.2
Ethyl acetate	6.2 ± 13.8	33.7	4.0 ± 8.3	20.7
Benzaldehyde	1.0 ± 4.3	9.5	3.9 ± 6.5	17.0
N-butane	5.1 ± 10.1	25.3	3.6 ± 4.5	12.5
m,p-Xylenes	1.8 ± 3.1	8.1	2.4 ± 11.0	24.4
Unknown siloxane	2.5 ± 10.6	23.8	2.4 ± 5.0	12.5
Trimethylsilanol	3.3 ± 7.7	18.7	2.3 ± 6.0	14.2
Dichlorodifluoromethane (CFC 12)	8.8 ± 27.2	63.2	2.1 ± 1.2	4.5
2-Methylpentane	1.1 ± 3.0	7.1	2.1 ± 6.9	15.8
2-Butanone (MEK)	2.4 ± 6.1	14.5	2.0 ± 3.6	9.3
N-hexane	40.1 ± 170.2	380.5	1.7 ± 6.3	14.3
Tetrachloroethene	2.2 ± 8.1	18.4	1.6 ± 7.7	16.9
Propane	1.0 ± 4.7	10.4	1.4 ± 3.3	7.9
N-nonanal	1.9 ± 8.5	19.0	1.3 ± 3.3	7.9
Norfluane	1.6 ± 6.2	14.1	1.1 ± 4.4	9.9
Methylene chloride	1.9 ± 4.0	9.9	1.0 ± 4.1	9.1
2-Ethyl-1-hexanol	0.7 ± 2.4	5.5	0.9 ± 3.0	6.8
1,1-Difluoroethane	1.3 ± 5.8	12.8	0.9 ± 5.9	12.8
Methyl alcohol	5.5 ± 8.0	21.4	0.9 ± 4.4	9.7
Trichlorofluoromethane	18.7 ± 76.5	171.6	0.9 ± 0.7	2.4
Chloroethane	5.1 ± 16.9	38.8	0.9 ± 4.7	10.3
Carbon disulfide	1.5 ± 3.7	8.9	0.8 ± 1.7	4.2
Ethylbenzene	0.4 ± 0.8	1.9	0.7 ± 3.4	7.5
D-limonene	3.4 ± 5.5	14.4	0.6 ± 1.5	3.6
O-xylene	0.5 ± 1.1	2.6	0.6 ± 2.5	5.5
Benzene	0.8 ± 1.3	3.4	0.5 ± 2.0	4.6
N-nonane	1.6 ± 4.8	11.2	0.4 ± 2.2	4.9
N-hexanal	0.2 ± 0.9	2.0	0.4 ± 1.0	2.5
α-Pinene	0.6 ± 1.9	4.3	0.4 ± 0.9	2.2
Styrene	0.5 ± 2.0	4.6	0.4 ± 1.2	2.8
Acrolein	0.8 ± 1.4	3.6	0.3 ± 0.6	1.5
Chloromethane	0.2 ± 0.7	1.6	0.3 ± 1.3	2.9
Tetrahydrofuran (THF)	0.6 ± 2.6	5.7	0.2 ± 1.1	2.4
N-dodecane	0.4 ± 1.8	3.9	0.2 ± 0.9	2.0
N-butyl acetate	0.3 ± 0.6	1.5	0.2 ± 0.8	1.7
N-undecane	0.5 ± 2.1	4.7	0.2 ± 1.0	2.2
1,2,4-trimethylbenzene	1.2 ± 2.6	6.4	0.2 ± 0.5	1.2
N-butanal	0.2 ± 0.8	1.8	0.2 ± 0.6	1.4
Total VOC	1323.2	3236.5	339.9	1213.9

ART, assisted reproduction technology.

Avoid introducing VOC into the laboratory

Painting the laboratory

Standard paints used to seal the wall and ceiling surfaces in laboratories may be a large source of VOCs. Paints are now available that claim to be low VOC or VOC-free (Gilligan, 2010). The addition of pigment at the point of sale should be avoided, as this could increase VOC content. The odour of these reduced-VOC paints is markedly different and less unpleasant. Hopefully, the reductions of VOCs in these paints makes them less toxic to embryos. Painting nearby rooms or hallways can lead to VOCs entering the laboratory. Therefore, it is wise to have all building personnel, e.g. facilities

Table 6 – Measured aldehyde levels in modern IVF laboratories built using cleanroom concepts (data from Alpha Environmental Inc., Emerson, NJ, USA).

Compound	Mean µg/m^3	SD	95% upper confidence limit µg/m^3
Formaldehyde	2.8	3.2	9.3
Acetaldehyde	1.8	1.9	5.7
Propionaldehyde	0.6	0.7	2.0
Crotonaldehyde, Total	0.0	0.0	0.1
Butyraldehyde	0.1	0.2	0.5
Benzaldehyde	0.1	0.1	0.4
Isovaleraldehyde	0.0	0.0	0.0
Valeraldehyde	0.1	0.1	0.3
o-Tolualdehyde	0.0	0.0	0.0
m,p-Tolualdehyde	0.0	0.0	0.0
n-Hexaldehyde	0.2	0.2	0.7
2,5-Dimethylbenzaldehyde	0.0	0.0	0.1
Total aldehydes	5.8		

managers and contractors, made aware of the effects of VOCs on embryos so that they discuss with the laboratory all VOC-containing building materials before use within the building. If painting is carried out within the laboratory or elsewhere in the building, it should be at a time when the laboratory is not carrying out embryo culture. Final VOC release after renovation can be enhanced by increasing room temperature and turning on the lights for a period, preferably days or weeks.

Selection of laboratory furniture

Laboratories generally include counter space used as workspace as well as cabinets used for storing supplies. Counter tops and cabinets constructed from manufactured wood products, e.g. MDF, contain binders that release formaldehyde into the space around them for a considerable period. Other wooden furniture may have varnished, shellacked or painted surfaces that could also release VOCs, although older wooden furniture that has been at the facility for a lengthy period may have off-gassed sufficiently to be non-toxic.

Stainless steel furniture is less likely to release VOCs, although the surfaces may be oiled during the construction process. These furniture items should be cleaned thoroughly with isopropyl alcohol to remove any superficial VOCs before introduction into the laboratory. The issue remains of grease used to lubricate hinges and drawer slides, which should be silicone-based.

Incubator commissioning

Concerns have been raised that the plastic seals (gaskets) around the edges of the external doors of large incubators were responsible for the release of substances that were embryotoxic. When an incubator, however, was commissioned and run at culture temperature with appropriate gas conditions for an extended period (roughly a month or two), toxicity associated with 'being new' disappeared (Jacques Cohen, personal communication). Most manufacturers probably select the components for use in construction of the units based on durability, cost and availability, rather than to eliminate VOCs and aldehydes. Incubators should be off-gassed whenever possible, e.g. running new units in a ventilated space (not the embryology laboratory) at high temperature for some time. Users should be careful when considering major repairs or servicing as replacement parts could re-introduce VOC-containing components.

Off-gassing plasticware

One of the most prevalent sources of VOCs in the laboratory is the plasticware, which is typically molded from polystyrene. One method to avoid introducing styrene, a major VOC associated with the production of polystyrene, into the laboratory is to off-gas the plasticware before use. It is best to carry out this off-gassing outside of the laboratory so that the released styrene does not contaminate the laboratory. Off-gassing is often carried out by opening sleeves of plasticware and allowing them to vent in a different room in which culture is not carried out. Note that it is critical to maintain sterility during this off-gassing process as open sleeves expose dishes to possible contamination. It is more difficult to carry out off-gassing with individually packaged plasticware (newer multi-well dishes and pipettes that are not packaged in plastic sleeves). One option is to open all dishes needed for the coming week and stack them in a laminar flow hood for off-gassing. The content of VOCs is lower in plasticware covered with filtered sleeves, which allow off-gassing before opening.

HVAC air intake

One of the primary goals of the IVF laboratory's dedicated HVAC system is to decrease the VOCs in the laboratory; however, at times, the 'fresh' air from outside the building will have extraordinarily high levels of VOCs. This can occur when the fresh air inlet is on the roof of the building and the roofing material (tar) is being replaced or repaired, when the region is exposed to high levels of smoke from a large fire, when a parking lot near the building is being repaved or painted, or when construction is taking place nearby with heavy diesel equipment, or even just an open hole in the ground. When such events occur, it might be possible to decrease the load of VOCs in the laboratory by switching the HVAC system to 100% recirculation rather than mixing fresh air with the recirculated air. Beware, however, this might have other undesirable consequences, e.g. on temperature, humidity and loss of overpressure in the IVF suite.

Smoking

Most healthcare environments now limit smoking to areas outside the building. Certainly, smoking should not occur in any area where it might affect the air quality inside the laboratory. Keep smokers (and internal combustion engine exhaust, including backup power generators) away from the air intake to the IVF laboratory's HVAC system. Laboratory personnel who smoke could introduce embryo toxins into the laboratory from their clothes, their skin, or possibly even from their lungs, via 'third hand smoke' exposure (Hang et al., 2013).

Cleaning the laboratory

The laboratory should be kept clean and free of microbial contamination, but consideration should be given to the types of products used to achieve these goals (Catt et al., 2013). Although ethanol is commonly used to provide a clean and relatively microbe-free work surface, it is a VOC and a known embryo toxin. Clearly, ethanol should only be used in situations in which its vapour cannot dissolve into cultures. Alternatively, some laboratories use very dilute sodium hypochlorite (Chlorox) solutions, or simply hydrogen peroxide. Many laboratories use water for cleaning and avoid the use of ethanol or other sanitizers, simply to avoid the possible consequences of embryotoxicity; others use hydrogen peroxide. Although products specifically for ART laboratories are available on the market, care must be taken to assure that they do not have oocyte- or embryo-toxicity.

Miscellaneous sources

It is possible to find toxicity with nearly everything that is used in the laboratory. Detergents used to launder scrubs, solvents in the felt-tip pens used to write on cryocontainers (referred to in some regulatory documents as 'specimen packaging devices'), shoe-polish, hairspray, cologne or deodorants are all things that must be considered and the benefits weighed against the toxicity and exposure.

Decrease ambient VOCs in the laboratory

Despite the best diligence to avoid their introduction, VOCs will exist in the laboratory. Two types of systems can help to reduce VOCs in the laboratory: the HVAC system and in-room purifiers. Both systems are designed to provide VOC-free air. Although HVAC-based VOC filtration systems are designed to virtually eliminate VOCs in the air supplied to the laboratories, in-room purifiers are limited by their capacity and so do not likely provide the same level of VOC removal as well-designed HVAC units.

The standalone HVAC system is constructed within and expressly for the laboratory or ART suite. Complete systems can regulate temperature (heating or cooling) and humidity, as well as air cleaning. To clean the air, the system should comprise HEPA filters to remove particulates (like bacteria and mould spores) and technology that can reduce the VOCs that pass through them. There are several approaches to effective VOC removal. The two most common technologies are activated carbon with potassium permanganate and ultraviolet photocatalytic oxidation. Both systems, when designed properly, provide sufficient capacity to remove VOCs while in the HVAC system. The size and type of filters should be designed for the laboratory's air volume providing sufficient turnover rate and exposure time to the carbon filter to remove VOCs from the room air. To perform as designed, the HVAC system must be maintained to assure sufficient air turnover rate and sufficient filter capacity (by changing filters on schedule). Equally important is the maintenance of humidity so that it is high enough to reduce desiccation of aqueous culture medium, i.e. increased osmolality, and low enough to prevent conditions that foster rampant growth of bacteria and moulds within the HVAC system (Swain et al., 2012; www.epa.gov/mold/take-mold-course). For systems that use activated carbon, potassium permanganate reduces the levels of oxidation/reduction reagents that could also cause problems for cultures.

The second approach is the use of a portable or within-laboratory device that will perform the same VOC-reduction tasks but may not regulate air temperature or humidity. Note that only units with carbon filtration or ultraviolet photocatalytic oxidation will reduce VOCs; HEPA filtration is not designed for, or capable of, reducing VOCs. An in-room filtration unit must have sufficient capacity for the size of the room and, like the HVAC, be maintained appropriately with appropriate filter replacement intervals to assure that it is reducing VOCs to levels as low as can be achieved.

Decrease VOCs in incubators

Source of purchased gases

The gases that are purchased in cylinders to supply the incubator chambers can be contaminated with VOCs (Mehta and Varghese, 2016). Cylinders for CO_2, O_2, and N_2 are available in different purity levels (grades). Manufacturers that provide gases for medical uses also supply other industries, such as welders. It is recommended that users should discuss the particular needs of IVF laboratories with representatives from the supply company. It might be helpful to insist that an IVF laboratory purchase new cylinders from the gas supplier for their exclusive use, thereby assuring that they are relatively clean when they are re-filled with gas, and should assure that the same cylinder was not used for acetylene, for example, before it was filled with carbon dioxide. Gas companies supply low-grade, food, medical or reagent grade gases. Medical grade gases are of a purity that can be used with patients (adults), but the purity required to avoid embryotoxicity may be much cleaner than is required by a living breathing patient with functioning detoxification organs. For many gas companies, 'medical grade' only means that the cylinders have only ever been used for medical gases at medical facilities, i.e. they have not been used for industrial gases or at industrial locations. An IVF laboratory might wish to pay more money to purchase gas that has much more stringent quality standards to ensure that the level of toxicants is less than some known specified level. Reagent grade gases can be manufactured to different contaminant levels and can be expensive. The cleaner the gas in the tank, however, the fewer toxicants it should release into the incubators.

In-line filters

Having the gases flow from the tanks through in-line filters can minimize VOC contaminant levels that reach the incubator. Some in-line filters contain only carbon as a filter material that absorbs VOCs. Others contain both carbon and a potassium permanganate stage to reduce VOCs as well as redox agents. Evidence is lacking on the effectiveness of the different types of in-line filters or the frequency of change-out, with monthly, quarterly or even less frequent changes being recommended. Of note, carbon filters can become saturated and then, depending on the external factors, can off-gas at variable rates that ultimately reduce air quality provided to incubators. One manufacturer has tested uptake using a known standard of VOC mixture at 1 ppm through a long commercial in-line filter and found that this type of filter effectively removed 100% of known VOCs. Testing was carried out by an independent laboratory (unpublished data from D Rieger, J Cohen and A Gilligan).

Incubator type

The type of incubator used can affect its susceptibility to different sources of VOCs. Traditional 'big-box' incubators, whether they are CO_2-only or tri-gas for reduced O_2, circulate room air through the incubator chamber and regulate the gas levels by the addition of CO_2 and N_2. Clearly, this style of incubator is susceptible to the inclusion of those VOCs that are in the room air. In contrast, modern benchtop incubators generally function by flushing the incubator chamber(s) with gas(es) provided in cylinders, either pre-mixed in the cylinders or mixed inside the benchtop incubator. As the incubator flushes the chamber(s) with the mixed gas, any ambient room air is flushed out of the chamber, thereby leaving the tanks and in-line filters as the only source of contaminants inside the incubation chamber.

Intra-incubator recirculating filters

Devices are available commercially that are placed inside a large incubator chamber (old style, 'big-box' incubators) and recirculate the chamber's gases through a carbon filtration system that will reduce the VOCs inside the incubator chamber. Time-lapse and benchtop incubators that mix gases recirculate the mixed gas continuously and usually include another inline carbon filter, thus effectively doubling the filtration compared with gas line filtration only. This approach,

however, is only effective when the incubation atmosphere is not humidified as liquid water on the surface of the carbon greatly impairs removal of airborne VOCs.

Decrease VOCs in cultures

Infiltration of VOCs into cultures can occur from gases inside the incubator or from brief exposure of the cultures to room air during procedures outside the incubator or by opening the incubator chamber and allowing room air into the chamber. Once VOCs are in the culture, it is difficult to remove them, but it is possible to reduce their concentration. VOCs can be either hydrophobic, e.g. benzene, styrene or hydrophilic, e.g. ethanol, acrolein, formaldehyde and glutaraldehyde.

Hydrophobic VOCs can partition into the embryos' membranes as they are also oil-like phases. Overlaying the culture medium with oil results in the hydrophobic VOCs partitioning preferentially into the oil phases, thereby reducing concentration in the aqueous medium. The relative solubility in oil of a compound is described by its oil–water partition coefficient, i.e. the concentration of the compound in the oil phase divided by its concentration in the aqueous (water) phase when the distribution between the two phases is in equilibrium. Compounds with a high partition coefficient are hydrophobic and will have a much higher concentration in the oil phase than in the aqueous phase at equilibrium.

Oil overlay is commonly used to decrease loss of carbon dioxide and desiccation of medium drops, and to minimize temperature changes of cultures when they are removed from the incubator for procedures. Although the latter two points are valid, the first has only a negligible benefit, as an oil overlay has been shown to cause substantial slowing of medium re-gassing (Blake et al., 1999; discussed in Mortimer and Mortimer, 2015). The added benefit of detoxifying the medium of VOCs is seldom considered but is likely to be a significant advantage of the use of oil overlay. An additional benefit is derived because not only do the VOCs tend to partition into the oil phase and at much higher concentrations in the oil, but also because the volume of the oil phase is so much greater than the volume of the aqueous phase that the VOCs are more greatly diluted in the oil phase. This has a major effect of decreasing the concentration of VOCs in the aqueous phase culture (medium) even further.

No evidence, however, is available on the use of oil overlay as an absolute barrier: once VOCs are dissolved in the oil phase, it is likely that some level of VOCs will be present in the medium based on their partition coefficients. If the level in the oil is high enough, the level of VOCs in the medium could reach a level that is toxic. Although no experimental evidence is available on diffusion and channel mechanisms across membranes for different types of VOCs, it has been hypothesized that hydrophilic VOCs could partition into the aqueous cytoplasm of oocytes and embryos, and would be present there at higher concentrations than in the oil phase (membranes and oil overlays) owing to their low oil–water partition coefficients. As hydrophilic VOCs dissolve less well in oil than in water, overlay oil acts as a substantial, but not necessarily absolute, barrier to hydrophilic VOCs diffusion in and out of the culture medium. Its main advantage is that it slows the attainment of kinetic equilibrium so that during brief exposures of oil-overlaid cultures to VOCs, a lower amount of hydrophilic VOCs will gain access to the culture medium, thereby decreasing the possibility of harming the embryos. It is likely, however, that, with sufficiently long-term exposure, the same equilibrium concentration of hydrophilic VOCs would be attained in the aqueous phases as without the oil. Oil overlay, therefore, is believed to help minimize VOC toxicity in culture medium by either a partitioning effect that tends to remove hydrophobic VOCs from the aqueous phase (and dilution of the hydrophobic VOCs in volumes of oil that are much greater than the volume of membranes); or by slowing the entry of hydrophilic VOCs into the culture medium and embryo cytoplasm.

A further consideration is that the rate at which VOCs are dissolved into the oil phase is dependent on the surface area of exposure. So, if we reduce the surface area exposed to the VOC-containing atmosphere, then we might reduce the level of VOCs that dissolve in the oil during a brief exposure. In typical culture dishes, the surface area of the exposed oil is large compared with the depth of the oil, favouring dissolution of atmospheric VOCs into the oil more rapidly. If the cultures are maintained with a deep column of oil over a small drop of medium (like a drop of medium in the bottom of a culture tube with a relatively tall column of oil covering the drop), then the surface area of the exposed oil is small compared with the depth and might be less likely to favour the dissolution of VOCs in the oil. Some of this volume effect could possibly be reduced by adding blank dishes filled with oil to act as an 'oil sink'.

Dissolution of carbon dioxide through the oil into the medium will also be affected in a similar manner, likely explaining the need for pre-incubation to achieve medium pH equilibration (Blake et al., 1999).

Consensus points

The participants agreed with the general statement that 'fair evidence derived from both animal and human studies indicates that controlling laboratory contamination positively impacts in vitro fertilization outcomes' (Esteves and Bento, 2016; Morbeck, 2015). On the basis of the reviewed material, it was recognized that level 1 evidence is mostly lacking, but it was also agreed that such evidence is hard to obtain owing to physical complications related to randomizing different atmospheres in the same laboratory space or incubator.

It was unanimously agreed that great effort should be taken to ensure that the ART laboratory has clean air. Therefore, many aspects of cleanroom design should be used in its construction. The ART laboratory, however, has critically different requirements to those of the type of high-level cleanroom that might be used for integrated circuit board or pharmaceutical manufacturing, or for transplant surgery. For example, while air quality should have particulate levels comparable to those in an ISO Class 6/GMP Grade B–C cleanroom, the number ACH required for such high-level cleanrooms is excessive for IVF applications as it can cause excessive cooling of the gametes and embryos and hence serious adverse effects on embryo development and clinical outcomes (Mortimer, 2005). The permitted background of Grade D under the EUTCD (<3,500,000 particles per m^3 'at rest', with <200 cfu/m^3 for micro-organisms was considered by the group to be insufficient when considering all the attendant risks when creating future generations of humankind. Therefore, the middle-ground of ISO Class 7/GMP Grade B 'in operation' / Grade C 'at rest' was taken as the target. This is easily achieved if HEPA filters are installed with sufficient ACH (10–15/h) and can also be achieved (at least at rest) using mobile air filtration units, although mobile units cannot create positive pressure within the laboratory.

IVF Procedure Rooms in which procedures such as oocyte retrievals, embryo transfers and percutaneous sperm retrievals are carried out, should be differentiated from invasive surgical facilities, which would usually be subject to separate regulation and licensing. Consequently, if the ART procedures room is to be used

for invasive surgical procedures, its HVAC system should be separate to the ART suite so as not to require unnecessary and potentially deleterious higher air quality criteria.

Assessing site suitability

Careful attention should be paid to the location of the building in which an IVF laboratory is to be constructed, recognizing possible sources of particulate and chemical pollution, e.g. parking garages, dry cleaners, foundries and petroleum processing facilities. This might include discussion with local environmental agencies about PM5 and PM10 data, i.e. the concentration of particulate matter of up to 5 µm and 10 µm diameter, respectively. If known pollution sources are identified, this might warrant additional measures to reduce those pollutants within the laboratory environment.

It is recommended that investigation of VOCs within the proposed building and its environs be undertaken, recognizing the limitations of snapshot testing. These analyses should consider specific VOCs rather than measuring total VOCs.

Basic design criteria (new construction)

As noted previously, these consensus points should be considered as aspirational benchmarks for existing ART laboratories and as guidelines for the construction of new ART laboratories. The ART laboratory should be supplied by HEPA-filtered air of a quality at least equal to that of an operating room.

Air quality

Particulates. Less than 352,000 particles larger than 0.5 µm to 10 µm per metre^3 (equivalent to <10,000 such particles per cubic foot)

Micro-organisms. Less than 10 cfu/m^3 and less than two spores/m^3 'at rest'.

VOCs. Total VOCs less than 500 µg/m^3 (~400–800 ppb total VOC, depending on molecular species); less than 5 µg/m^3 aldehydes.

Air changes. Fifteen total air changes per hour, including three fresh air changes per hour, i.e. 20% outside air. Type of VOC filtration and filters' manufacturer instructions concerning ACH should also be considered when setting the fresh to recirculated air ratio.

Overpressure. Ideal target is +38 to +50 Pa in the IVF laboratory (recommended minimum +30 Pa). This can be attained through a cascade of overpressure across several rooms, e.g., external space to access vestibule to IVF laboratory, or recovery area to access vestibule to procedure room to IVF laboratory, to avoid too great a pressure differential between immediately adjacent rooms.

Temperature. Temperature control of the ART suite should be effected by the HVAC system. This means that the total heat output of the laboratory equipment and staff need to be considered. Working temperature in the laboratories should be stable and maintained in a range comfortable to the staff, typically within the range of 20–24°C (depending on region). Keeping the temperature within a narrow range facilitates equipment calibration and operation.

Humidity. Room relative humidity should be between 40% and 45%. Higher levels will promote growth of moulds, lower values are uncomfortable, if not unhealthy for humans. Lower humidity also causes high levels of evaporation during dish preparation, which will affect osmolarity of the culture medium and be deleterious to embryos in culture (Swain et al., 2012).

General design and construction criteria

The AHU must be dedicated to supplying the ART suite to avoid contamination of the recirculating cleanroom air with non-clean air from adjacent spaces. The HVAC system must run constantly, not just during working hours. Although it might be financially impractical to provide generator back-up for the entire HVAC system, it is recommended that, at a minimum, the HVAC fans should be on generator back-up, but such a decision must also take into consideration maintaining adequate temperature control within the facility. The fresh air intake must be located away from obvious sources of noxious fumes, and incoming air be appropriately pre-filtered to optimize the functional life of filter systems.

An ART Laboratory has different requirements compared with other medical and laboratory facilities, in particular the extreme sensitivity of embryos to VOCs, especially aldehydes. Therefore, the AHU and ductwork must be thoroughly cleaned to remove any corrosion prevention treatments (such as mineral oil and anti-rust agents) before assembly, using either isopropanol (2-propanol) or ethanol (USP grade or equivalent). Ductwork sections must be cleaned and sealed off-site, then assembled on-site in a stepwise manner. All ductwork joints must be sealed, e.g. using water-based silicone, and sealed externally using metal tape.

The system needs to be designed with a high level of recirculation of the cleaned air to minimize VOC levels while reducing energy costs in terms of heating and cooling.

It is strongly recommended that contractors should be educated on the unusual requirements of an IVF suite compared with other medical and laboratory facilities.

Sealing. Sealing of the IVF laboratory should be stressed. The materials specified in the design should be the materials used in the construction. Under ideal circumstances the worksite should be inspected frequently, ideally every day, by the client or their representative.

Activated carbon/potassium permanganate filter. Regardless of the VOC elimination technology used, an activated carbon or potassium permanganate filter should be included in the HVAC system, downstream of all air handling but before the HEPA filter. The residence time in this filter should be 0.2–0.35 s.

Heating and cooling of incoming fresh air. Adequate provision must be made for heating and cooling of the incoming fresh air according to local climatic requirements.

Isolating external air. The HVAC system should be capable of being totally isolated from the outside air in case of an emergency, e.g., extreme temperature inversions, forest fires, high ozone days, local construction work that generates high levels of VOCs, such as resurfacing roads or parking lots and ash clouds. When running in such a 'submarine' mode, the over-pressure will be lost owing to the lack of make-up fresh air, but this is preferable to damaging the filter systems or allowing the highly toxic exterior air into the ART laboratory.

Air supply vents and return ducts. Air supply vents should be located in the ceiling and return ducts should draw from close-to-floor level. Attention must be paid to the possibility of drafts from incoming air vents affecting the operation of some pieces of equipment.

HEPA filters. HEPA filters should be located centrally, to avoid the need for access to multiple locations within the ART suite when changing them.

Air quality. To achieve optimum air quality, the system should be re-circulating with typically only 20% fresh air (see above) to create the necessary over-pressurization.

Pressure sensors. Ideally, there should be pressure sensors and differential pressure displays installed on each side of each doorway into, out of, and between areas within the ART suite cleanroom space.

'Sealing the box'

Achieving and maintaining effective over-pressure within the embryology laboratory, so that the direction of air flow is always out of the space, means that the cleanroom suite must be built with minimum opportunity for air loss.

Slab-to-slab. The exterior walls of the cleanroom must go from the concrete floor up to the underside of the concrete of the floor above (often described as 'slab-to-slab'), and all perforations must be completely sealed.

Ceiling. The ceiling must be composed of a contiguous, solid material, e.g. plasterboard, gypsum panels, Gyprock, Sheetrock®, not tiles, and the need for any access panels must be minimized. Essential access panels must have air-tight, silicone gaskets.

Light fittings. Light fittings must be air-tight, designed for cleanrooms, so no air leakage occurs into the plenum void above the ceiling. Light fittings can be surface-mounted provided that the cable access is sealed, and there is no horizontal rim or flange where dirt can accumulate.

Electrical, gas and data conduits. All electrical, gas and data conduits must be sealed where they enter or leave the clean room to prevent air loss through them (including behind light switches); within the suite, use steel 'Dado' trunking attached to the wall for the distribution of power, data and gas lines.

Doors. Doors must be tight-fitting with bottom 'sweeps' and perimeter seals (top and edges). Any view panels must be mounted using gaskets to make them air-tight.

Pass throughs. Pass-throughs must be air-tight to preserve room air pressure differentials.

Construction materials

The following aspects must be recognized when selecting the materials to be used in the construction of an ART suite, comprising therapeutic laboratories and possibly procedure rooms.

Walls. Suitable materials for walls are true cleanroom modular panels with powder-coated, metal, gasketed interfaces, or plasterboard coated with zero VOC paint.

Floors. Sheet vinyl with impervious sealed joints must be used for floors. In areas in which large volumes of liquid nitrogen are used, consideration should be given to using a non-thermally-fragile floor covering, such as stainless-steel tread plate.

Countertops. Non-porous materials that do not release VOC should be used for countertops. Suitable materials include epoxy, Corian®, and Trespa®.

Ceilings. Inspection or access panels must be kept to an absolute minimum and should be gasketed and sealed at the interface to the ceiling and at the door access panel frame.

Windows. Windows should preferably be glass, gasketed and small, and primarily mounted in doors. Any windows to the outdoors should have spectral filters to exclude ultraviolet wavelengths. If considering large, laboratory observation windows, the difficulty in creating and maintaining an effective seal for these windows should be considered.

Cabinets. Cabinets (under and over benches) should be powder-coated metal or stainless steel.

Wood products. Use of manufactured wood products, such as MDF, Formica®, linoleum or oil-based paints is not recommended, as they have all been demonstrated to be embryotoxic.

Plumbing

Handwashing facilities. Handwashing facilities should typically be located in vestibules rather than in laboratories. Their waste pipes will require traps.

Noxious and corrosive chemicals. Noxious and corrosive chemicals should not be used in IVF laboratories, so there should be no need for drench showers. In the case where these are required by local building codes, great care should be taken to prevent the trap from drying out.

Fire suppression system. If installed, the fire suppression system, i.e. sprinklers, in the laboratory should be protected from accidental triggering from outside the laboratory.

Open flame. If a laboratory system includes an open flame, a risk assessment of the likelihood or risk of fire, and of exposure to the products of combustion should be undertaken.

Plumbing. Avoid the presence of unnecessary plumbing in the ceiling plenum space as it represents a source of flooding and potential contamination. This is especially the case when renovating an existing building as plumbing is often discovered during renovation and must be redirected outside of the ART suite plenum.

Laboratory commissioning and ongoing VOC management

'Burn-in'

A newly constructed or renovated laboratory should be given adequate time for off-gassing of construction materials. The period required for this will depend upon the location and materials used

and might require several weeks; a minimum of 2–3 weeks should be allowed for this in the construction schedule. Verification should be established by specific VOC testing to provide comparison with a baseline. Bioassays such as human sperm survival test are not sufficient for this purpose.

'Deep clean'

A newly constructed or renovated laboratory must be subjected to an intensive cleaning before being validated for clinical use. Every surface, including all hard-to-reach corners, inside cupboards and drawers, and all equipment, is cleaned with products capable of removing all expected contaminants, and then cleaned again to ensure no trace of the cleaning agents remains.

Servicing

A clean room HVAC system must be serviced annually, or more often if performance fails to meet expectations. The principle is to ensure continued performance as per specifications and fitness-for-purpose and practices can be based on the ISO 14644 family of standards. Typical pleated sheet particulate filters can be changed at 90-day intervals. HEPA filters typically last 2–4 years and the pressure differential across these filters should be monitored by pressure gauges, e.g. Magnehelic®. Changing chemical filters at a set time interval is not optimal as they can be rapidly exhausted under conditions of excessive solvent use, fires and construction activities. While routine chemical tests of the filters can pose practical and financial problems, building a history of a facility's rate of consumption of the chemical filter media would generate a rational schedule for changes.

Infection control. Infection control measures in routine use for other applications in hospitals might not be appropriate and might even be detrimental, i.e., embryotoxic. Consequently, infection control products, e.g., hand sanitizers, must be evaluated before introduction into the ART suite.

Aldehyde-based cold sterilizers. Aldehyde-based cold sterilizers must not be used anywhere within the IVF suite, as the vapour is embryotoxic. Therefore, units such as Glutaraldehyde User Stations must be located where there is no risk of their vapours contaminating the IVF suite's air.

Cleaning agents with chlorine dioxide (bleach) are not appropriate for IVF laboratory cleaning during clinical operations. Cleaning with 6% H_2O_2 is to be encouraged as a replacement for cleaning with 70% ethanol or isopropyl alcohol (methanol is not recommended due to its toxicity to humans) because it oxidatively destroys microorganisms and readily breaks down and can be removed easily by sterile water. Alcohols only denature or 'fix' proteins *in situ*. Several alcohol-free alternative cleaning agents are also available on the market, e.g. one containing quaternary ammonium compounds, which are considered safe and could potentially replace all the above. Care must always be taken not to expose embryos to the sterilizing agent or its residues.

Plasticware. As discussed above, plasticware clearly off-gasses VOCs. Polystyrene, in particular, will release non-polymerized styrene, which is highly embryotoxic. It is recommended that all plastic cultureware should be off-gassed, outside the laboratory if possible (while maintaining cleanliness and sterility of the products). The necessary duration of off-gassing remains unknown and the group encouraged prospective studies to confirm this. It is suggested that manufacturers should use breathable packaging to support off-gassing.

Packaging materials. Packaging materials that have been exposed to the outdoor environment should never enter the ART suite. For example, cardboard packaging is a source of fibres, particulates, dirt, fungal spores, etc. Passage of interior cardboard, paper packaging and all other paper into the ART suite should also be minimized.

Cleaning and cosmetic products. Use of cleaning products, air fresheners, cosmetics, grooming products, such as perfume, aftershave and nail polish, which release VOCs, must be avoided. This includes handwashing products, hand sanitizing products and hand lotions.

Perfume-free environment. Many clinics already operate as a perfume-free environment; therefore, if clinical procedure rooms are part of the ART suite, then patients must not wear any skin care, cosmetics or grooming products.

'Third-hand smoke'. There is evidence for harmful effects of 'third-hand' smoke, i.e., that coming from the hair and clothing of people who have either been smoking or exposed to others who smoke (Hang et al., 2013). It is, therefore, recommended that necessary steps be taken to prevent such third-party smoke contaminants from entering the IVF laboratory or ART suite.

Operators as sources of VOCs. It should be remembered that the operators are themselves significant potential sources of VOCs and other contaminants. This should be borne in mind when undertaking risk assessments.

Scrubs and equipment. Scrubs and other items of personal protective equipment, such as hair and shoe covers, are selected as per facility policies should be non-shedding, non-static, and colour-stable under conditions used for their washing, drying and sterilization.

Detergents. Laundry detergents used for cleaning should not release VOCs.

Photocopiers and printing equipment. Photocopiers, laser (and possibly inkjet) printers should not be used within the ART suite, as they emit unwanted chemicals, such as ozone, solvents and particulate containing toner dust (Barrese et al., 2014; Maddalena et al., 2011). Placing them in sealed cabinets under continuous negative pressure might be a solution, although this would be difficult to manage without prejudicing the cleanroom air supply.

Computers. Desktop computers in operation are known to emit VOCs and formaldehyde in the microgram range with increasing power consumption, and time-related off-gassing of ageing computers has been described (Bakó-Biró et al., 2004; Maddalena et al., 2011; McKone et al., 2009). A minimum number of computers should be used in the ART suite and they should be switched off when not in use. It is recommended that new computers be off-gassed by running them for 10 days outside the ART suite. Lower powered computers, such as laptops, generate less VOCs, whereas tablets and smartphones produce no or limited amounts of VOC and aldehyde (Funaki et al., 2003; JEITA, 2014). Each laboratory should undertake its own risk assessment regarding the use of computing equipment within the cleanroom laboratory.

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