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INVITED RESEARCH HIGHLIGHT

Male Reproduction

Air quality control in the ART laboratory is a major determinant of IVF success

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A recently published article described how a fertility center in the United States implemented air quality control to newly designed *in vitro* fertilization (IVF) laboratory.[1] A highly-efficient air filtration was achieved by installing a centered system supplying filtered air to the IVF laboratory and related critical areas, combining air particulate and volatile organic compound (VOC) filtration. As a consequence, live birth rates were increased by improvements in air quality. This article highlights the key aspects of air contamination in the IVF context. The topic is important not only to IVF specialists but also to Andrologists due to the great number of male infertility patients referred to assisted reproductive technology (ART) treatments. The evidence is growing that laboratory air quality is paramount importance for improved IVF outcome.

IMPORTANCE OF LABORATORY AIR QUALITY TO EMBRYO DEVELOPMENT *IN VITRO*

Both animal and human studies have suggested an association between poor laboratory air quality conditions and impaired embryo development, resulting in decreased implantation and pregnancy rates. The deleterious effects of poor air quality to embryo development and implantation and how controlling laboratory air quality can minimize such effects have been investigated over the last 15 years (**Table 1**).[1–17] Recognizing the importance of laboratory air quality to the safety of IVF treatments, regulatory directives in the European Union and Brazil dictate specific requirements for air quality control within reproductive laboratories.[18,19] Such regulatory directives aim to safeguard public health in line with the precautionary principle, but they require different strategies to mitigate the air-related risks (revised in Esteves and Bento, 2013).[2] Little attention has been given, however, to how IVF laboratories should implement air quality control.[2,20]

A RISK ASSESSMENT IS CRITICAL BEFORE INSTALLING AIR FILTRATION SYSTEMS

Is air particle filtration enough or do we need to combine it with volatile organic compound (VOC) filtration? Are commercially available stand-alone filters sufficient or is it necessary to implement centralized built-in air filtration systems? How often do we have to replace the filters? What periodic testing is needed to ensure conformity? Will the implementation of air filtration change IVF outcomes? With so many uncertainties but recognizing the importance of laboratory air quality, many of us working in this field have chosen to install commercially available filtration systems without proper risk assessment and validation procedures. Little attention is given, for instance, to other critical issues that affect indoor air quality, such as laboratory premises (e.g., age and size of laboratory, equipment/furniture and construction materials atmospheric air pollution, and proximity to anesthetic gases), room humidity and temperature, disposable materials and cleaning agents used inside the laboratory, and personnel (number per workspace and use of protective clothing and cosmetics).

IMPORTANCE OF AIR QUALITY CONTROL IN ASSISTED REPRODUCTIVE TECHNOLOGY (ART) LABORATORY

One of the goals of air filtration in the IVF environment is to decrease the number of air particles through the use of high-efficiency filtration systems. This is important because microorganisms can attach themselves to these particles. Removal of airborne particulates is achieved by forced movement of air using positive air pressurization through a series of filters of increasing efficiency.[21] On the other hand, VOCs are much smaller than the effective pore size of high-efficiency particulate air (HEPA) filters and cannot be trapped by HEPA filters.[22] Volatile organic compounds, which are constantly generated by materials and cleaning agents used in the laboratory, react with the indoor ozone. These chemical reactions produce submicron-sized particles and harmful by-products that have been associated with poorer IVF outcomes.[3–8,17] In the IVF setting, VOCs can be found in CO_2 gas cylinders, insulation used in air handling systems, refrigerant gases, cleaning agents, plastic ware, constructing materials, and furniture.

HOW TO IMPLEMENT AN EFFICIENT AIR FILTRATION SYSTEM – LESSONS LEARNED FROM NOVEL RESEARCH

VOC removal should be an integral element of air cleanness in IVF. Removal of VOCs is achieved by potassium permanganate-impregnated, pelletized coconut shell-based activated carbon filters. The spaces between the carbon particles contain a cloud of delocalized electrons that acts as electronic glue, thus forcing the chemical contaminants to bind to the

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Table 1: Summary of evidence assessing the impact of laboratory air quality in IVF outcomes

First author and reference	Year	Study design	Study population	Method	Outcome
Little[3]	1990	Observational analytic cohort study	*In vitro* cultured rat embryos	Cellular protein and DNA damage analysis	Aldehyde (acrolein) is incorporated to the yolk sac and causes embryotoxicity
Cohen[4]	1997	Descriptive qualitative study	None	Air sampling and VOC determination in human IVF laboratories	Higher levels of VOC (mainly toluene and isopropyl alcohol) in HEPA-filtered laboratory ambient air and incubators compared to outside unfiltered ambient air
Schimmel[5]	1997	Descriptive qualitative study	None	Air sampling and VOC determination 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[6]	1998	Combination of descriptive qualitative and observational analytic cohort studies	*In vitro* cultured mouse embryos	Air sampling and VOC determination in human IVF laboratories; acrolein bioassay using 2-cell 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
Mayer[7]	1999	Prospective randomized crossover study	129 IVF and ICSI cycles	Assessment of IVF outcomes after embryo culture in incubators with and without VOC filtration	Higher pregnancy rates in couples whose embryos were cultured in incubators equipped with VOC air filters
Racowsky[8]	1999	Observational analytic cohort study	467 IVF and ICSI cycles	Assessment of IVF outcomes after embryo culture in laboratories and incubators with and without VOC filtration	Reduction in miscarriage rates in IVF cycles performed in laboratory and incubators equipped with carbon-activated filters
Boone[9]	1999	Observational analytic cohort study	275 infertile couples undergoing IVF	Air sampling and IVF outcomes after construction of a cleanroom with centralized particle filtration for IVF, oocyte retrieval and embryo transfer	Reduction in air particles and increase in the number of high-quality embryos for uterine transfer
Worrilow[10]	2001	Descriptive qualitative study	None	Air sampling in a newly designed IVF laboratory equipped with a centralized highly purified, HVAC system and VOC filtration	All areas within the IVF laboratory and accompanying procedure rooms qualified as Class 100 areas. No VOCs were found at concentrations above detectable limits or greater than 0.1 parts per billion
Worrilow[11]	2002	Observational analytic cross-sectional study	IVF cycles*	Outside ambient air and indoors (IVF lab) air sampling for particles and VOCs over a 2-year period. Assessment of IVF outcomes performed in a cleanroom laboratory with VOC filtration	Levels of outside air VOCs serving the IVF lab air control system varied according to seasonal humidity and temperature, which affected implantation rates
Esteves[12]	2004	Observational analytic cohort study	468 ICSI cycles in an unselected IVF population	ICSI outcomes in cleanroom facilities (equipped with centralized particle and VOC air filtration for embryo culture, gamete retrieval and embryo transfer) compared with an IVF lab equipped with stand-alone air filtration system	Increase in high-quality embryos and clinical pregnancy rates, and reduction in miscarriage rates in cycles performed in cleanroom facilities compared with IVF lab with stand-alone air filtration system
von Wyl[13]	2004	Descriptive qualitative study	None	VOC and air particle determination in an old IVF laboratory and in a newly built facility with positive-pressure air filtration for particles	Air concentrations of the measured compounds were lower in the new over pressurized IVF laboratory
Esteves[14]	2006	Observational analytic cohort study	399 ICSI cycles in couples whose male partners had severe male factor infertility	ICSI outcomes in cleanroom facilities (equipped with centralized particle and VOC air filtration for embryo culture, gamete retrieval and embryo transfer) compared with an IVF lab equipped with stand-alone air filtration system	Increase in high-quality embryos and clinical pregnancy rates, and reduction in miscarriage rates after oocyte/sperm retrievals, ICSI and embryo transfers performed in cleanroom facilities compared with IVF lab with stand-alone air filtration system
Knaggs[15]	2007	Observational analytic cohort study	Infertile couples undergoing IVF/ICSI cycles*	IVF/ICSI outcomes in a newly designed and constructed laboratory facility meeting the European Union tissues and cell directive. Analysis of key performance indicators in a period prior to and after the move into the new embryology facility	Implantation and pregnancy rates increased after the move into the cleanroom
Khoudja[16]	2013	Combination of descriptive qualitative and observational analytic cohort studies	1403 infertile couples undergoing IVF/ICSI cycles	IVF outcomes in IVF laboratories equipped with stand-alone and centralized particle and VOC air filtration systems. The latter was designed and constructed by incorporating a novel air purification method involving specially treated honeycomb matrix media with a Landson™ system	VOC levels decreased and overall air quality improved after installation of a novel air purification method. Significantly better fertilization, cleavage, blastulation, pregnancy, and implantation rates were observed with this new technology
Esteves[2]	2013	Combination of descriptive qualitative and observational analytic cohort studies	2315 ICSI cycles in unselected IVF population	ICSI outcomes in cleanroom facilities (equipped with centralized particle and VOC air filtration) for embryo culture, gamete retrieval and embryo transfer. A historical cohort in which IVF cycles were carried out in an IVF lab equipped with a stand-alone air filtration system was included for comparison	Negligible levels of VOCs in the cleanroom IVF lab; Cleanroom facilities classified as ISO 5 (IVF lab), ISO 7 (operating theater) and ISO 8 (embryo transfer room). Significantly higher rates of high-quality embryos and live birth rates, and lower miscarriage rates, in cycles carried out in cleanroom facilities

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Table 1: Contd...

First author and reference	*Year*	*Study design*	*Study population*	*Method*	*Outcome*
Munch[17]	2015	Observational analytic cohort study	524 fresh and 156 cryopreserved IVF cycles	IVF outcomes in a lab equipped with carbon filtration	Fertilization, cleavage, and blastulation rates for fresh cycles declined during the period of absent carbon filtration and restored after reintroduction of carbon filtration
Heitmann[1]	2015	Combination of descriptive qualitative and observational analytic cohort studies	820 IVF/ICSI cycles in unselected IVF population	IVF/ICSI outcomes in a cleanroom IVF lab (equipped with centralized particle and VOC air filtration) compared with an IVF lab equipped with a stand-alone air filtration system	Air quality testing demonstrated decrease in total VOC concentrations in the new IVF lab compared with the previous facility, which was associated with significantly higher implantation and live birth rates

*Number of cycles not described. ISO: international organization for standardization; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection; VOC: volatile organic compound; HEPA: high-efficiency particulate air; HVAC: heating, ventilation and air conditioning

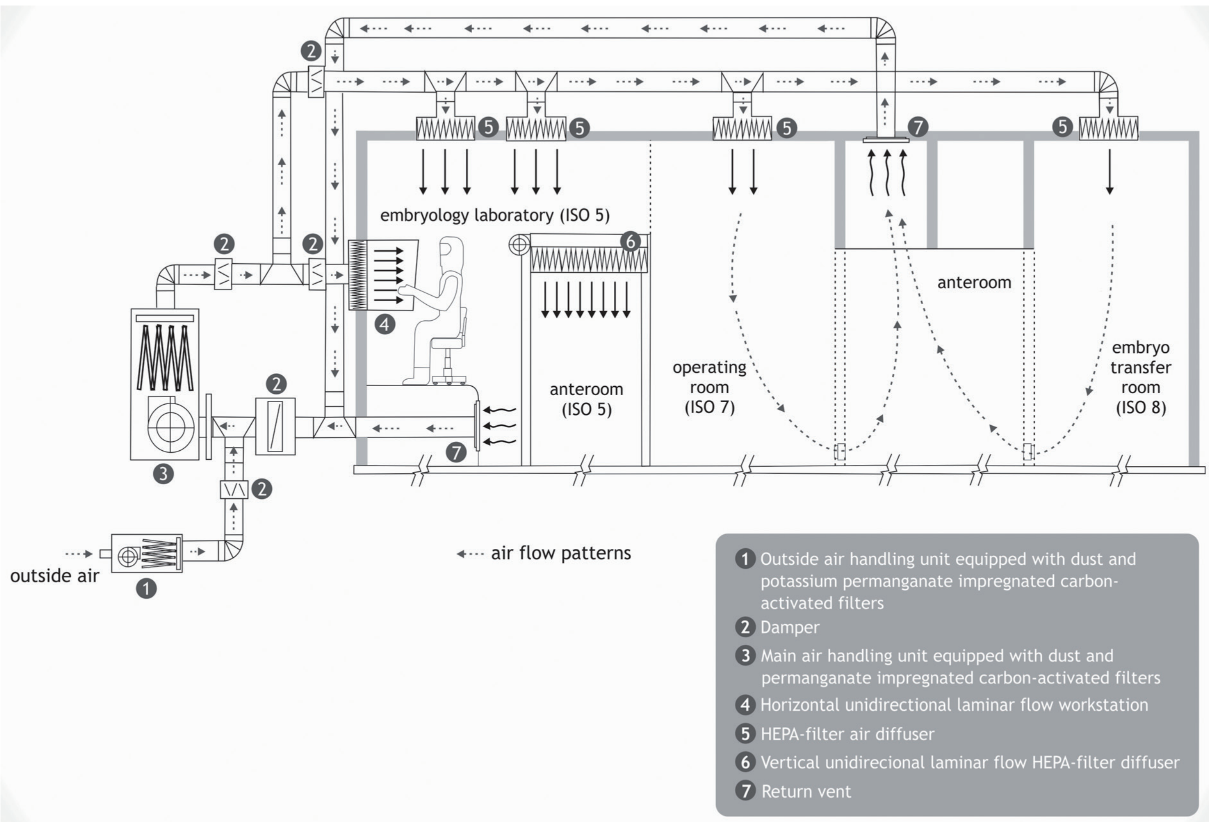

Figure 1: Schematic representation of cleanroom IVF facilities, including airflow patterns and filtration units. The air handling ventilation unit room has a roof-top air-handling unit that draws outside air through coarse and charcoal prefilters before it enters into the main ventilation unit. A free-standing main ventilation unit pulls prefiltered outside air and cleanrooms' return air through coarse filters, past a 16-unit potassium permanganate impregnated pelletized coal-based activated carbon filters, and then through fine dust filters. Lastly, filtered air enters the cleanrooms through high-efficiency particulate air (HEPA) filter diffusers. 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 from Esteves and Bento, *Reprod Biomed Online* 2013; 26: 9–21, with permission from Elsevier.

carbon.[23] Alcohols and ketones that are not normally removed by the pore structure of coconut shell-based carbons can be oxidized, and thereby detoxified by potassium permanganate.[6]

In vitro fertilization laboratories aiming to control air pollution should integrate both air particle and VOC filtration. An example of a laboratory with the aforesaid combination is depicted in **Figure 1**. Evaluating results over 9-year period, we demonstrated the benefit of operating under these optimum environmental conditions, which resulted not only in an increase in live birth but also reduction in miscarriage rates.[2] Along these lines, a better definition for IVF cleanrooms would be "a room, in which the concentration of airborne particles and VOC is controlled and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles and VOCs, and in which, temperature, humidity, and pressure are controlled." Equally important are the methods set up for training laboratory personnel and validating/monitoring the installations while in operation, that is, during normal routine workload. In general, expensive filters, such as HEPA, are not replaced unless they show nonconformance during periodic inspections. VOC filter efficiency is monitored periodically by sending chemical module samples to the manufacturer to determine remaining chemical bed activity, thus guiding how often filters should be replaced. Filter saturation levels depend on outside air quality and levels of indoor VOC generation, and replacement of filters by analyzing objective data helps minimize operational costs.

Heitmann and colleagues also contributed a detailed description of their filtration system and construction methods, which included removal of both particulate matter and VOC.[1] Better air quality conditions were associated with significantly higher embryo development, implantation, and live birth rates in couples undertaking treatment in their new facility. In both aforementioned studies, an air filtration system controlling indoor particulate and VOC was implemented using a centralized system supplying filtered air to the IVF laboratory and adjacent critical areas.[1,2]

Installation of centralized air filtration such as the highlighted ones is costly. A less expensive but yet to be proven effective alternative, particularly for existing IVF laboratories, would be to incorporate portable freestanding commercial units. However, it is unlikely that portable units would provide the same air quality than a robust, centralized air filtration system. Notwithstanding, risk minimization and quality management should be considered equally powerful tools to improve IVF laboratory air quality.[22]

In conclusion, accumulating evidence indicates that laboratory air quality plays a significant role in IVF outcome, which is of broad interest for practitioners dealing with male infertility and patients alike. Implementation of air quality control by the combination of particulate matter and chemical filtration seems sound, but guidelines on the target limits and best practice statements on how to implement air quality control to IVF are still lacking. At present, built-in systems supplying filtered air to the IVF lab and adjacent areas seems to be the best alternative to mitigate the risks of poor IVF outcomes related to laboratory air quality. Good laboratory practices are also critical for improved IVF outcomes.

COMPETING INTERESTS

The authors declared that they had no conflict of interest.

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