

Opinion: Ambient Air and its Potential Effects on Conception *In-Vitro*

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Abstract

Incidences of chemical air contamination or CAC are common in assisted reproductive technology, but not reported in peer review format. Justified fear of car and industrial emissions clearly exists among reproductive specialists, but standards for air contents and off-gas limits have not been reported. Here, we describe air sampling methods and assay systems which can be applied to any laboratory or laboratory item. It was found that unfiltered outside air may be cleaner than HEPA filtered laboratory air or air obtained from incubators due to accumulation of volatile organic compounds derived from adjacent spaces or specific laboratory products such as compressed CO₂, sterile petri dishes and other materials or devices known to off-gas compounds. Specific groups of products such as anesthetic gasses, refrigerants, cleaning agents, hydrocarbons and aromatic compounds such as benzene and toluene are described. The latter were shown specifically to accumulate in incubators. Isopropyl alcohol was the most dominant product found, though it was not used by the laboratory staff. Levels of this agent were very low in incubator air indicating that it was probably absorbed by the water in the pan or by culture medium. Measures to counter CAC are proposed, including the use of activated carbon filters and oxidizing material placed in the central air handling systems, in separate free standing units or even inside the incubators.

Introduction

The physical laboratory environment for gametes and embryos has changed very little in the past 35 years (Brinster, 1963; New and Stein, 1963). Conditions were initially adapted from systems used by cell tissue culture laboratories (Whitten, 1956). Recently, commercial interest in metabolic and chemical requirements for gamete culture has increased, resulting in numerous new types of growth media and the introduction of helper cell systems (Edwards and Brody, 1995). But other major environmental factors have not been addressed, such as changing temperatures during meiosis, fertilization and

embryonic development, optimal cellular pH, effects of infection control, air and water quality. The purpose of this essay is to illustrate the delicate balance between hitherto unknown factors in the changing organic chemistry of ambient laboratory air and the potential effect this may have on conception in-vitro. Though environmentalists have studied some of the consequences of volatile organic compounds on animals and humans, such toxicology has been in general limited to studies on post-implantation embryos and effects on potential offspring. Indeed, most regulatory groups such as the Environmental Protection Agency and Food and Drug Administration in the USA require data from multigenerational studies in-vivo (Francis and Kimmel, 1988; Klinefelter and Gray, 1993). Yet differentiated mammals are protected to a major degree against their immediate ambient environment through their immune, digestive and epithelial systems. But none of the required classical toxicology investigations apply to oocytes and free-living embryos, where passive and active absorption mechanisms are mostly indiscriminate.

Questionable air quality: definitions, thresholds and standards

There has been no statistically valuable toxicological evaluation of air and its effect on fertilization and development outcomes, during and after human assisted reproduction. Nevertheless, there is evidence that non-infectious contamination or chemical air contamination (CAC) may inhibit any stage of the procedure; but this evidence is largely derived from anecdotal exchange of information between reproductive professionals, rather than peer review reporting. Ambient air studies are notably lacking from the scientific and medical literature on assisted reproduction. Thus, many reproductive specialists are not aware of ambient air concerns, and frequently IVF laboratories are casually placed in unchecked buildings in polluted residential or semi-industrialized areas.

It is not often realized that incubators obtain 94-95% of their ambient air directly from the laboratory room, through the opened door or via an inlet port in the rear and 5% is from often deteriorated gas bottles, which are unfiltered and not routinely checked for levels of organic compounds, or metallic contaminants. Using a desiccation method, the incubator atmosphere can be replaced by gas from pre-mixed bottles. Although this may appear sensible in principle, factories producing pre-mixed gas mixtures are also unaware of the high laboratory standards needed by reproductive specialists. Though industrial norms for compressed air and other gases do exist, such as OSHA standards in the USA, these guidelines apply to adult humans, based upon earlier Threshold Limit Values (TLV's) established by the American Conference of Governmental Industrial Hygienists (Bertin and Werby, 1993). The standards are designed to cover workers representing on-going conditions under which it is believed nearly all workers may be repeatedly exposed day after day without adverse effects (Cralley and Cralley, 1982). These criteria are not designed for cultured and largely unprotected cells. Additionally, routine medical grade (USP Grade) materials can contain low levels (micrograms/ cubic meter) of organic compounds. A typical analysis of USP grade carbon dioxide from our source in New Jersey (Matheson, USA) shows the presence of benzene, freons, alcohols, and chlorinated organics (Table I). All concentrations are expressed as micrograms per cubic meter.

Some of these compounds were found at lower levels in incubators and traces could be detected in other areas of the laboratory.

The use of pressurized rooms using HEPA filtration is recommended in our specialty, but in addition it should be applied in environments with air-tight walls and ceilings, achieving the standards applied to pharmaceutical clean rooms. The supposition that HEPA filtration retains gaseous organic and inorganic molecules efficiently is false, although CAC levels may change after HEPA treatment. Other filters used for treatment of culture media and placed in CO₂ lines have pores thousands of times larger than organic and inorganic molecules of low molecular weight. Hence, whatever is found in the ambient air in and around the laboratory, will also be largely present in incubators, petri dishes, culture flasks and test tubes. There are many anecdotal reports of effects of paints, adhesives and car exhaust fumes on embryo development and pregnancy frequency after assisted reproduction. As far as we know, none or very few of those have been verified by environmental toxicologists, because most reproductive specialists are unaware of the low detection limits of modern clean-air surveys, which can now measure most low molecular weight molecules in micrograms per cubic meter, one thousand times below many OSHA standards (Table II). The effects of ambient air during assisted reproductive technology are extremely complex and most environmental incidents cannot be verified, since active measures to prevent environmental disturbances are either not taken, or are too easily influenced by other changes in laboratory methodology, which by themselves may also have affected results. Based on some clear environmental hazards presented here, it would be useful to introduce Ambient Air Embryology as a new sub-specialty.

The four most common air pollutants (Locke, 1990) are; (I) volatile organic compounds (VOC). A typical range for low molecular hydrocarbons in an urban area is shown in Table III. (II) Small inorganic molecules such as N₂O, SO₂ and CO (Moore and Moore, 1976), and (III) substances derived from building materials. Examples are organic compounds such as aldehydes from flooring adhesives. Another example are substituted benzenes, (1,2,4-Trimethylbenzene), phenol and n-decane released from vinyl floor tile (DeBotoli and Colombo) Other polluting compounds (IV) may be released by pesticides or by aerosols containing butane or iso-butane as a propellant. Finally, (V) liquids such as floor waxes may contain heavy metals. Examples of these compounds effects on cultured gametes and embryos are incidental, but nonetheless dramatic. Volatile organic compounds are produced by industry, a variety of cleaning procedures, vehicle and heating exhausts, and are therefore most apparent in urban and dense suburban areas. Volatile organic compounds may also be produced by specific instruments such as microscopes, television monitors or furniture. Some of these are from material used in making the instrument, such as polymers containing traces of residual monomers, plasticizers, antioxidants and mold-releasing agents. Factors which may dilute these effects are (I) the air flow in the immediate laboratory area, (II) legal (such as National Ambient Air Quality Standards in the USA) and industrial measures regarding emission control (background), (c) the height, location and design of the building and the laboratory's level in it, (d) the use of over-pressured, mechanical systems and central filters, and (e) separate laboratory equipment such as ionization units, HEPA filters and

solid absorption systems containing media such as activated carbon or activated carbon supplemented with potassium permanganate. Such measures are not often able to be taken in laboratories with small budgets. One author's in-vitro fertilization laboratory moved from a suburban Naples (Italy) location to a downtown facility some years ago. The new laboratory had no special air handling and was built directly above one of the busiest streets in Naples, a city known for its vehicular emissions, stagnant air and poor industrial emission control. The move resulted in an immediate drop in pregnancy rates from the higher 20th percentile to zero. A small pump and a water-filtered gas bottle were installed outside the incubator, and air was pushed through the rear inlet into the incubator's cabinet. Pregnancy results were immediately restored to normal levels. The same system was successfully installed in incubators used in two other IVF programs in southern Italy after those clinics had experienced extended periods with low success. In one of these programs repeated floor waxing unwittingly resulted in a dramatic decline in pregnancy, which was restored after installation of the simple filtration system mentioned above. Analysis of the wax revealed lead levels of one part per thousand.

An example of seasonal air pollution is the yearly crop burning of fields in Cambridgeshire in the UK. For many years this has resulted in decreased results at local IVF clinics during the month of August (Elder, unpublished results). More common are the hazards associated with building and restoring new laboratory spaces, where ambient pollution is caused by a process of 'out-gassing' or 'off-gassing' from the constituent materials of new equipment and interior finishing. In the summer of 1995 we temporarily performed research in a regular office room immediately adjacent to the space in which a new research and permanent laboratory was being built at Saint Barnabas Medical Center in New Jersey. The temporary laboratory became an inadvertent six-week long experiment in effects of off-gassing on early mouse embryos. Nearly all construction events appeared lethal in one way or another. Very few of the events inhibited fertilization, but all paints, dry wall preparations and floor adhesives inhibited embryo development, in spite of the extensive use of free-standing ionization and HEPA filtration units in the temporary laboratory. This was before we installed a solid media filtration unit, which appears effective in absorbing volatile off-gassing materials from new construction. Permanent floor adhesive was the most aggressive, arresting all embryos after fertilization prior to the 4-cell stage.

Environmental equipment and assay systems

VOCs levels can now be determined by extracting compounds from fixed filtration media such as activated carbon and potassium permanganate. It is nevertheless more appropriate to quantify VOCs using a vacuum capture system and a flow controller. Quantification of VOC's and documentation of air flow are crucial in the determination of pollutant sources and their impact on closed environments. We have assayed solid carbon and potassium permanganate media pellets (Purafil, USA) which were housed in a free-standing filtration unit of industrial design (Eco-Care Co., New York, USA) placed in the middle of our culture laboratory for several months. The intention was to remove off-gasses following construction of the new space. This unit effectively removed adhesive and paint smells within a few days, and other pollutants. Since that time, our clinic has an

overall clinical pregnancy rate that has been in excess of 50% in 1400 patients for 16 months and with occasional sustained periods of 60% fetal heartbeat or more. The implantation rate per embryo varied from 22% to 36% during this same period. Although the amount of air exchanged was unknown (the unit was not always turned on and flow output was occasionally reduced), our evaluation of the absorbed organic compounds found over 300 different environmental toxins, mostly VOCs such as toluene (12% of all absorbed material), Octane (4.2 %), as well as a variety of Freons, gasoline additives (Methyl-tert-Butyl Ether 0.6%), Xylenes (2.2%) and a diversity of chlorinated solvents. We also found enflurane (4.0%) which is an anesthetic agent. We had in fact only expected to find methanol, a compound we reluctantly and sparingly use for cleaning counter-tops, but it was actually at low levels. Over 300 VOC's were detected in spite of (a) centralized 99.9% HEPA, (b) generic but centralized carbon and pre-filtration, as well as (c) numerous ionization units placed at strategic points in the laboratory. It should be stressed that this assay method was used to determine total adsorption on activated filtration media with an oxidizer added to destroy odorous sulfur compounds, and is therefore qualitative and dependent on many factors, such as (I) air flow, (II) the ability to extract the chemical from the carbon media, (III) stability of the chemical on an oxidizing media (IV) use of plastics and disposable plastic ware, (V) activities in adjacent areas and (VI) general use of equipment known to produce off-gasses. This laboratory is in a generally clean non-industrial suburban area, indicating that many of the VOCs could be from passenger car emissions. The presence of MIBE, a gasoline additive, was assayed as a marker of car emissions. The air inlet is 300 meters from a two-lane road and 150 meters from the building's parking lot. Car exhausts alone cannot explain two serious fluctuations in results, illustrated in Figure 1. Quality control was measured in consecutive series of 20 procedures (ICSI and IVF combined) and plotted as a cumulative result of clinical pregnancy and fetal heart beat per embryo (implantation rate) in order to compensate for varying numbers of embryos being transferred. Two decreases in quality control measures were found; the first was an abrupt threefold decrease over a three-week period indicated by the first arrow in Figure 1. The second decrease (second arrow) evolved over a longer period and was not as severe. Both periods showed a significantly lower pregnancy and implantation rate than preceding and subsequent periods (chi square test: $P < 0.01$) without a significant change in fertilization rate, embryo development or morphology. The frequency of embryologists or physicians performing procedures in both periods was unaltered. Media and water batches and changes in catheters and other disposable products were not correlated with the events. The only plausible explanations concerning the fluctuations were changes in air quality. Unknown to us, the adjacent spaces were repeatedly fumigated with aerosols containing pesticides preceding and during the first decline in success rate in response to a serious local infestation of ladybugs in the fall of 1995. Surprisingly, this repeated use of pesticides was not reported to the embryologists. The likelihood of penetration of the aerosol product is high since over-pressured areas are only aimed at removing microbial organisms. Leaks in walls and HVAC duct system as well as products carried in by staff all contribute to exchange of air. The second reduction in success rate corresponded to the redesign and installation of advanced carbon pre-filters.

In response to the absorption data on the filter material and the two fluctuations described in Figure 1, and although the overall pregnancy rate remained satisfactory, we decided to pursue detailed and costly quantification of ambient air samplings at different points in the laboratory. We also sampled two routine incubators, three laminar flow units, two adjacent hallways and areas outside the building using the methods described in the section below.

Volatile organic compound quantification and specific gaseous sampling

Sampling was performed using a calibrated pump for sulfur dioxide and nitrogen oxides and specialized absorbent media. Organic materials were sampled by evacuated, chemically clean, stainless steel sampling canisters over an 8-hour period at a controlled flow rate in order to obtain a total sample volume of six liters. All tubing connectors were new Teflon tubing which had been previously purged with 99.999+% nitrogen to ensure that there was no residual material. The analysis was performed in accordance with the methodology outlined in US EPA Method TO-14 from the Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, EPA 600/4-84-041 (1984/1988). The instrument used was Gas Chromatography/Mass Spectrometry (GC/MS) (Hewlett Packard Model 5989, USA) with an Cryogenic Concentrator (Entech 7000, USA). A 100% dimethyl-polysiloxane capillary column was used to achieve chromatographic separation. A blank sample was also run to verify the instrument baseline. All of the standards were less than the detection limit on the blank sample. The tests were performed during opening hours while the laboratory and its incubators were in normal operating mode. All compartments of the IVF facility were sampled, including the procedure room, retrieval flow benches, cryogenic and media preparation areas, as well as two IVF incubators. External areas immediately adjacent to the facility were also sampled, such as hallways to nearby cleaning areas.

Nitrogen dioxide and nitric oxide samples were collected and analyzed in accordance with OSHA guidelines ID 182 and 190 (Ku, 1991). In this method a known volume of air is drawn through a sampling tube at a flow rate of 0.1 liters per minute. The air flow was measured with a calibrated rotometer, analyzed by ion chromatography (Lachat QuickChem 8000, USA). For sulfur dioxide determination, samples were collected and analyzed in accordance with OSHA method 104 (Wilczek and Zimowski, 1989). Collection is achieved by drawing a known volume of air through a sampling tube at a flow rate of 0.1 liters per minute. The air flow was measured with a calibrated rotometer. The samples were analyzed using the ion chromatograph. Sulfur dioxide was calculated stoichiometrically from the mass (micrograms) of the sulfate found and the volume in liters of air sampled. Traces of these inorganic gases compounds were measured in parts per billion. The results of this testing showed the facility had a non-detectable level of nitrogen and sulfur oxides.

Other instruments can directly determine total VOCs, CO₂ and CO using a new photo-acoustic infrared detection method. This new method is used to estimate off-gassing from new elements, permitting VOC source emission to be performed on new furniture and equipment as well as on floors, walls and paint. Air flows can be determined using Sulfur

Hexafluoride (ASTM method E741-93). This gas is colorless, inert and odorless and can be monitored at very low levels. The use of tracer gas studies allows a mass balance to be developed. Sources of outside contamination can be identified and quantified.

Specific findings and recommendations

Before reviewing the data, it is necessary to have an appreciation of general concentration levels and measurement methods. Routine indoor air quality measurements usually show low molecular weight concentrations (benzene, toluene, hexane) of 1 to 15 milligrams per cubic meter of air. Chlorinated hydrocarbons can also be as high as 1 to 10 milligrams per cubic meter of air (Hodgson, 1995). With this as a background, it is crucial to realize that the data is presented in micrograms per cubic meter of air or 1000 times less than more conventional measurements. Also, the environment is not an office or home location, but an outpatient center carrying on a myriad of cleaning, preparation and medical procedures using a variety of chemicals. Surprisingly, NO, N₂O, CO and SO₂ were not detected in any of the assays.

There is extensive overlap in the data between the quantitative and qualitative tests performed on the filter system (Purofil). The Purofil data is based upon a charcoal adsorption and then desorption followed by GC/MS analysis. It shows a high percentage of high molecular weight hydrocarbons that start from benzene through C-12 alkanes. It does show similar amounts of some materials such as the anesthetic gas enflurane. It is important to note that the Purofil data does not state the concentration but rather the percentage of volatiles found on the carbon. It is different from the quantitative test data which is expressed as a concentration.

The presence of enflurane is clearly related to other surgical activities, since the agent is not used in our facility. Subsequent air sampling showed that it was widely distributed in and around the IVF facility (Figure 2). Enflurane is an anesthetic gas which is not used in the laboratory or egg collection rooms, but was nevertheless present in all of the sampling locations at measurable quantities. It was even found in an incubator of the research laboratory, two floors above the IVF laboratory space. The lowest level detected was in the mixed return and incoming air at 70 micrograms/cubic meter. Enflurane is a synthetic material and its presence in the incoming air shows that the air conditioning system's pressurization capabilities are obviously inadequate. After the initial round of tests, further inspection of the ceiling space above the IVF laboratory showed numerous breaches in the walls for pipes and cables.

It is clear that the IVF facility's air is directly influenced by all the activities of the adjacent day surgery center. The sample closest to the day surgery center, indicated as "hallways" in Figure 2, had the highest enflurane concentration of 600 micrograms/cubic meter, plus the anesthetic gas Halothane at 200 micrograms/cubic meter indicating that an appropriate sampling plan may pinpoint the source of contaminants. Additionally, sampling around the facility showed a pattern of refrigerant leakage or solvent loss at every point tested. The graph in Figure 3 illustrates the sum of freons dichlorotetrafluoroethane, chloroethane and dichlorofluoromethane found. A possible

source may be leakage from the air conditioning system. A second source of refrigerants is the use of compressed gasses for the cleaning of optical or electrical systems. Other significant substances are present at reduced amounts such as iso-propanol or iso-propyl alcohol (IPA). IPA was the material most commonly found in our VOC study. It is possible that the qualitative Purofil analyses failed to show this, since it would be oxidized by the mixed permanganate. The VOC air samples revealed considerable amounts of high molecular weight hydrocarbons such as C10-11-12 aliphatic hydrocarbons. These are possible combustion products, diesel fuel emissions, or off-gassing products from lubricants, and since their presence in the intake air was less than the IVF laboratory air, the source is unknown. The summation of these components is shown in Figure 4. Internal sources and pollutant pathways for external vehicular emissions (such as loading docks, stairwells, and elevator shafts) should not be disregarded. The results range from several hundred micrograms to approximately 1000 micrograms per cubic meter of air.

Elevated levels of IPA were detected in most of the sampling locations. These levels are largely due to the common use of IPA as a hospital disinfectant. It should be noted that while IPA is not used in the laboratory, it is occasionally used in the egg collection room and that the two laboratory incubators showed significantly lower levels of IPA compared with other laboratory spaces (Figure 5). The culture media, or hydrating water are possibly acting as a sink for this highly water soluble alcohol. A similar trend can be seen for other soluble alcohols. Benzene, Toluene, Xylenes, and other hydrocarbons such as hexane were present throughout the facility (Figure 6). Surprisingly, the highest concentrations were found in the incubators. The source was later confirmed by separately assaying the compressed gas from the bottles, showing levels of benzene as high as 100 micrograms per cubic meter. Other compounds found in the gas bottles were freons (probably residual material from the cleaning of the cylinders, regulators, etc.), isopropanol, acetaldehyde, acetone and toluene. The total concentration of VOC was in excess of 551 micrograms/cubic meter. The incubators also revealed significant concentrations of silicones such as Trimethylsilanol and hexamethylcyclotrisiloxane. Possible sources of contamination are the mold release agents for the plastic ware and the sealing gasket used in the incubator. Another source for low level silicone contamination may be the adjacent outpatient surgery center. Indeed one sample in the corridor leading to the outpatient surgery also had an elevated level of Trimethylsilanol.

The incubators contained detectable levels of styrene, a reactive material which is a carcinogen, presumably off-gassed from plastic-ware, made from polystyrene. The levels were 12-45 micrograms/ cubic meter. Subsequently samples of the laboratory plastic-ware were run in a glass chamber study and found to off-gas styrene. The plastic-ware was examined in a glass chamber for emissions and these were then subjected to GC/MS analysis. Table IV shows the results in nanograms per petri dish.

Other compounds which may enter incubators without their direct use in the laboratory are volatile detergents used in standard hospital and office rooms. We detected a background level of d-Limonene, a perfuming, wetting and dispersing agent. It is found at a low level throughout the laboratory (10-20 micrograms /cubic meter). This agent is

used in most common household detergents, but was not an ingredient of any the products used in the laboratory, again indicating that low molecular weight molecules may traverse into over-pressured areas.

Discussion

The specific results of the incubators used for embryo culture varied significantly (i.e., high in silicone materials, low molecular weight aromatics, and very low in IPA). The low IPA may be due to absorption by the culture media or mineral oil. Tests showed that benzene, xylenes and some freons were derived from the carbon dioxide tanks. The source of the styrene appeared to be derived from petri dishes (Nunc, Denmark), which possibly indicates a limited off-gassing period after manufacturing. Some manufacturers use uni-directional filter wraps which may eliminate build-up of gasses. The source of the high molecular weight aliphatics requires further investigation. The mineral oil and surgical lubricants may explain some of the levels found, particularly in the incubators, but the distribution of molecular weights suggests that a larger, unknown source is active.

The results of the testing are partially explained by comparison of the VOC's with components used in various materials in and around the IVF facility. During the study Material Safety Data Sheets (MSDS) were obtained from all cleaning, lubricants and disinfectants used in the area of the facility. Reviewing this listing helped to explain the sources of several components. For example, surgical implements are lubricated with an aerosol containing mineral oil, a source for the higher molecular weight hydrocarbons. The inventory also showed the use of 1,1,1,2-tetrafluoroethane in the laboratory "Dust-Off." Regardless of an IVF laboratory's budget, a compiled inventory of MSDS sheets could be used to spot potentially questionable ingredients. Finally the storage of surgical supplies, disinfecting operations and basic management of HVAC controls could be reassessed to reduce potential problems.

The current evaluation suggests that the effects of CAC should be studied in detail using an animal model and purposefully designed laboratory spaces and incubator systems. But this type of pre-implantation toxicology does not yet exist. Our laboratory at Saint Barnabas Medical Center where these tests were performed, uses bovine uterine epithelial cells for co-culture, when the first attempt at IVF by the patient-couple shows abnormal embryo development (Wiemer et al, 1996). Although the exact method is unknown, it is likely that co-culture operates by removing water soluble traces from the immediate environment of embryos. The current findings suggest that the use of helper cells is recommended in laboratories with suspected high levels of inorganic and low molecular weight organic traces. Other recommendations are a detailed re-evaluation of all materials used in and outside laboratory spaces, an inspection of heating and air conditioning systems and resultant air flow and the incorporation of equipment aimed at removing compounds from ambient air.

Conclusions

Ambient air determinations have revealed dynamic interactive processes between air handling systems, spaces, tools, disposable materials and all items unique to our IVF laboratory. The levels of substances found can be considerably different from those of adjacent spaces and may demonstrate an interaction between water soluble and lipid soluble solid phases such as those in incubators. In this study, it was shown for instance that IPA may be absorbed by culture media, but this may be counteracted by providing a larger sink such as a humidification pan in the incubator. Mineral oil may act as a sink for other components. Manufacturers of compressed air and incubators appear largely unconcerned with the specific clean air needs of reproductive specialists. Air extracted from a brand new incubator tested recently (unpublished data), revealed levels of VOCs more than a 100 times higher than those obtained from testing used incubators from the same manufacturer indicating that off-gassing of new laboratory products is crucial. Numerous other tests will need to be conducted in order to provide acceptable limits of VOCs and other inorganic and organic compounds in ambient IVF laboratory air. From our preliminary findings, it is clear that relatively VOC-free outside air may in fact be a cleaner source than inside air, since each of the fixed and transient laboratory components used may produce off-gasses. Air handling systems should probably be designed with these findings in mind. Extensive cleaning with detergents and alcohol based scrubs should be reconsidered since their efficacy is dubious, especially if they are implemented in order to substitute for poor sterile technique. This is even more important since microbial infections are uncommon in HEPA filtered spaces with the exception of contaminants derived from seminal plasma. The latter can be largely circumvented by using antibiotics prophylactically and following appropriate instructions during semen collection.

Finally, if extensive quality control measures have been found to be unsuccessful in terms of increasing success rates and in spite of a high level of experience of a laboratory team, one should seriously consider designing new culture spaces or even relocation to areas less affected by environmental hazards. Primary consideration should be given to design of culture spaces and adjacent areas, because it appears impossible to prevent pollution inside the laboratory from the surroundings with current air conditioning technologies. Thus, when an experienced team has undertaken extensive quality control measures and failed to improve low average success rates in an existing facility, the only alternative left may be to renew culture spaces or relocate to an area less affected by environmental hazards. Alternatively, industry-specific development of active filtration units placed inside cell culture incubators (Coda, GenX, Connecticut, USA) or in the laboratory spaces itself (Eco-Care, New York, USA) may prove sufficient to avoid most hazards. The effect of poor ambient air on the success of assisted reproductive technology cannot be determined with any accuracy because it varies widely in each facility; nevertheless it is likely to be one of the most important factors plaguing the overall low success rates in our industry.

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Table 1. Composition of VOC's detected in compressed CO₂ used for clinical gamete and embryo culture.

Volatile organic compound	m g/m ³
Benzene	100
Unknown Freon	100

Isopropanol	80
n-Pentane	50
Acetaldehyde	50
n-Butane	30
Isohexane + Acetic Acid	30
Acetone	24
Ethanol	20
Toluene	12
n-Heptane	10
C9H12 Alkyl Benzene	10
n-Undecane	10
C7H16 Alkane	9
C12 H26 alkane	7
Trichloroethene	4.7
m- & p-Xylenes	3.8
Ethylbenzene	1.6

Table II. Standard OSHA Threshold Limit Values (TLV'S) and current analytical capability using the TO-14 detection system in parts per million (ppm).

Material	OSHA TLV (ppm)	TO-14 detection (ppm limit)
Benzene	1	0.00031
Toluene	100	0.00027
Styrene	50	0.00024
Tetrachloroethene	25	0.00015
Ethyl Chloride	20,000	0.00038
Isopropyl Alcohol	400	0.00041
Freon 113	4500	0.00013
n-Hexane	5000	0.00028
2-Hexanone	5000	0.00024

Table III. Range for low molecular hydrocarbons in typical urban areas.

Component	Minimum Part Per Billion	Maximum Part Per Billion
Methane	1,200	15,000

Ethane	5	500
Propane	3	300
Isobutane	1	100
n-Butane	4	400
Isopentane	2	200
Propene	1	100
Isobutylene	<1	20
1,3 Butadiene	<1	10
cis-2-Buten	<1	10
Acetylene	<1	20

Table IV. Compounds released from cell tissue culture grade petri dishes

Material	>50ng/sample		=<50ng/sample
Styrene	920.00	n-Pentane	50
Toluene	180.00	3- Methylpentane	50
Acetone	150.00	Nonanal	50
2-Butanone	130.00	Butanal	40
Acetaldehyde	100	3-Pentanone	40
n-Butane	100	n-Hexane	30
Benzaldehyde	100	Butene Isomer	30
Hexanal	70	Benzene	23.00
Ethylbenzene	64.00	n-Octane	20
2-Hexanone	58.00	n-Nonane	20
		Decanal	20
		Cumene	10
		Propylbenzene	10
		Octanal	10
		m- & p- Xylenes	7.5
		o-Xylene	5.80

Figure 1

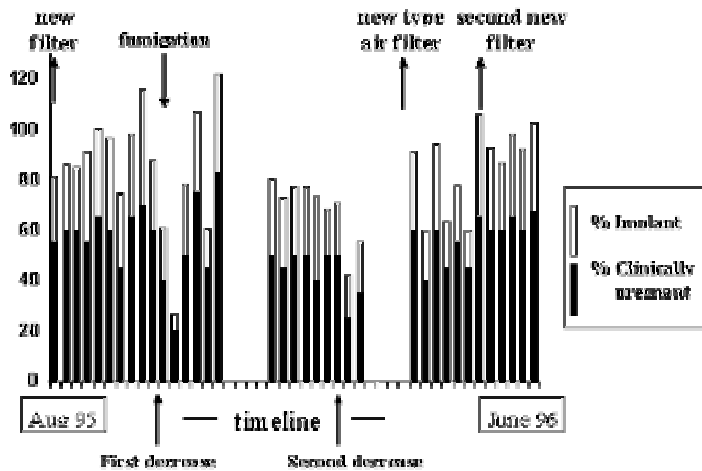


Figure 1. Fluctuations in pregnancy results during a ten months period. Each bar represents 20 egg retrievals. Pregnancy and implantation rates are cumulatively expressed in order to amplify any subtle change in success. Two significant decreases are noted and correlated with changes in air quality.

Figure 2

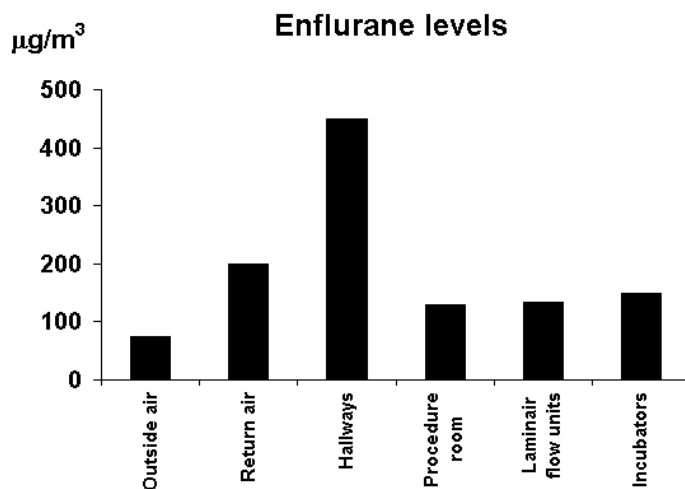


Figure 2. Enflurane, an anesthetic agent, not used in the laboratory and procedure rooms was found at all sampled locations. Elevated hallway levels indicate an outside source.

Figure 3

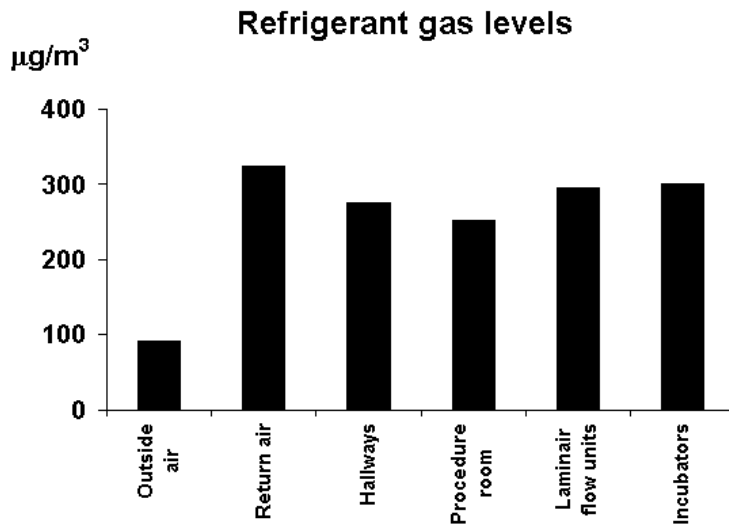


Figure 3. Refrigerants found at the IVF facility. These gases are a combination of dichloro-tetrafluoroethane, chloroethane and dichlorodifluoromethane.

Figure 4

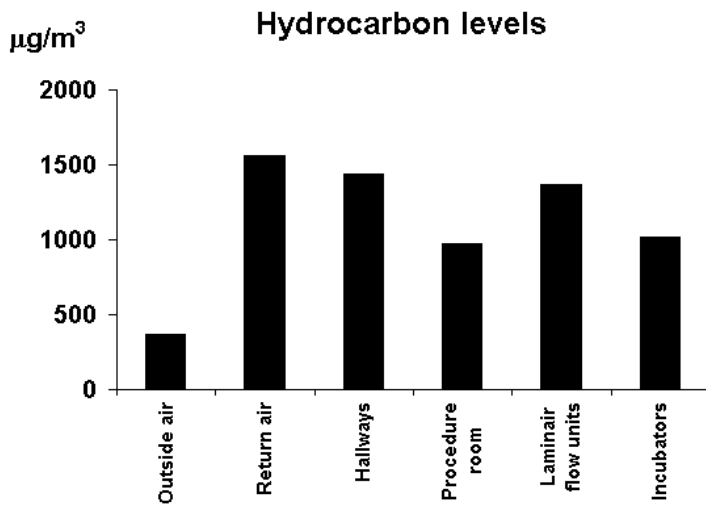


Figure 4. High molecular weight aliphatic hydrocarbon exposure in the IVF laboratory. These hydrocarbons are a combination of C10, C11 and C12 branched alkanes.

Figure 5

Isopropyl Alcohol Exposure

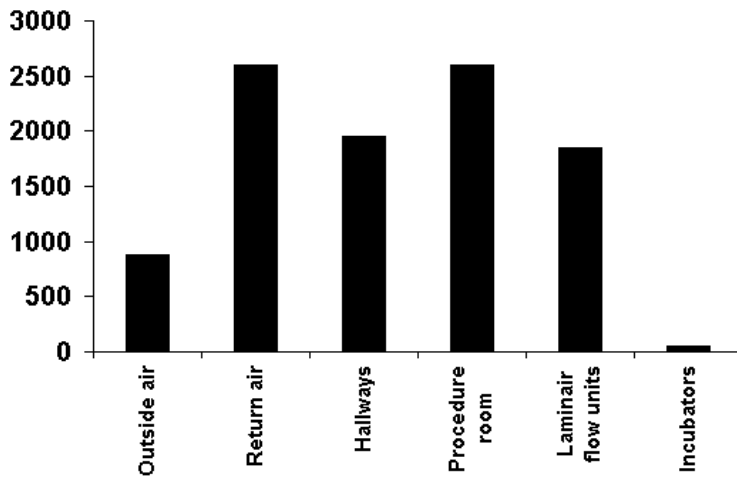


Figure 5. Isopropyl alcohol exposure in the IVF laboratory. This product is not used by laboratory and clinical staff and is presumably derived from general use in adjacent clinical spaces. Note the absence of this alcohol from incubator air.

Figure 6

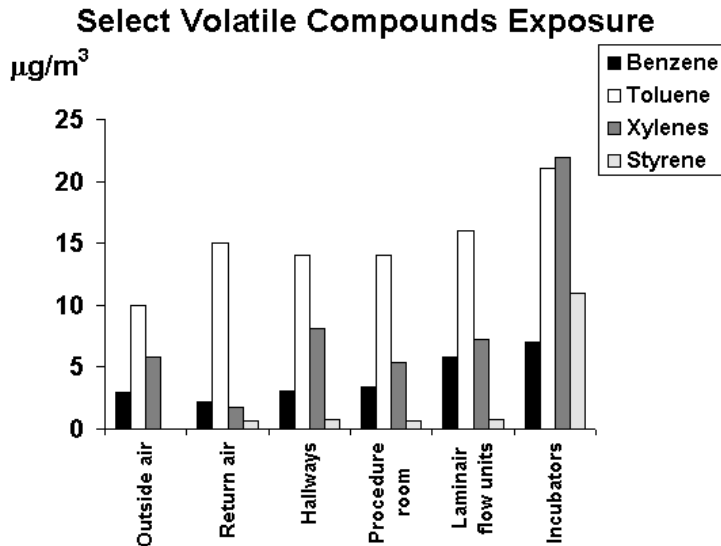


Figure 6. Aromatic compounds detected in the IVF laboratory. Accumulation was most notable in one of the two incubators assayed. Styrene is presumably derived from sterile petri dishes, whereas benzene is a byproduct of compressed CO₂.