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Science

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VOC Levels in a New IVF Laboratory With Both Central and In-Laboratory Photocatalytic Air Purification Units

Introduction

Over the past decade, practitioners of Assisted Reproduction Technology ("ART") have become increasingly aware that the quality of the air in their laboratories and clinical procedure rooms can have enormous adverse effects upon embryo quality, and hence clinical outcomes of ART treatment.

Historical fears of toxic fumes from motor vehicles and industrial sources, and anecdotal reports among ART scientists of diminished success rates associated with building renovations, have now been confirmed and expanded into a solid evidence base. The earliest peer-reviewed publication on such issues was by Cohen *et al.* (1997) and led to the development of various products designed specifically to reduce the impact of toxic vapours inside incubators.

Attention has focussed upon volatile organic compounds (VOCs), many types of which can be detected by simple absorption and gas chromatographic or flame ionization detection and analysis. Aerosolized pesticides, small inorganic gaseous molecules such as nitrous oxide (N₂O) and sulphur dioxide (SO₂), as well as heavy metals such as lead, are common air pollutants that can settle onto work surfaces or the surfaces of tissue culture plasticware and also dissolve in aqueous solutions such as embryo culture medium.

Motor vehicle exhaust is a common contaminant of "fresh" air

intakes for building air conditioning systems in urban areas (see Cohen *et al.*, 1997: p.1744, re Dr B Dale's IVF laboratory in Naples), containing high levels of VOCs (e.g. Varshney & Padhy, 1998) as well as lead (although greatly reduced in recent years). Industrial emissions are also common contaminants of "fresh" air intakes for building air conditioning systems in urban areas, containing very many different VOCs, nitrous oxide, sulphur dioxide and other acidic gases, carbon monoxide, hydrogen sulphide and heavy metals. Road surfaces, i.e. tarmac and tarmac sealant (coal tar derivatives) contain acrolein, which is highly toxic to mouse embryos *in vitro* (Hall *et al.*, 1998).

Construction materials represent a major source of VOCs in IVF labs. Particle board and other wood-based panels such as medium density fibreboard ("MDF" or "craft wood") release formaldehyde (Brown, 1999). PVC flooring materials (Lundgren *et al.*, 1999; De Bortoli *et al.*, 1999) and carpet (De Bortoli *et al.*, 1999) release VOCs, as does dry wall (plasterboard) and its filler. Paints (Sparks *et al.*, 1999, Srivastava *et al.*, 2000; De Bortoli *et al.*, 1999) and adhesives (especially vinyl floor tile adhesive: Cohen *et al.*, 1997) release numerous VOCs, including aldehydes. Many cleaning products are also sources of VOCs, e.g. vinyl floor liquid wax which can contain lead (Cohen *et al.*, 1997), ammonia-based products such as glass cleaners, and aerosol propellants such as

butane or iso-butane. Pesticides include many poisons and even known teratogens, as well as aerosol propellants if delivered via that route.

Plastic components of medical equipment can out-gas residual monomers, plasticizers, antioxidants and mould-releasing agents, and electronic components can emit various VOCs when warm.

In addition to common laboratory chemicals such as ethanol, methanol, iso-propyl alcohol, xylene and related substitute solvents, toluene, benzene, ethers, aldehydes and ketones, fixatives and sterilizers are obviously toxic, especially glutaraldehyde (e.g. *Cidex*). Ethylene oxide ("EtO") can also be an issue if ART products such as catheters that were sterilized using EtO were not properly ventilated for at least 4 weeks and submitted to mouse embryo bioassay testing before supply to customers. Chlorhexidine (e.g. *Hibitane*) is known to be toxic to human sperm. Anaesthetic gases can dissolve in aqueous culture media and impair embryo metabolism. Also, many products emit VOCs when heated, hence autoclaved materials (e.g. drapes, instrument packs) can release VOCs when packs are opened for use. Cosmetics, especially perfumes, colognes and aftershaves, are highly toxic to embryos *in vitro*, primarily due to evaporation of their solvent bases (Johnson *et al.*, 1993), and hence many IVF labs – even whole clinics – are now "perfume-free" zones.

Finally, cigarette smoke contains several hundred volatile compounds including recognized carcinogens and mutagens (Stillman *et al.*, 1986), and high levels can contaminate "fresh" air intakes if improperly located.

Objectives

1. To assess the levels of construction- and product-related VOC off-gassing in a newly constructed IVF laboratory with three ZAND-AIR 200 units (Zander Medical Supplies, Vero Beach, FL, USA) installed in series in the HVAC system.
2. To assess the performance of floor-standing zIVF-AIRe 100 air

purification units in reducing ambient VOC levels in an IVF laboratory.

Materials & Methods

The air handling system for the Pacific Centre for Reproductive Medicine ("PCRM") was designed as a recirculating, over-pressure cleanroom system to supply HEPA-filtered air to the Embryology Laboratory and Procedure Room with a maximum of 15% fresh air per passage. In addition, three ZAND-AIR 200 photocatalytic units were installed in the return air flow where the velocity was lower. These photocatalytic units were chosen because of their much lower operating cost compared to large charcoal/permanganate filters. According to the manufacturer (see www.zandair.com) each unit removes approximately 15% of the circulating VOCs per passage, hence three units in series will eliminate almost 40% of the VOCs per passage. In addition, floor-standing in-laboratory units were installed in both the Embryology Laboratory (2 × zIVF-AIRe 100C units) and in the Andrology Lab (not supplied by the cleanroom HVAC system: 1 × zIVF-AIRe 100P unit). These zIVF-AIRe units were chosen due to their lower purchase and operating costs compared to passive filtration systems. Prior to their introduction into the laboratories, the zIVF-AIRe units were run outside the laboratories for three days to eliminate residual particle and odours from the units and filters.

Immediately after completing construction of the laboratories (i.e. no "burn-in" period), the VOC levels were measured at various locations within the laboratories, as well as at external reference areas, both before (week of 18 September 2006) and after (week of 25 September 2006) the introduction of the in-laboratory units. VOC levels were measured using a RAE Systems model ppbRAE analyzer (RAE Systems, San Jose, CA, USA) which was provided on loan from Zander Medical Supplies. The ppbRAE unit was calibrated before each use as per the manufacturer's recommendations using a calibration

gas of 10 ppb isobutylene.

Fourteen locations were established as VOC measurement points and measurements were taken at those points at the same time each day:

- One outdoor reference location for external air: A.
- Three Andrology Lab locations (no HVAC system): B–D.
- Five Embryology Lab locations: E–I as well as one location inside the laboratory cabinetry: N.
- Four indoor locations in the PCRM facility that were not in the labs J–M.

VOC measurements were taken at the identified locations for five consecutive days prior to introducing the zIVF-AIRe units, and for four consecutive days after the units were activated and allowed to run for 24 hours in the Andrology and Embryology Labs.

Clinical efficacy of the new laboratory was evaluated over the first 30 treatment cycles, which commenced in October 2006. Laboratory systems were optimized as per Mortimer & Mortimer (2005), and included an IVF Chamber workstation (HD Scientific, Wetherill Park, NSW, Australia), the Cook Sydney IVF sequential media system and Cook K-MINC-1000 benchtop incubators (Cook Canada, Stouffville, ON, Canada) supplied with pre-mixed gas (6.0% CO₂ / 5.0% O₂ / balance nitrogen).

Results

Figures 1 and 2 show the measured VOC levels before and after the introduction of the zIVF-AIRe units into the PCRM laboratories respectively.

The VOC levels in the laboratories were already low (typically 20 to 80 ppb, i.e. <1 ppm VOCs) before the introduction of the zIVF-AIRe floor-standing units, demonstrating the effectiveness of the three ZAND-AIR 200 units installed in the HVAC system supplying the cleanroom zone, combined with apparently good quality local ambient air. The air coming directly out of a zIVF-AIRe

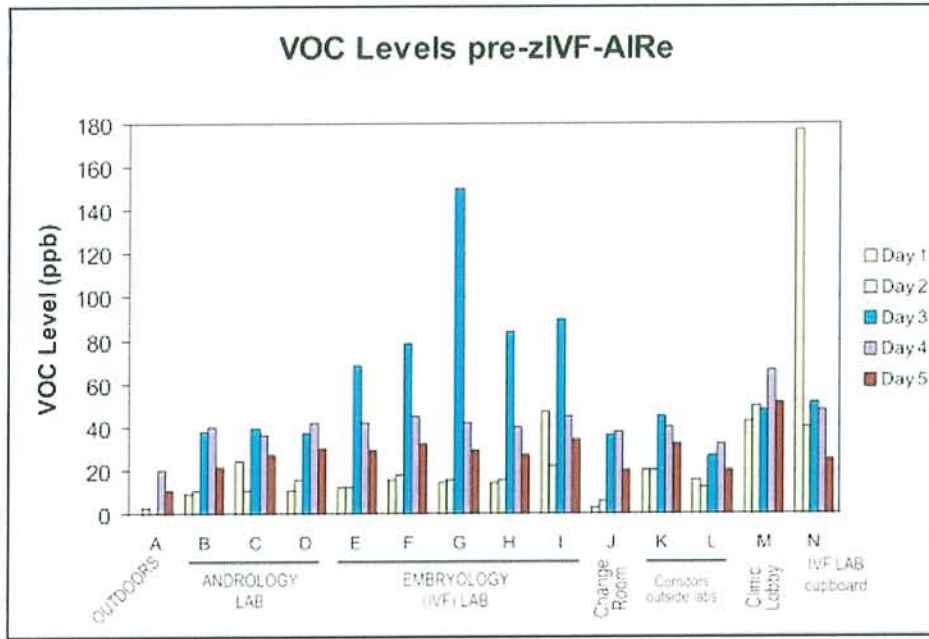


Figure 1

100C was measured at 14 ppb VOCs.

Introducing the zIVF-AIRe units reduced the fluctuations in VOC levels measured in the Embryology Lab (locations E-I in the Figures 1 and 2). Although the mean values were not statistically different for each location before and after the introduction of the zIVF-AIRe units (Student's *t*-test), the standard deviation values were much smaller for readings made after the introduction of local air polishing (see Table 1).

On Day 3 post-zIVF-AIRe there were very high VOC levels in the corridor immediately outside the laboratory suite's main doors (location M) due to a wooden door being varnished there: 9971 ppb, i.e. almost 10 ppm. However, in spite of this, there was no elevation of VOCs at any of the laboratory measurement locations, confirming that the overpressure in the Embryology Lab was effective in preventing contaminated from entering the clean room area.

Clinical Efficacy

The results for the first 30

treatment cycles performed in the PCRm laboratory are shown in Table 2. Clinical pregnancy and implantation rate results are based on 7-week ultrasounds.

Discussion

The present results illustrate not only that attention must be focussed on reducing VOCs in the air supply to an IVF Lab, but that employing additional, in-room air purification

consideration.

Woodwork: Construction materials such as particleboard and MDF ("craft wood"), and also furniture will release VOCs, especially formaldehyde, at considerable levels for months (Brown, 1999). VOC emissions are higher for particleboard than MDF, and even higher for laminate office furniture, although MDF remains a low level source of hexanal for several months post-construction. For these

will help eliminate locally-generated VOCs within the laboratory (Cohen *et al.*, 1997; Hall *et al.*, 1998; Boone *et al.*, 1999; Elder & Dale, 2000). Moreover, paying particular attention to the choice of construction materials can also reduce VOC emissions, not only during the initial period post-construction/installation but also during the prolonged period of secondary emissions that can continue for months thereafter (Wolkoff, 1999). Consequently, we believe that when designing a modern ART laboratory the following issues should be taken into

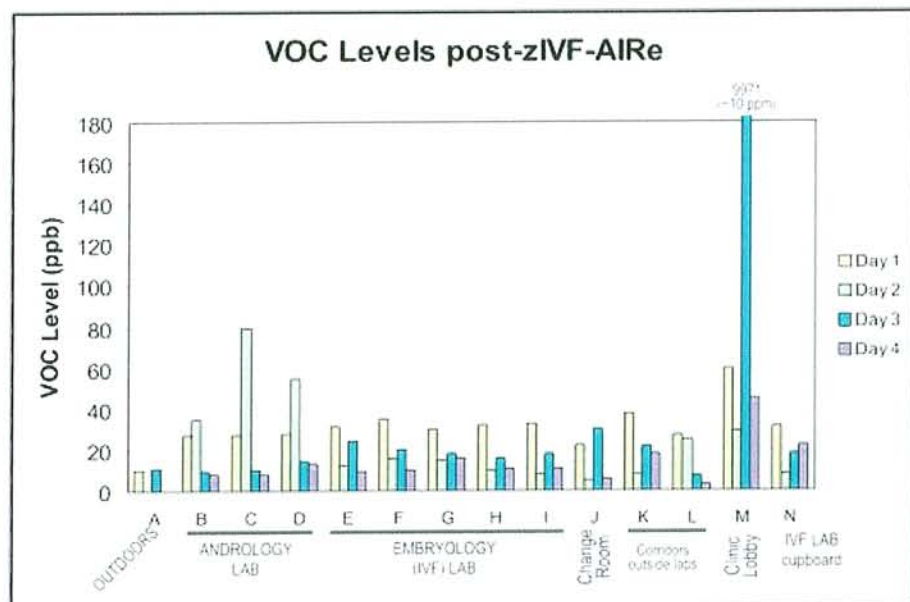


Figure 2

Table 1: VOC measurements in the Embryology Lab before and after introduction of the zIVF-AIRe units. Values are in parts-per-billion (“ppb”) as mean ± SD. Note that the size of the SD values indicate substantial, non-Normal variation in the distribution of the individual readings.

Location	Before	After
E	33 ± 23	19 ± 10
F	38 ± 25	20 ± 11
G	50 ± 57	20 ± 7
H	36 ± 29	17 ± 10
I	48 ± 26	18 ± 11

reasons, all woodwork in the PCRM laboratory was sealed on all surfaces to prevent out-gassing.

Floors: While emissions of many VOCs from some PVF flooring are undetectable by 26 weeks after manufacture, some flooring materials show only minimal decreases in their emission rates beyond 6 months (Lundgren *et al.*, 1999). **Floor tile adhesive** is one of the most aggressive VOCs tested so far on embryos, arresting >90% of mouse embryos at the 2-cell stage – even though it was only used in an area adjacent to the ART laboratory (Cohen *et al.*, 1997). Consequently, a high grade solid vinyl sheet with bonded urethane surface and welded joints was used in the PCRM laboratories.

Paints: The powerful odour of many paints, from the slow evaporation of the organic solvents that comprise their liquid phase, is well-known, hence latex paints are increasingly used when painting medical and

laboratory areas because they have lower odours than traditional oil-based paints. Even some “low-VOC” latex paints can have significant VOC emissions, which can be measured for over 200 days (Sparks *et al.*, 1999) and the performance of some low-VOC latex paints is not as good as conventional latex paints (Chang *et al.*, 1999). All paints used at PCRM were Dulux *Lifemaster* products, which have zero VOCs when white, although small amounts of VOCs are introduced when tinted.

While professional codes of practice or accreditation guidelines for ART laboratories do not yet include specific air quality standards for VOCs, there are specifications for particulates and micro-organism contamination of clean room air in the EU Tissue Directive 2006/86/EC (European Union, 2006) – although there are concerns about the risk of decreased ART success rates with excessive air flows (Mortimer, 2006).

Nonetheless, ensuring that poor quality air (which must clearly include low VOC levels) does not have a detrimental impact upon outcomes should be a general consideration of professional responsibility. Numerous guidelines and standards promulgated by regulatory authorities and professional bodies clearly place an obligation upon professionals working in ART Centres to ensure that everything that comes into contact with gametes and embryos is not toxic and will not cause any deleterious effects upon outcomes. Even if air quality is not yet mentioned specifically, failure to recognize – and eliminate – such an adverse factor could be seen as a breach of professional responsibility, and perhaps preclude the unencumbered accreditation or licensing of an ART Centre.

Conclusions

The results of our study confirm the efficacy of including photocatalytic VOC removal units in the HVAC system supplying the Embryology Laboratory. Moreover, the results also illustrate the value of having additional in-laboratory photocatalytic “air polishing” units to help eliminate locally-generated VOCs. Finally, the results of the first 30 treatment cycles demonstrated that a “burn-in” period for a new Embryology Laboratory is not needed when it has been carefully planned and constructed.

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Table 2: Summary results for the first 30 IVF/ICSI treatment cycles performed in the PCRM laboratory.

Indicator	Results
Age of female partner	26–40 (mean = 34)
Number of embryo transfers	29
Average number of embryos transferred	2.0
Positive β-hCG	18 / 30 = 60% per cycle
Clinical pregnancy	14 / 30 = 47% per cycle
Implantation rate	38% (22 sacs from 58 embryos)

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