Exposure of COCs and embryos to VOCs (Volatile Organic Compounds) affects embryo quality and outcomes

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Disclosure

- **Director NewLifeAid – Global AB, Consulting**
- Lecturer, Auditor, Design of Clean Room ART Laboratories, Introduction and Optimization of Old & New Techniques
- **International Scientific Advisor – LifeAire, USA***
- **Mentorship Veroxlabs, Sri Lanka**
- **Affiliated Scientific Director in ART Clinics**

Learning Objectives

- Impact of the Environment on Outcomes
- VOCs (Volatile Organic Compounds)
  - Where do they come from?
  - How can we reduce – avoid that VOCs are taken up in the cultures? How will this affect outcomes and improve the expectations of the patients?

*Do you have unexplained seasonal variations in outcomes ***

A solid foundation to stand on in order to get consistent high outcomes

- Prevent the exposure of COCs and embryos to **environmental embryo-toxic pollutants** from incoming air or from within the clinic to ensure that the couple's gametes and embryos are given an **optimal chance to develop into top-quality embryos** with high implantation and low miscarriage rates.

Air Contaminations

- **Nonviable particulates**
  - Classification of ISO and EU GMP Class rating
  - Non-infectious but serve as "transporters" of infectious viable particulates
- **Viable particulates** – EU GMP
  - Biological and viral particulates
  - Microbial and fungal pathogens
- **Volatile Organic Compounds (VOCs)**
  - Ethanol, styrene, toluene, aldehydes + > 90.000+

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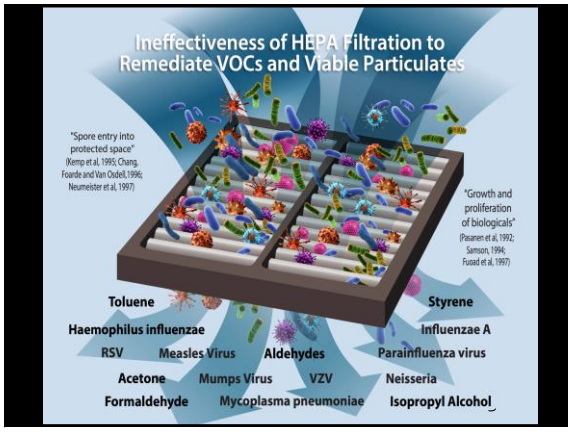
• General IVF does not seem to require the traditional cleanroom or ISO 5/6 environment?

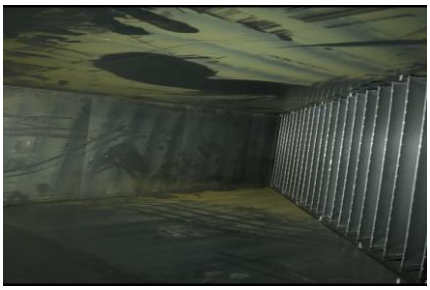
- The traditional cleanroom focuses on nonviable and viable particulates, **NOT** the level of **VOCs** that must be controlled in order to optimize the in vitro culture environment for the human embryo.

Three Sources of Air in the Laboratory

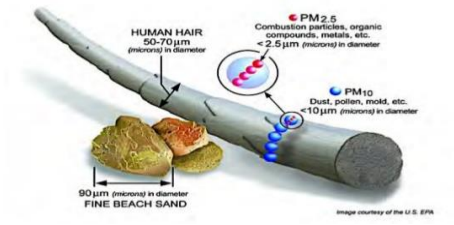
- Outside air serving the HVAC (inlet)
- Air provided by the HVAC system*



HVAC duct system may contain mold that produces toxic VOCs!



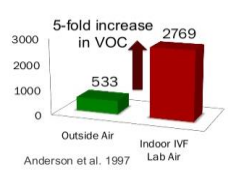
VOCs are not visible! You might smell them or just see the effects of them!

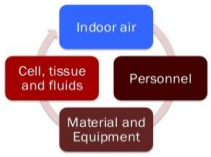


Three Sources of Air in the Laboratory

- Outside air serving the HVAC (inlet)
- Air provided by the HVAC system*
- Recirculated air within the space to be protected – dependent upon the building material, disposables, detergents and staff of the laboratory!

REPRODUCTIVE LABORATORIES





Esteves et al., 2017

A defect ventilation system together with "the indoor air" affects the outcomes!!!!!

Cell Culture Environment
Common Constituents

- **HVAC / refrigerators**
  - Chloroethane
  - Dichloro-tetrafluorethane
  - Dichlorodifluoromethane
- **Medical gases for incubators***
  - Freon
  - Xylene
  - Acetaldehyde
  - Isovaleraldehyde
  - Benzaldehyde
  - Formaldehyde
- **Personnel bioburden**
  - Benzene
  - Particulate – not enough staff
  - Viruses
  - Bacteria

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Mobiles – Why should they not be used in the laboratory?

Bodena et al., 2019

Cell Culture Environment
Common Constituents

- **Tissue culture ware ***
  - Styrene
  - Toluene
  - Acetone
  - 2-butanone
  - Alkenes
- **Disinfection - SOPs**
  - Isopropanol
  - Ethanol
  - Chlorhexidine
- **Equipment off-gassing ***
  - Formaldehyde
  - Glutaraldehyde
  - Trimethylsilanol
  - Hexamethylcyclosilicone

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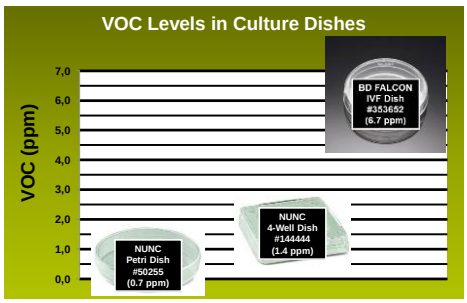
VOC and Disposables

- Volatile organic compounds (VOC) are hydrocarbons with high vapour pressure = evaporates easily?
- VOCs can also be found in plastic ware (Cohen, Gilligan et al. 1997) and therefore also in incubators.
- VOCs lowers fertilisation rates, embryonic development and pregnancy outcomes (Hall, Gilligan et al., 1998; Perin, Maluf et al., 2010, Morbeck et al 2015, Cairo Consensus, 2018).
- Outgassed over time?

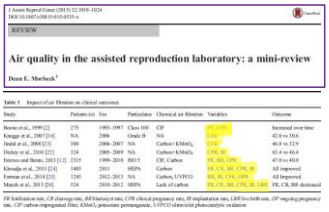
*VOC Levels: Dishes**



MS Project, Monash, 2016?

Preparation of Dishes? Outgassing and washing?

Optimizing blastocyst cultures



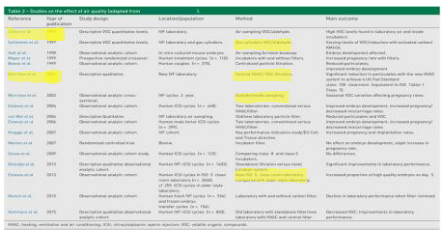
Air quality in the assisted reproduction laboratory: a mini-review

Dean E. Morbeck[1]

Environment / air quality

"… clear that air quality impacts IVF outcomes…. little evidence that particulate filtration alone improves outcomes, … evidence suggests that filtration systems should focus on robust VOC filtration."

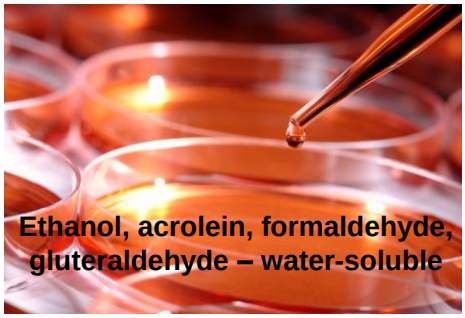
Morbeck 2015



- **Cairo consensus on the IVF laboratory environmentand air quality: report of an expert meeting, RBM Online 2018**
- D. Mortimer, J. Cohen, S. Mortimer, M. Fawzy, D. McCulloh, D. Morbeck, X. Pollet-Villard, R. Mansour, D. Brison, A Doshi, J. Harper, J. Swain, A. Gilligan.

What Controls the Actions of Individual VOCs?

- The action of individual VOCs can depend on their oil and/or water solubility and vapor pressure
- Entry into the media or oil can occur within a fraction of a second.
- Once present in the media or oil, the VOCs are a permanent resident in the culture environment, and thus a permanent threat to the gamete or embryo.
- **VOC compounds and their embryotoxicity, ASRM 2019 – VOCs accumulate within the culture media! (Sawicki et al, 2019)**



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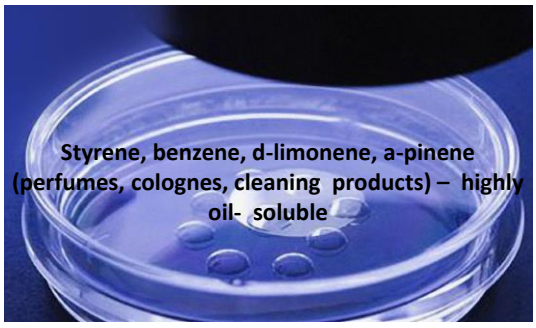

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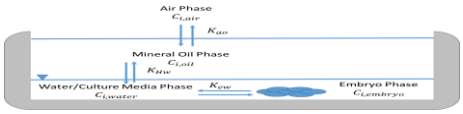
Modelling of the equilibrium partitioning of low level airborne volatile organic compounds in human ART.
Fox et al, 2022

- ALPHA Meeting, Seville, 2022: Explains how VOCs in the air phase can exert their negative influence on the cultures.
- Seven compounds (acrolein, formaldehyde, phenol, toluene, acetaldehyde, ethanol, and isopropanol) should be of great concern for the IVF clinics.
- High air-phase concentrations of VOCs will generate toxic levels of VOCs in the culture media which have a negative impact on the embryo quality and are indicators of poor clinical outcomes.



Mechanisms of VOC Cytotoxicity: Mammalian Cell Culture and Physiology

- Perturbed cellular membrane – leakage and disturbed homeostasis
  (McDermott et al, 2006, Pariselli et al, 2008)
- Increased intracellular Ca2+ - leads to cell injury and death (McDermott et al, 2006)
- Impaired mitochondria function
  (McDermott et al, 2006, Pariselli et al, 2008)
- Decreased spindle formation and chromosome alignment (Jeelani et al, 2016)

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VOCs vary in their polarity, molecular weight and biochemical structure

- Require different mechanisms of removal.
- Must target all biochemical families.
- Increase the effectiveness.
- Carbon will **only** adsorb non-polar HW and KMnO4 will oxidize some of the polar low MW VOCs.

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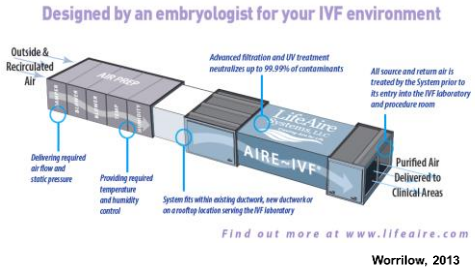
LIFE-AireIVF Ventilation System



Worrilow, 2013

First European Installation of the LifeAire System at King's Fertility in the United Kingdom - Exceptional Clinical Outcomes

"The LifeAire System has substantially contributed to the high-quality environment in our culture laboratory and provides air purity beyond the EU requirements for ART. Our staff is proud of the increased clinical outcomes and our patients are satisfied with the results of their treatment." Dr. Ippokratis Sarris, Director, King's Fertility, United Kingdom

Clinical Results Following the Aire~VOC Installation at King's Fertility

The results listed below are from 821 patient cycles immediately prior to the LifeAire installation and 746 patient cycles immediately following the LifeAire System installation.

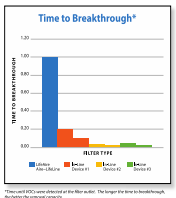
Parameter (All Maternal Ages)	% Change Following LifeAire Installation
Single Embryo Transfer (SET)	15.9% ↑
Clinical Pregnancy Rates per Embryo Transfer (CPR/ET)	14.3% ↑
Implantation Rates (IR)	24.4% ↑
Miscarriage Rates	17.5% ↓

Improving blastocyst development, ongoing study 2022-2023

Aire~LifeLine™

LifeLine Embryo Culture Study Results – Prospective Analysis

Facility	Filter	Stim Cycles N	Average Age	Blastocyst Conversion Percentage Point Increase
IVF Program #1	LifeAire	10	34.8	20.2%
	In-Line Device #1	11	34.8	
IVF Program #2	LifeAire	14	35.4	9.6%
	In-Line Device #1	19	36.7	
IVF Program #3	LifeAire	29	36.4	14.5%
	In-Line Device #1	46	38.3	
IVF Program #4	LifeAire	17	35	7.0%
	In-Line Device #1	40	36	
IVF Program #5	LifeAire	6	36.3	6.3%
	In-Line Device #1	6	36.3	



*Time until VOCs were detected at the filter outlet. The longer the time to breakthrough, the better the removal capacity.

"We had great blast conversion rates while using LifeAire's Aire~LifeLine. We had multiple repeat patients that previously had poor results in incubators connected to other in-line filters. The same patients had great conversion and more normal embryos in incubators with the LifeLine filter." *Suzanne Dopkins, The Center for Reproductive Medicine, Mobile, AL*

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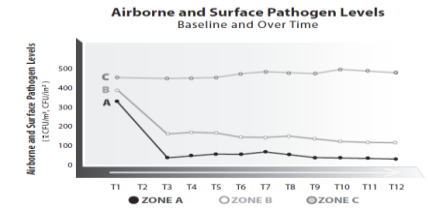
St. Lukes Hospital- AireIVF

Sawicki et al, Surgery 168, 2020,



"LifeAire has increased the throughput and capacity of my current SLUHN real estate while reducing length of stay, improving patieint care and our healthcare economics."

CIO, Chair of Innovation, St. Luke's University Health Network

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Take Home Message

- VOCs affects the quality of oocytes and embryo and increase miscarriage rates.
- VOCs are taken up in both oils (heavy or light) and culture media.
- MEA tested disposables are NOT evaluated for VOCs.*
- Are the media product companies producing their products in a VOC free environment? What about their vials - bottles?

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