SRBT WEBINAR

Take Control - OWN Your IVF Laboratory Environment

Presenter:
Kathryn Worrilow, PhD
Founder and CEO, LifeAire Systems, LLC
Professor,
Pennsylvania State University Lehigh Valley

Moderator:
Sue Gitlin, PhD
Chairman SRBT Education Committee

September 10, 2014

Sponsored by cryoport

Kathryn Worrilow, PhD

- PhD - UVa in anatomy and cell physiology
- Post-doc Reproductive Physiology and Infertility U Penn School of Medicine
- Over 20 years experience as IVF lab director
- Interests
  - Molecular signaling sperm/oocyte
  - Paternal contribution to the embryo
  - Impact of ambient air
- Founder and CEO, LifeAire Systems, LLC
- Professor, Penn State University Lehigh Valley

Take Control: <u>OWN</u> Your Environment!

Society of Reproductive Biologists and Technologists Webinar Series
September 10, 2014

Kathryn C. Worrilow, Ph.D.

Founder, LifeAire Systems
Adjunct Professor,
Pennsylvania State University Lehigh Valley

Our Objectives Today

- Define the key considerations/components involved in providing the optimal laboratory and clinical environment to support the *in vitro* culture of the human gamete and embryo.
- Discuss the specific role of ambient air quality in successful preimplantation embryogenesis - which airborne pathogens are critical to evaluate and which are less important to the process.

Our Objectives Today

- Discuss the common sources of airborne threats to the laboratory environment - there are as many within the IVF laboratory as there are in the outside source air serving your laboratory and clinical space.
- Recognize what can you do - what questions can you ask - to assure that your lab design, HVAC system, upstream equipment and protocols are supporting an optimal and consistent laboratory environment?

How Many of Us Have Had Our Clinical Pregnancy Rates Fluctuate?

- If an increase, celebrate!
- If consistently increasing, celebrate more!
- If a sudden decrease, what do you focus on first, second, third?

Where to Begin?

- Media, protein, oil, incubators?
- Lots of materials used?
- Adherence to protocols?
- Patient etiology?
- Stimulations? ET protocol?
- The integrity of the *in vitro* culture environment?
- Air?

Can you really control any of these variables?

Outside Influences Out of Your Control

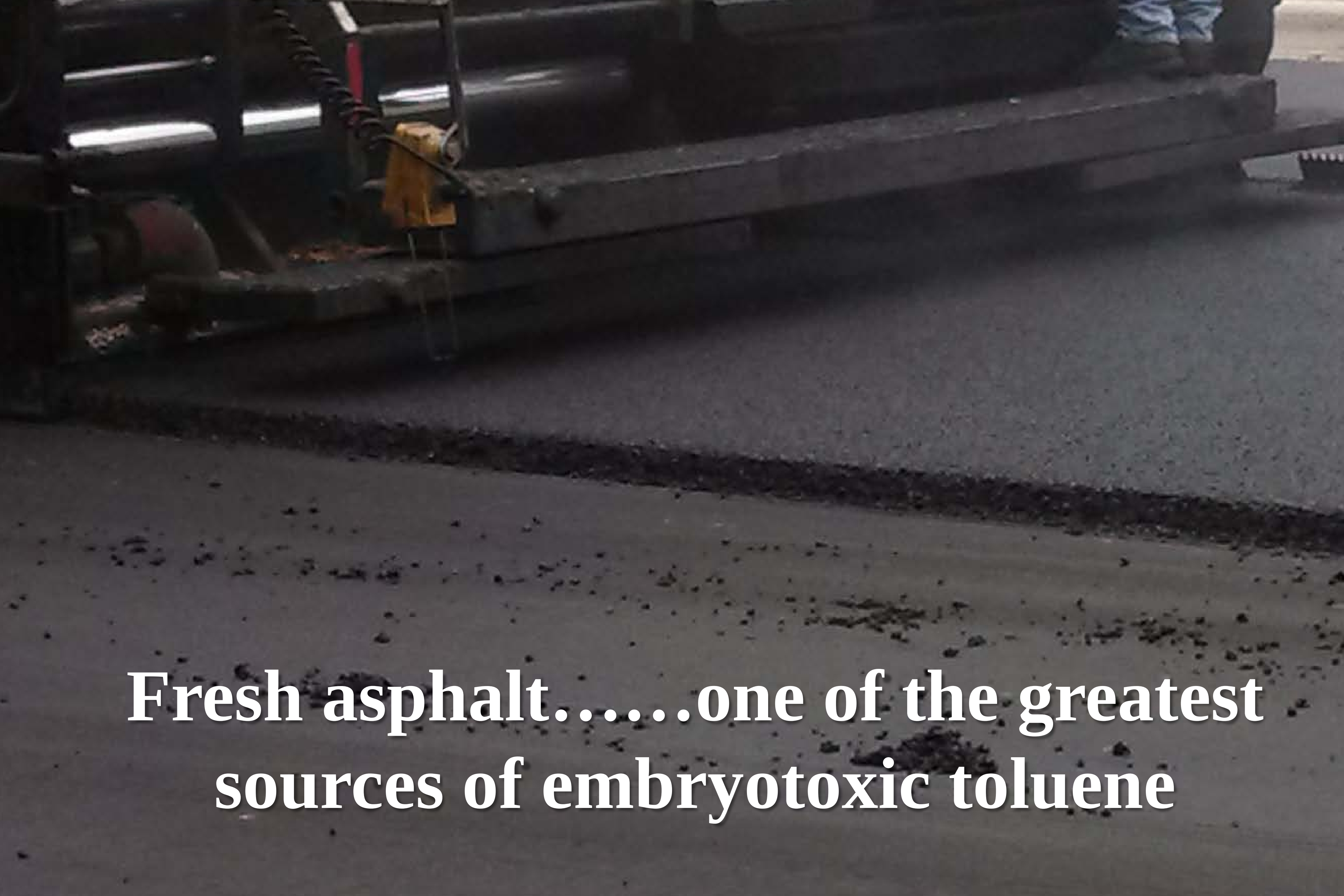
- Road resurfacing
- Rooftop resurfacing
- Construction
- Idling engines, exhaust
- Waste management, restaurant, generator exhaust direction
- Accidents, tire fires
- Seasonal pollutants


Great. Disturbed soil = ketones, aldehydes, nitriles, benzenes, alkenes, tetrachloroethane, chloroform, methane, toluene, xylene, vinyl chloride

Pressed board, wood = formaldehyde, acetone, methane methylene chloride, propane, acrolein, benzene, propionaldehyde, chloroethane, particulates

Placement of cement foundation: toluene, styrene, methylene chloride, particulates

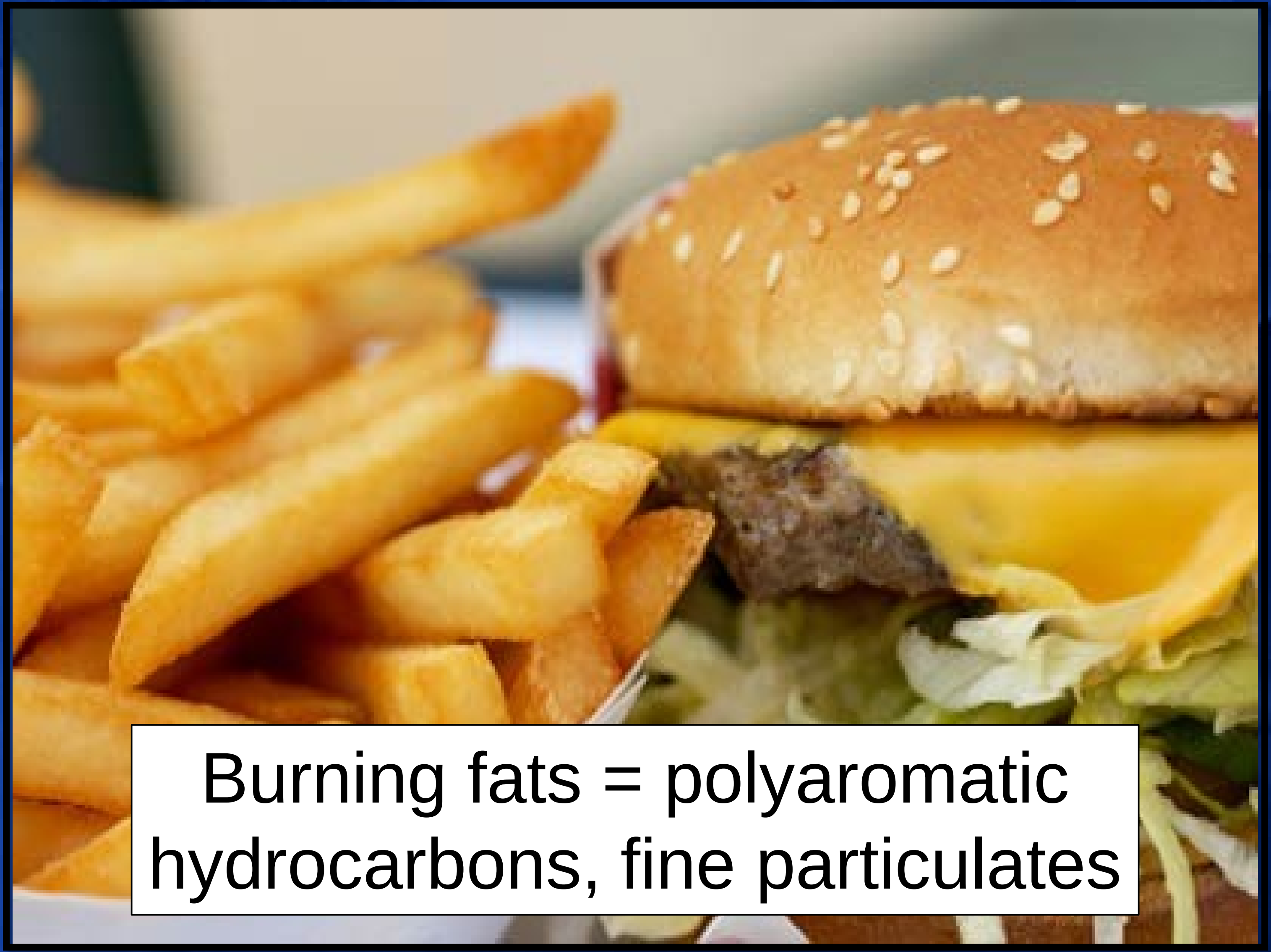

Road, rooftop
resurfacing


Fresh asphalt……one of the greatest
sources of embryotoxic toluene

Healthcare traffic, idling ambulance engines

Car, ambulance, medevac exhaust……sulphur dioxide, formaldehyde, nitrogen, ethylene, toluene, nitrogen oxides, benzene

Restaurant Exhaust



Hospital Generator Exhaust

Our neighbor, the SPU, saw a few bugs and called George. Pesticides = carbon tetrachloride, chloroform, 1,1 dichloroethane, tetrachloroethane, trichloroflurormethane

We think that these airborne contaminants/pathogens remain outside….

Acrolein

Biologicals

Gluteraldehyde

Formaldehyde

Isopropanol

Bioaerosols

Acetaldehyde

Styrene

Toluene

Methylene Chloride

Particulates

Three Sources of Air

- Outside air serving the HVAC system
- Recirculated air within the space to be protected
- Air provided by the HVAC system

Contributing Factors to Outside Air

- Traffic – all mechanisms of transport
- Industrial emissions – fossil fuel combustion
- Area construction
- Local road repairs, resurfacing
- Seasonal pollutants
- Accidents, spills, weather

Components of Outside Ambient Air

- Airborne particulate
- Ozone, nitrogen dioxide, sulphur dioxide, carbon monoxide
- Water & oil soluble contaminants – styrene monomers, aldehydes
- Airborne hydrocarbons – terpenes
- VOCs – gas, vapors, exhaust, benzene, toluene, acetone
- Construction aldehydes – formaldehyde
- Microfauna – viruses, bacteria, mold spores

Your Neighbors and Their Actions: The Generated VOCs and Viable Particulates Have Access to You…

Three Sources of Air

- Outside air serving the HVAC system
- Recirculated air within the space to be protected
- Air provided by the HVAC system

The IVF Laboratory:
Common Constituents of Recirculated Air

- Tissue cultureware
  - Styrene
  - Toluene
  - Acetone
  - 2-butanone
- Isopropanol
- Equipment off-gassing
  - Trimethylsilanol
  - Hexamethylcyclosilicone

The IVF Laboratory:
Common Constituents of Recirculated Air

- HVAC / refrigerants / compressed gasses
  - Chloroethane
  - Dichloro-tetrafluorethane
  - Dichlorodifluoromethane
- CO2 tanks
  - Freon
  - Benzene
  - Xylene
- Personnel bioburden
  - Particulate
  - Viruses
  - Bacteria

Some of my best friends are embryologists…however…

	Shedding Rate
VOC: Acetone	50,000 ug/day/person
VOC: Acetaldehyde	6,000 ug/day/person
VOC: Diethyl Ketone	21,000 ug/day/person
VOC: Ethyl Acetate	25,000 ug/day/person
VOC: Ethanol	45,000 ug/day/person
VOC: Methanol	75,000 ug/day/person
VOC: Toluene	7,000 ug/day/person
Biologicals, microbials	3,000 – 50,000 cfu/minute/person
Nonviable particulates	100,000 particles $\geq$ 0.3 um/minute

Three Sources of Air

- Outside air serving the HVAC system
- Recirculated air within the space to be protected
- Air provided by the HVAC system

HVAC System-Specific Organisms

- Pathogens – viruses, bacteria, fungi
- Allergens – bacteria, mold
- Toxins – endotoxins, mycotoxins

Common Fungal Embryotoxic VOCs

- Acetone
- Ethanol
- Isopropanol
- Toluene
- Styrene
- Methylene chloride
- Hexane
- 2 – Heptanone
- Hexanol
- 2 Pentanol
- Methyl acetate
- Benzene
- 2 – Propanyl acetate
- 2 – Pentanone
- 2, 2 – dimethylpropanol
- Acetic acid
- Ethyl acetate
- 1, 4 – Pentadiene
- Octanol

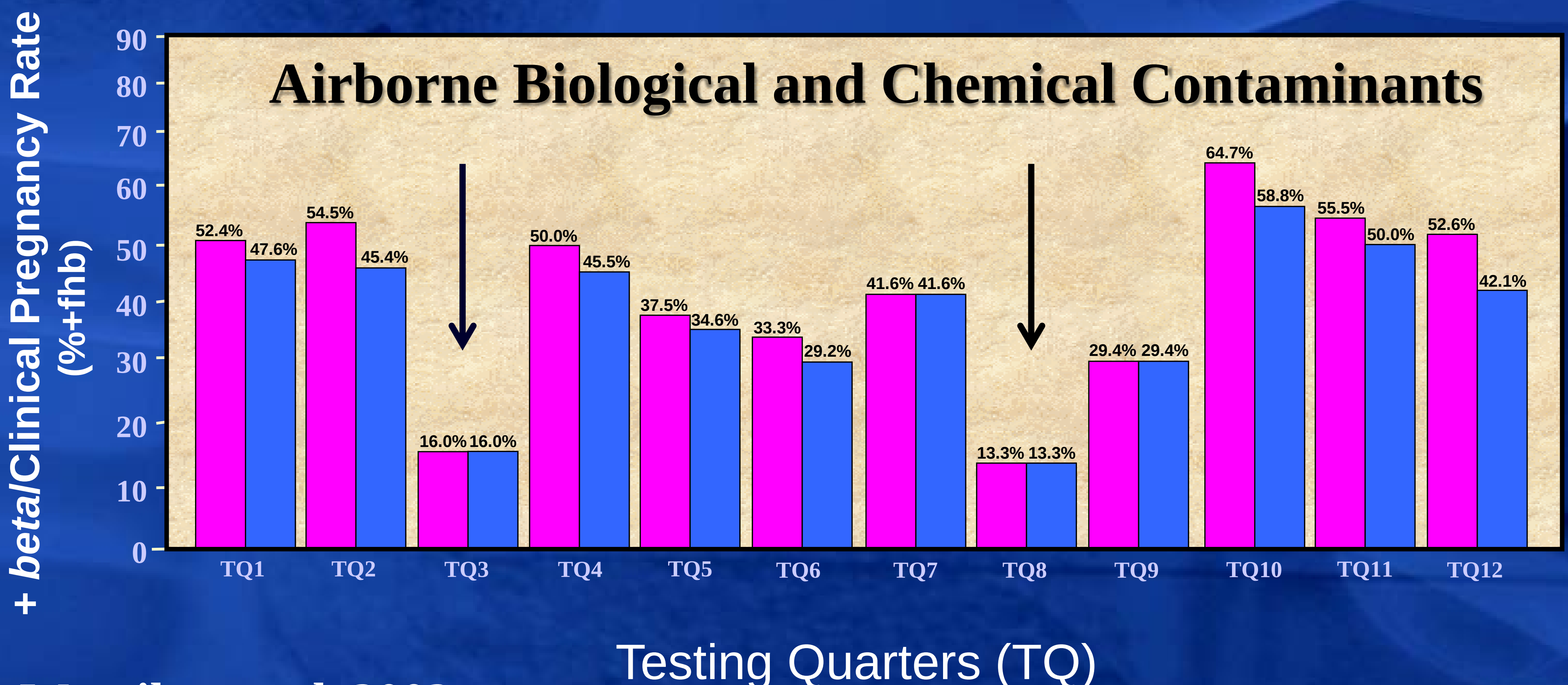
To Remove the Variable of Air (Source and Recirculated), We….

- Designed and built an ISO 5 cleanroom, custom air purification system
- Placed 300 lbs. of virgin carbon and 150 lbs. of $KMnO_4$ in our HVAC system
- Used a dedicated AHU to the lab
- Used a dedicated steam dehumidifier
- Placed UV lights throughout the air stream
- Placed ULPA filters above our IVF laboratory and clinical procedure rooms


HV 2472
Operation within an ISO 5 cleanroom – thus removing the variable of air

How effective was the IVF laboratory cleanroom and HVAC air filtration system as designed?

Analysis of CPR, TVOC and Biological Loading within the IVF Laboratory



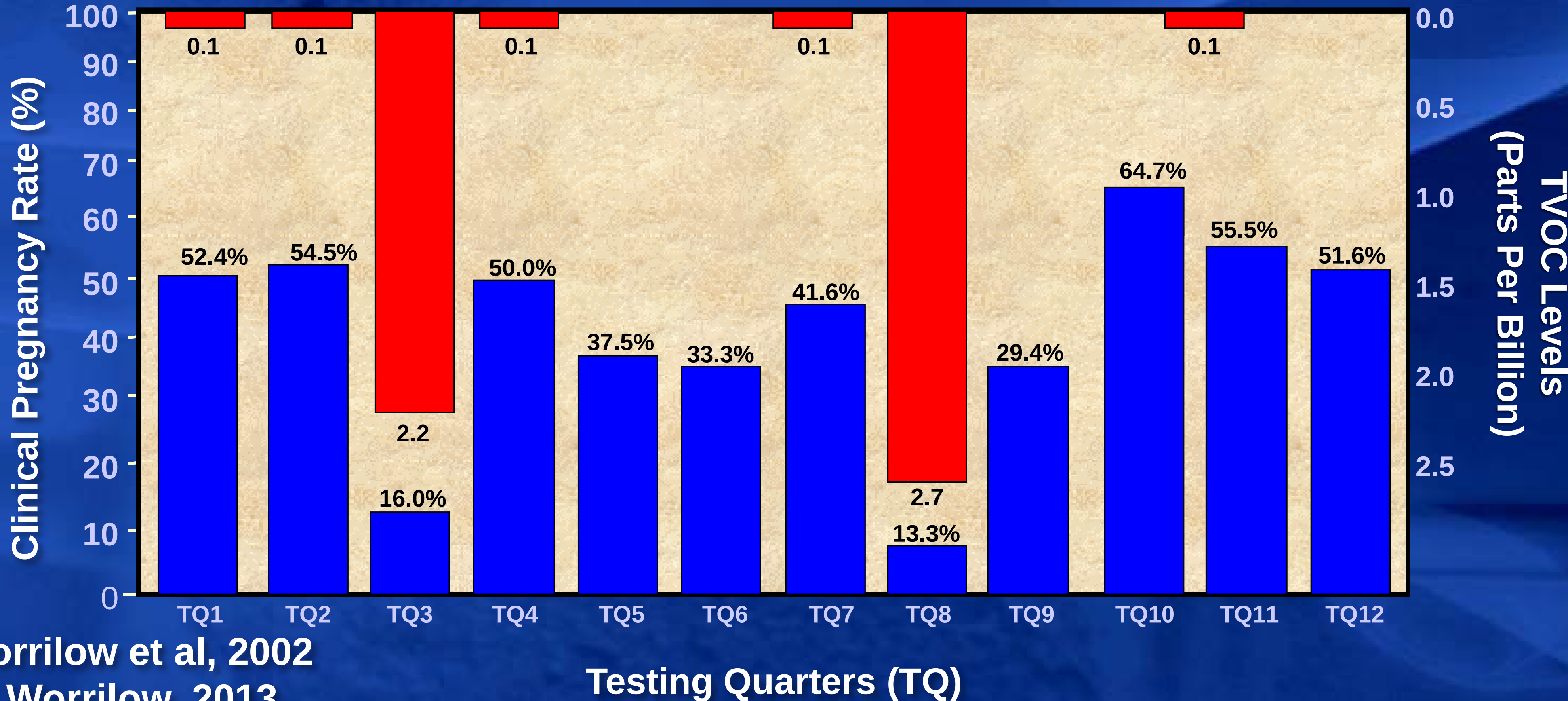
Worrilow et al, 2002
Worrilow, 2013

Confidential

What did the study of preimplantation toxicology, human embryogenesis, ambient air quality and clinical outcomes tell us? The study clearly delineated and defined the problem of the variable of ambient air and the optimal culture environment, and the specific role that they played in our processes.

THM #1: The impact of subtle levels of VOCs on our process and clinical outcomes

Analysis of CPR & TVOC Loading within the IVF Laboratory



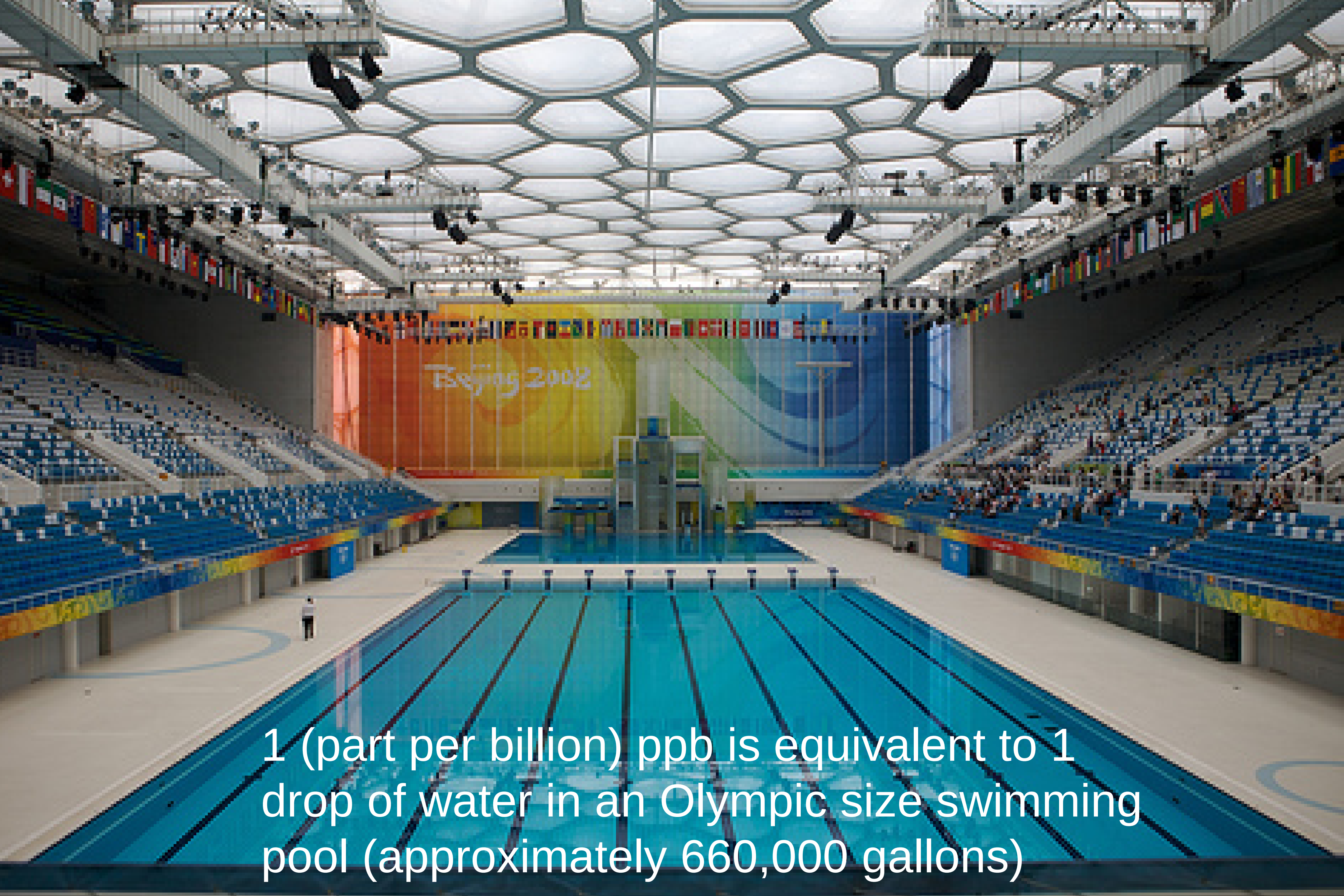
Worrilow et al, 2002
Worrilow, 2013

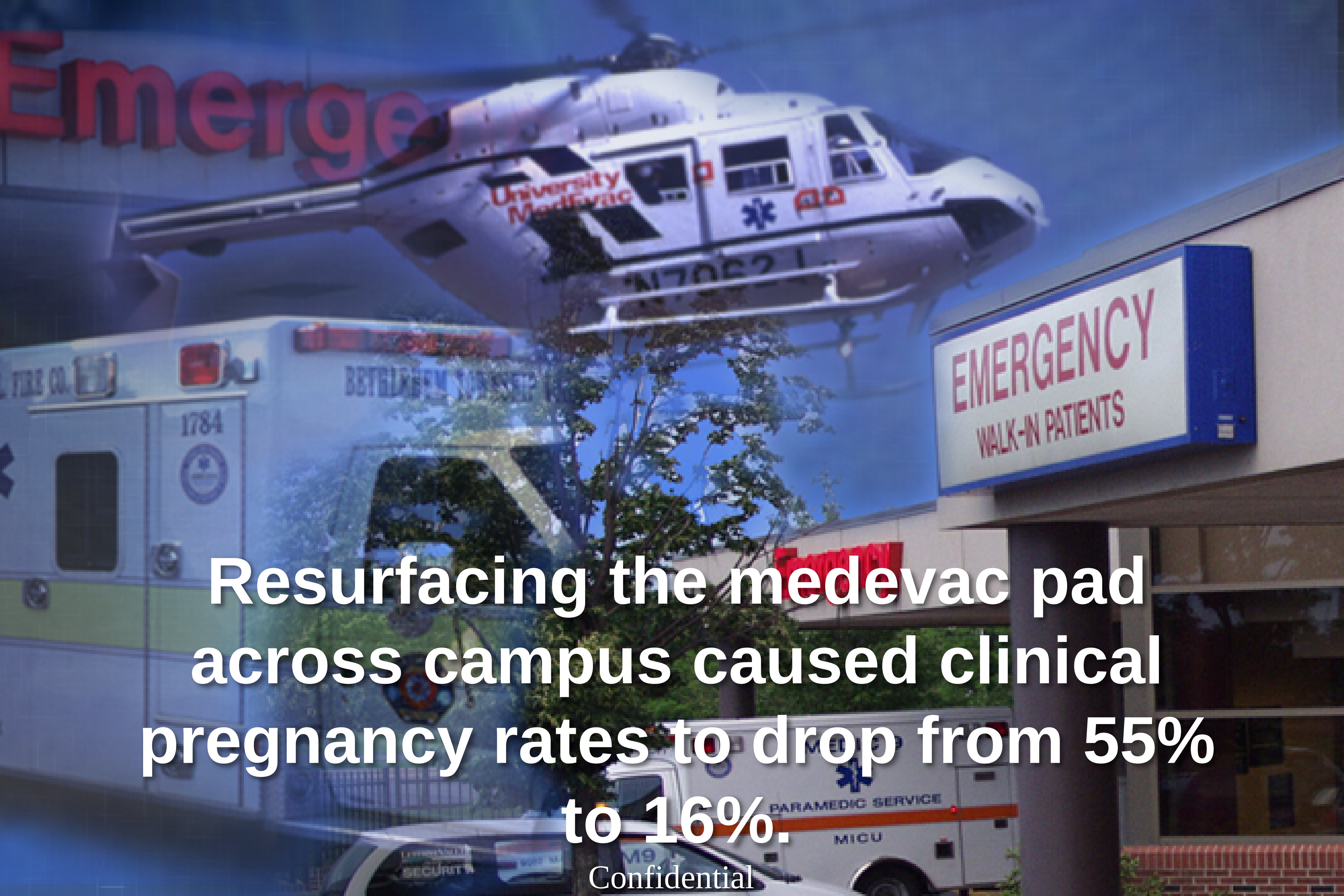
The presence of ppb VOCs severely compromised clinical outcomes

What Do These Numbers Represent?

2 → 22

1 → 660,000


Beijing 2008
1 (part per billion) ppb is equivalent to 1 drop of water in an Olympic size swimming pool (approximately 660,000 gallons)


EMERGENCY
WALK-IN PATIENTS
1784
PARAMEDIC SERVICE
MICU
Resurfacing the medevac pad across campus caused clinical pregnancy rates to drop from 55% to 16%.
Confidential

FAQ: Can’t we simply add more carbon and $KMnO_4$ to fix the problem?

No.

VOCs Common to the IVF Laboratory

- Polar VOCs – isopropanol, ethanol, aldehydes, esters, ketones, acetones
- Nonpolar VOCs – benzene, toluene, styrene, hexane
- Low MW hydrocarbons – isobutane, methane, acetylene, propene
- High MW hydrocarbons – ethylbenzene, styrene, aldehydes, acrolein, formaldehyde, gluteraldehyde

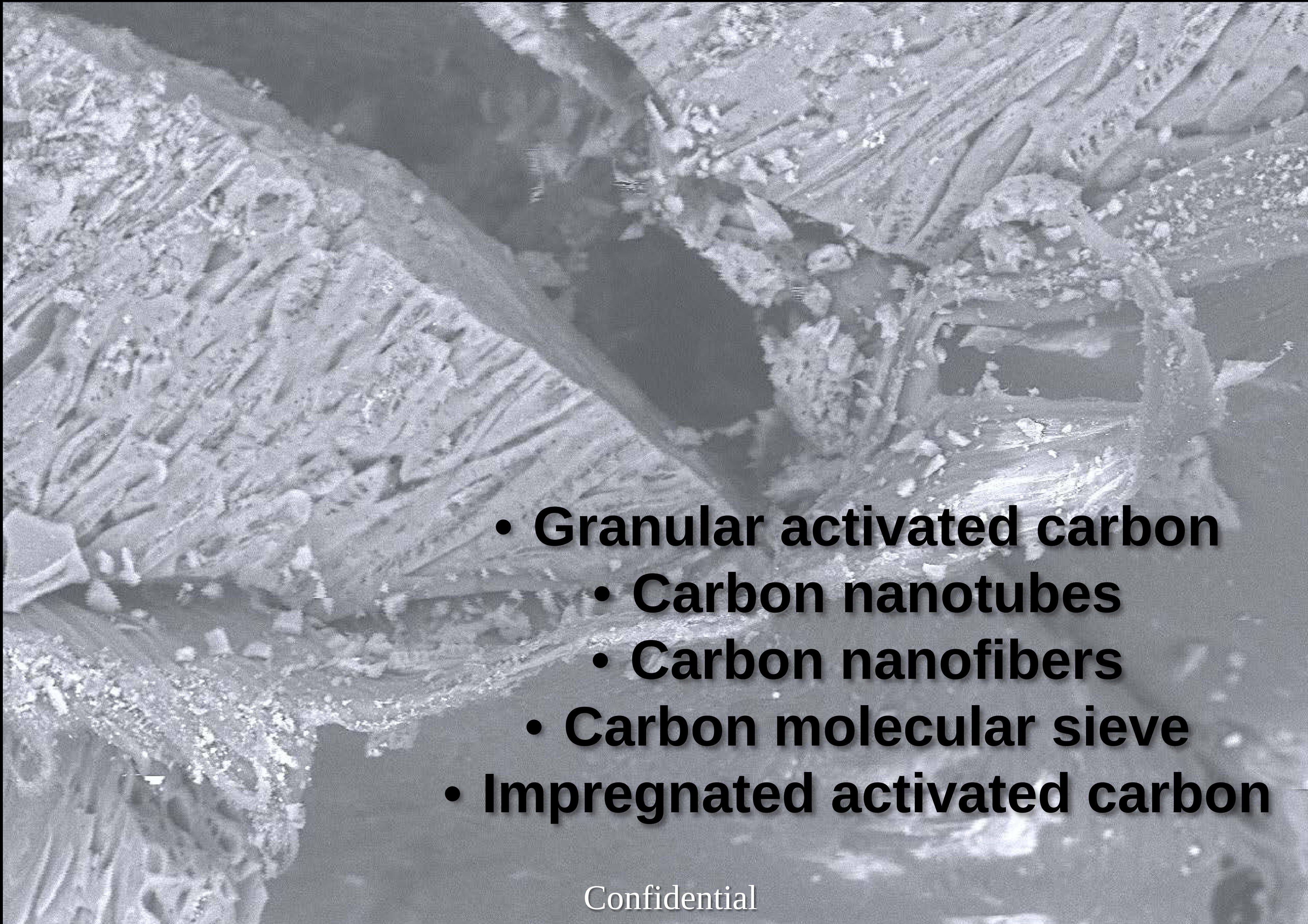
VOCs Common to the IVF Laboratory

- Fungal VOCs – 2-heptanone, 1,8-cineole, 3-methyl-butanol
- Microbial VOCs – alcohols, aldehydes, amines, ketones
- Biogenetic VOCs – terpenes, isoprene, limonene, sulphur-based VOCs

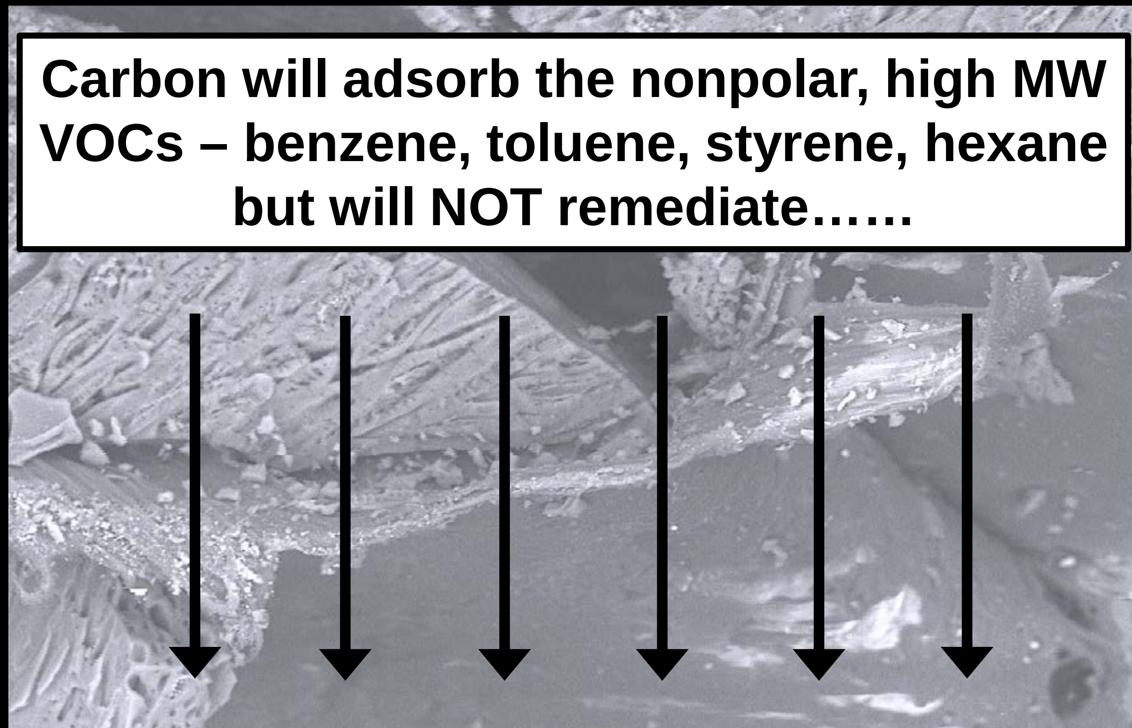
Filtration Physics: Mechanisms of VOC Remediation

- Physical adsorption
- Chemisorption
- Oxidation
- Persulfate oxidation
- Thermal oxidation
- Photo-Fenton oxidation
- Ultraviolet photocatalytic oxidation (UVPCO)
- Ultraviolet UVV wavelength
- Molecular sieve
- Transition metal impregnation
- Fixed bed adsorption
- Surface, contact condensation

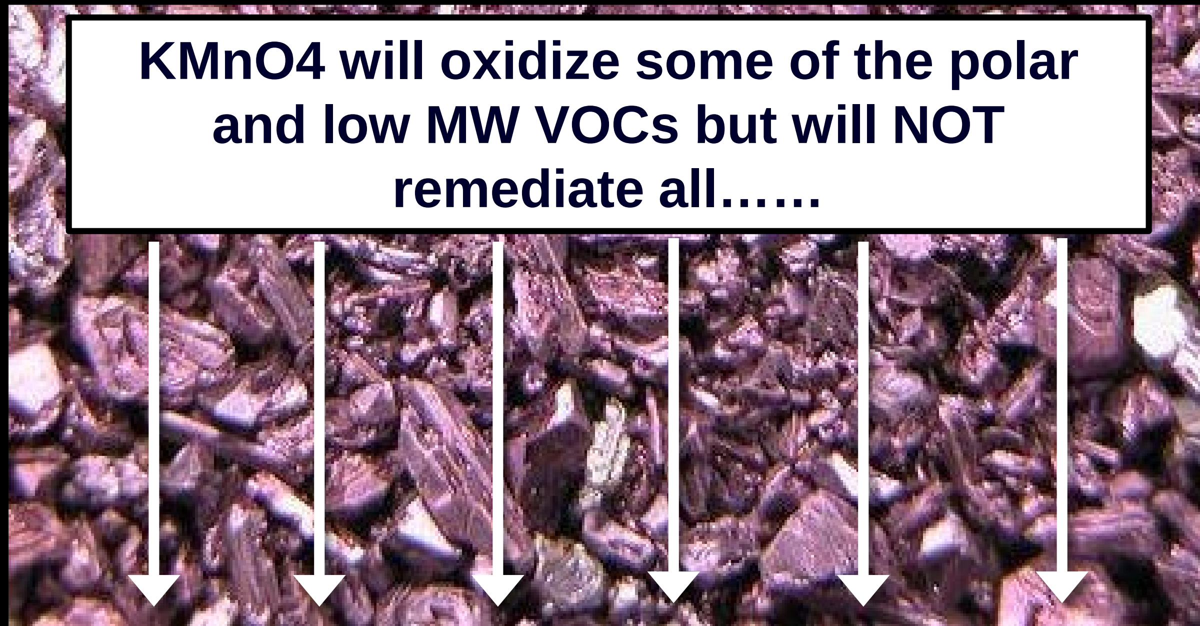
The most commonly used media for VOC remediation in IVF is that of carbon and KMnO4.



- **Granular activated carbon**
- **Carbon nanotubes**
- **Carbon nanofibers**
- **Carbon molecular sieve**
- **Impregnated activated carbon**



1. Polar VOCs – isopropanol, ethanol, aldehydes, esters, ketones, acetone
2. Low MW hydrocarbons – isobutane, methane, acetylene, propene
3. Fungal VOCs – 2-heptanone, 1,8-cineole, 3-methyl-butanol
4. Microbial VOCs – alcohols, aldehydes, amines, ketones
5. Biogenetic VOCs – terpenes, isoprene, limonene, sulphur-based VOCs



1. Polar VOCs – isopropanol, ethanol, aldehydes, esters, ketones, acetone
2. Nonpolar VOCs – benzene, toluene, styrene, hexane
3. Low MW hydrocarbons – isobutane, methane, acetylene, propene
4. High MW hydrocarbons – ethylbenzene, styrene, aldehydes, acrolein, formaldehyde, gluteraldehyde
5. Fungal VOCs – 2-heptanone, 1,8-cineole, 3-methyl-butanol
6. Microbial VOCs – alcohols, aldehydes, amines, ketones
7. Biogenetic VOCs – terpenes, isoprene, limonene, sulphur-based VOCs

Beyond Carbon and KMnO4: Comprehensive Remediation of TVOCs

- VOCs vary in their polarity, molecular weight and biochemical structure and thus require different mechanisms of removal.
- VOC media must target all biochemical families.
- Application of VOC media to the substrate critical to effectiveness.
- Resonance and dwell time paramount.

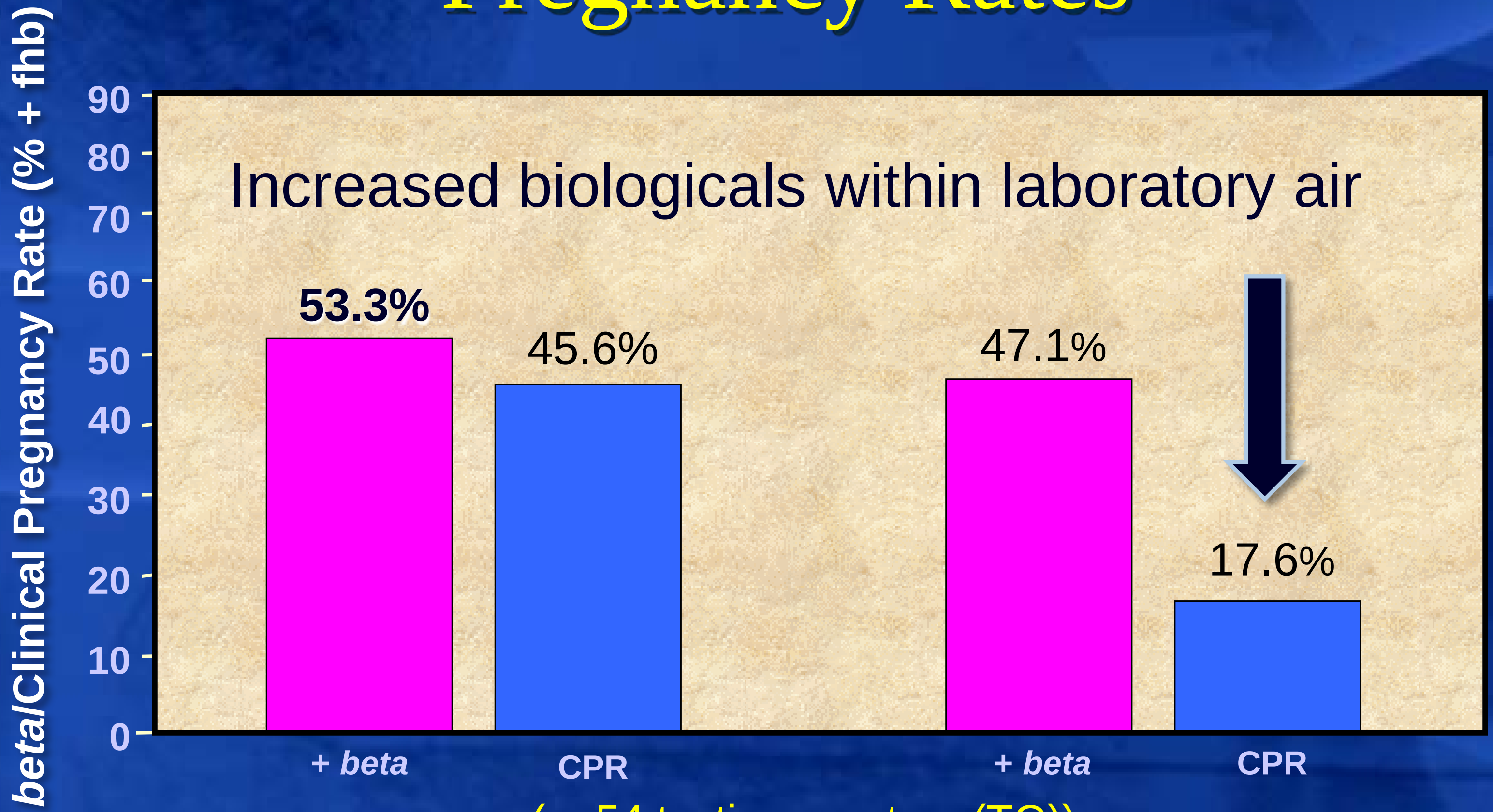
“I culture under oil, use tabletop incubators, time-lapse imaging, etc. VOCs cannot enter my media or affect my embryos.”

We wish this were true.

If oil soluble, VOCs can easily enter the oil overlay and if water soluble, the media. Once present in the media or oil, the VOCs are a permanent resident in the culture environment, and thus a permanent threat to the embryo.

THM #2: The impact of subtle levels of biologicals on our process and clinical outcomes

The Impact of Subtle Levels of Airborne Biological Pathogens on Clinical Pregnancy Rates



(n=54 testing quarters (TQ))

Loss of power to the ballast boxes supporting the UV lights in our HVAC system coincided with an increase in our clinical loss or miscarriage rate

Confidential

What Did We Learn?

- UV must be present in the air purification system serving the IVF laboratory.
- UV will destroy the biologicals and remediate particular biochemical families of VOCs.

Microbial Susceptibility to UV

Most susceptible

↓

Vegetative bacteria

Mycobacteria

Bacterial spores

Fungal spores

↓

Least susceptible

Filtration Physics: UV

- Critical to successful human embryogenesis and clinical outcome is the intensity, lamp coordinates, level and longevity of the UV output.
- Selection of the right UV source and assurance of maintenance SOP is paramount.

THM #3: What HEPA filtration does and does NOT do for our culture environment

Filtration Physics: HEPA, ULPA

- HEPA filtration is designed to remove or capture particles greater than 0.3 microns in diameter at a 99.97 – 99.99% effectiveness rating.
- If air bypass or filter leakage are present and/or if inadequate maintenance and change-outs, the capture rate can drop to 99.94 – 99.95%.
- Such decrease in efficiency can allow, in the course of a single day, 7.2M particles to penetrate the HEPA filter and contaminate the IVF. laboratory and clinical procedure rooms.
- HEPA and ULPA filtration remediate by “capture.”

We create the perfect environment for growth…..

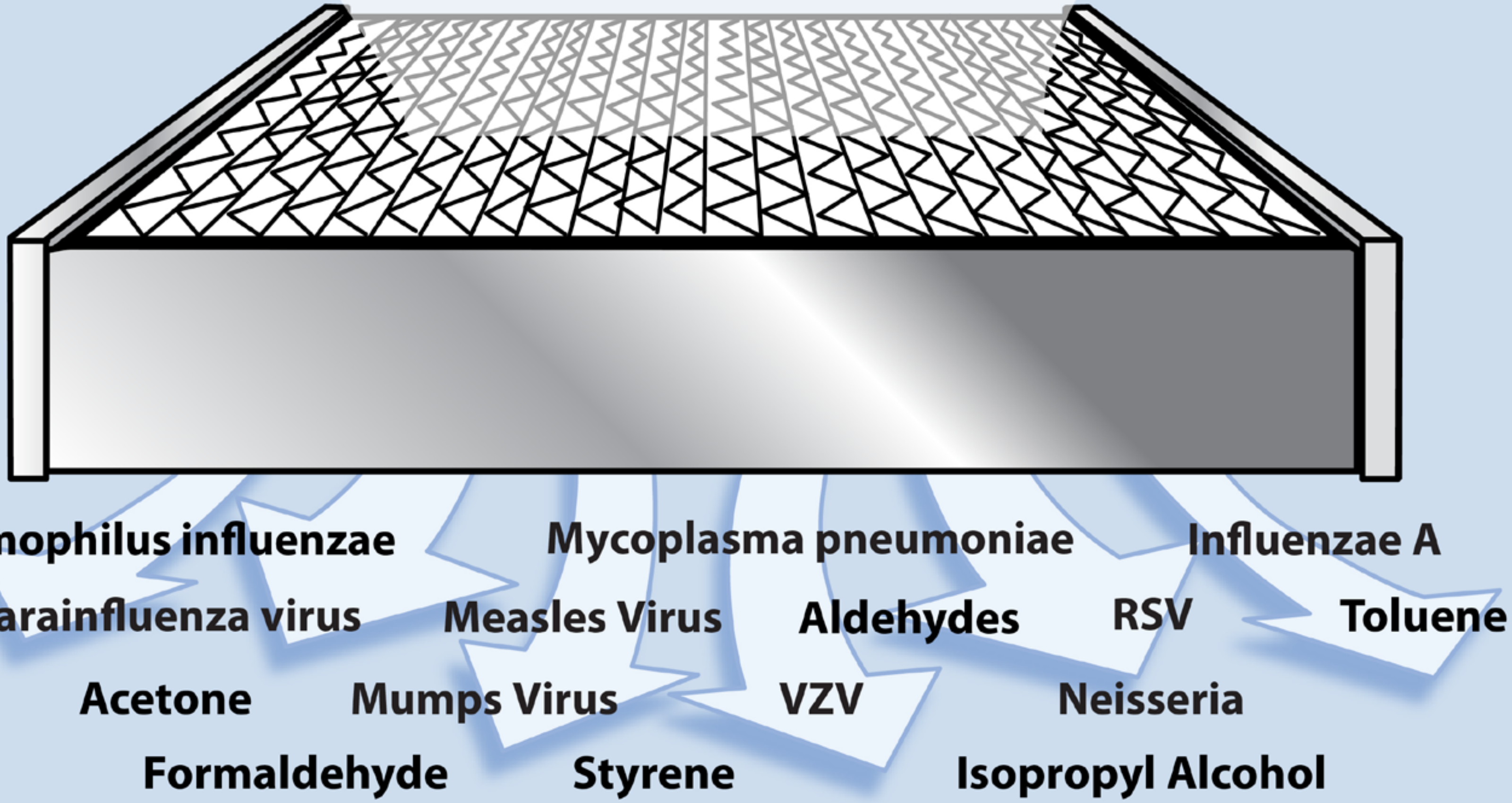
IVF laboratory room temperature, humidity and HEPA/ULPA filter substrate = proliferation of bacterial and viral spores, mold and biologicals

HEPA/ULPA Final Filter Above IVF Laboratory

Ineffectiveness of HEPA Filtration to Remediate VOCs and Viable Particulates

"Growth and proliferation of biologicals"
(Pasanen et al, 1992; Samson, 1994;
Fuoad et al, 1997)

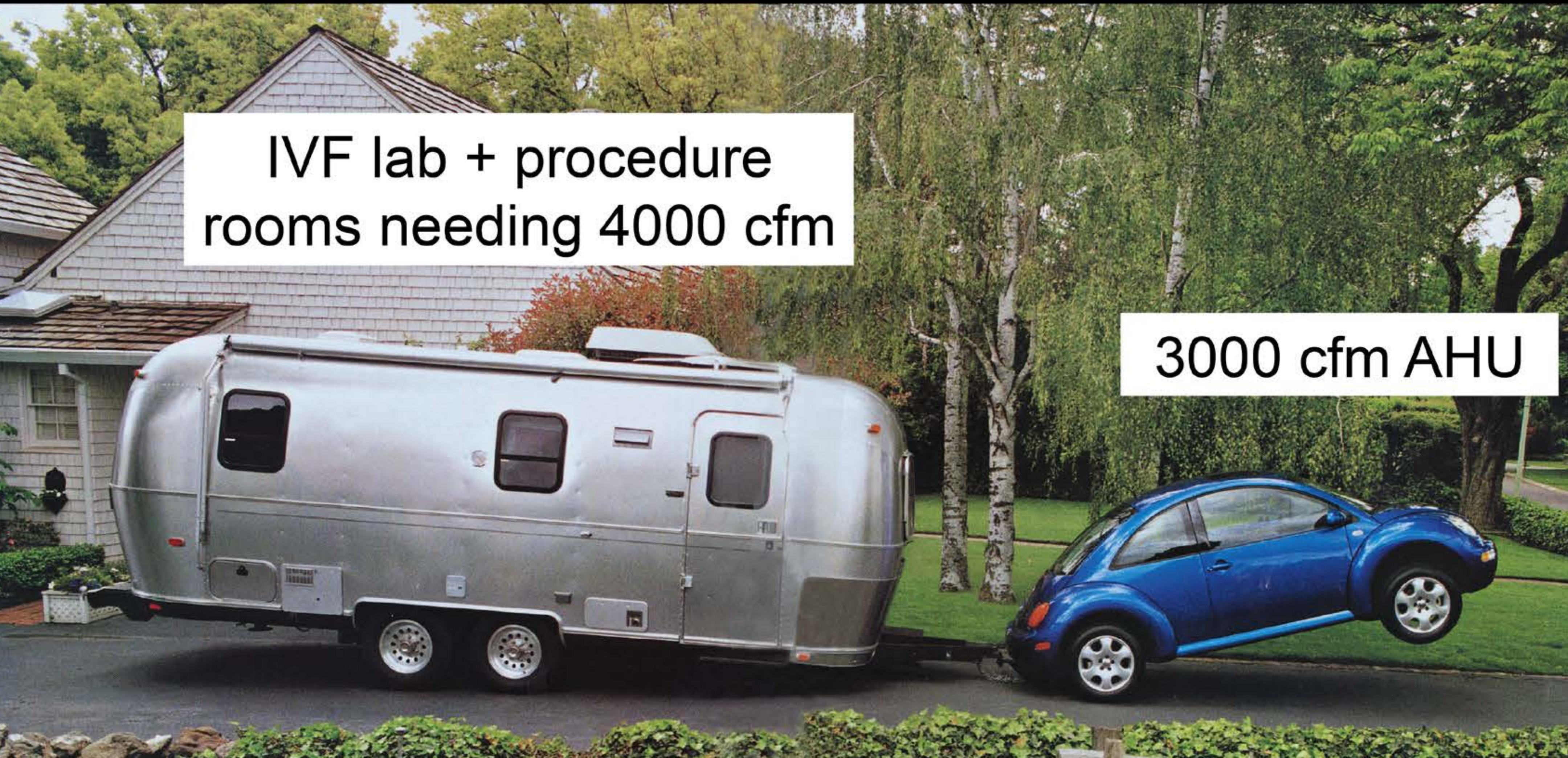
"Spore entry into protected space"
(Kemp et al, 1995; Chang, Foarde and Van Osdell,1996;
Neumeister et al, 1997)



Know what you have in your HVAC system, pop the ceiling tiles and ask questions….lots of questions……

Critical Components: HVAC System

- Location of air intake relative to outside activity – anticipate change in source air
- Dedicated air handling unit (AHU), proper sizing to deliver positive pressure and adequate air changes/hour
- Water and gas pipelines – cooling effect, dampness, mold growth = microbial growth and fungal VOCs
- Upstream filters – no electrostatic filters
- Chiller – water or air cooled


IVF lab + procedure rooms needing 4000 cfm
3000 cfm AHU

Critical Components: HVAC System

- Water and gas pipelines – cooling effect, dampness, mold growth = microbial growth and fungal VOCs
- Upstream filters – no electrostatic filters
- Chiller – water or air cooled
- Dedicated humidity system, DI water only, continual flushing, proper dispersion, moisture = microbial growth
- Proper dehumidification, cooling and heating equipment

Critical Components: HVAC System

- Non-shedding ductwork, externally insulated only
- Comprehensive air purification system
- Proper return and final diffuser location relative to critical points of process
- Dedicated return air – do not share with other clinical areas if possible
- AHU, air filtration/purification system, temperature, humidity, BMS/DDS/BACNET on generator

Air Quality Component and Management

- Vertical unidirectional air flow with low returns
- Proper air changes per hour
- Proper air velocity across filter face
- Proper air velocity into space – achieve proper velocity without generating turbulence in space
- Appropriate volume of outside source versus volume of recirculated air
- Proper volume of exhaust

Air Quality Component and Management

- Consideration of energy efficiency
- Proper air flow with no/minimal leakage from adjacent areas; plenums, service ducts, identification of “still” pockets, etc.
- Placement of positive pressure manometers to reference areas of lesser relative pressure
- Comprehensive testing air balancing (TAB) and in advance of MEA testing
- Proper maintenance and service of your HVAC and air filtration system per your SOP

Quality Control: Air Testing

- Nonviable particulate assessment: laser particle counting of 0.3 micron particulates within 1 cubic foot of air
- Viable particulate assessment: microbial settle plates, impaction sampling, membrane aspiration, swabbing/micro-culture of surfaces
- Volatile organic compounds (VOC) assessment: Total VOC assessment, individual VOC assessment via TO-15 (non-aldehyde VOCs) and TO-11 (aldehydes) assays

Additional Environmental Components to Consider…..

- Walls, flooring and ceiling - low/no VOC materials, wipeable, heat-welded floors, no adhesives, dovetail edges, no corners
- Proper sealant for concrete foundation
- Seamless design in walls, countertops and flooring
- Proper lighting, lab furniture, storage and offgassing protocols
- Proper SOP in place for maintenance of clean space, instill a "culture" in your team
- Proper "bake-out" of new materials or renovated, expanded or new laboratory or clinical space


There is always someone willing to do it for cheaper!

When HVAC Solutions Are Proposed: Ask for Data! Proof of Technology!

- Do they understand the environment that you are protecting, the vulnerability of the human embryo?
- Can they assure consistency and performance of your environment?
- Do they understand the requirements needed by your upstream HVAC equipment?
- Is the upstream HVAC equipment meeting your criteria?
- How will maintenance and SOP protocols be followed to assure consistency?

Quality Control: Operational Recommendations

- Off-gas tissue culture dishes (embryotoxic styrene)
- Avoid storage of particle or cardboard in laboratory spaces
- Careful placement of all media, tissue cultureware in proper storage areas
- Minimal use of isopropanol for cleaning, place lint-free cloths with isopropanol outside of laboratory/clinical procedure rooms
- Follow best practice material and personnel flow

Quality Control: Clinical and Laboratory Staff Recommendations

- Use of non-particulating scrubs, cover scrubs when leaving area, change of scrubs if exposure to environment outside of IVF laboratory/clinical procedure rooms
- No use of scented products by staff or patients; cologne, perfume, scented deodorant, hairspray, facial foundation, nail polish, body wash or powder, etc.
- Test gloves and scrubs via touch and culture of microbial settle plates

Identification of The Problem Led to A Comprehensive Understanding

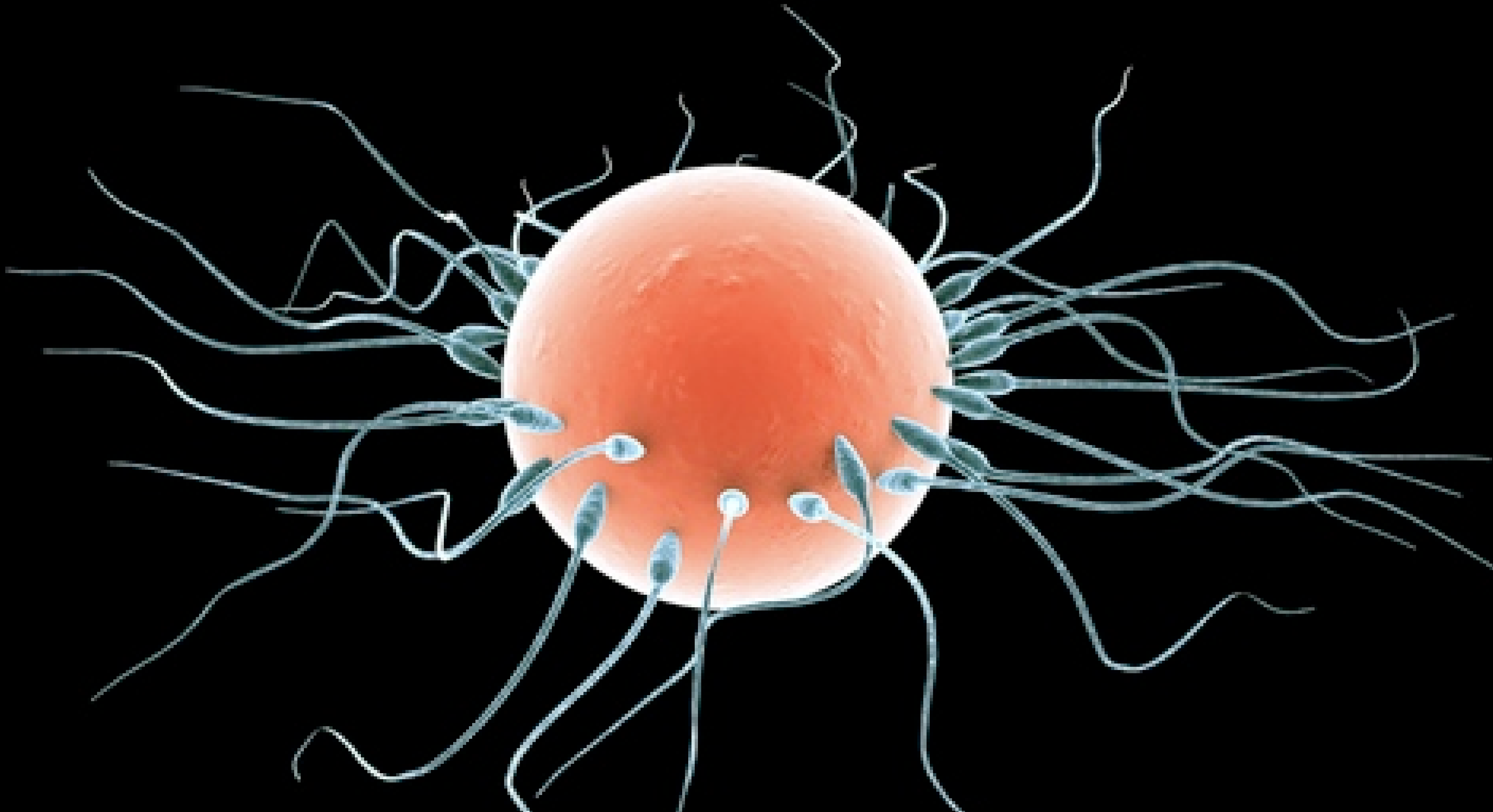
~ Understanding the source of and impact of subtle levels of VOCs and airborne biologicals on our processes and defining the optimal *in vitro* culture environment led to a comprehensive understanding of the critical nature of the environment that we provide ~

In Summary

- The air serving our *in vitro* culture environment is dynamic in nature and is influenced by the outside source air, materials used in the laboratory, laboratory protocols, staff, the HVAC design relative to your critical points of process, and all associated HVAC upstream equipment.
- Critical to the successful maintenance of the *in vitro* culture environment is the identification and QC of the proper upstream equipment serving your laboratory; AHU, humidifier, cooling coils, ductwork, air purification system, etc.

- The development of proper SOPs and culture to maintain the environment is paramount to a proper operation.
- Subtle levels of airborne VOC and biological contaminants can be devastating to successful human embryogenesis and clinical outcomes – mechanisms of filtration physics are available to remediate and control the airborne pathogens.
- It is critical to control the quality of the ambient air serving your *in vitro* culture environment for successful preimplantation embryogenesis and improved levels of patient care.

We all have the same goal – to provide the optimal *in vitro* culture environment - control and OWN your environment!



Thank you for your time, attention and discussion!

Kathryn C. Worrilow, Ph.D.
kcworrilow@lifeaire.com