

Quality Control Part One: Your IVF Environment

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The goal of the IVF laboratory is to provide an environment that optimizes the *in vitro* culture of the human embryo – to minimize and avoid, if possible, exposure of the gametes and embryos to adverse external factors. Exposure to these factors can cause physiological stress and such cellular stress can result in alterations in embryonic gene expression, regulation, imprinting and epigenetic effects.

(Wale and Gardner, 2016)

Variation in Protocols

- ✓ Controlled O²
- ✓ Tabletop incubators
- ✓ Upright CO² incubators
 - ✓ Continuous culture
 - ✓ Sequential culture
- ✓ ICSI, PICSI, PGD, PGS
- ✓ Day of cryopreservation/ vitrification
 - ✓ IVF clinical and laboratory staff

✓ Awareness/Control of
your IVF Environment

A Call From Attorneys –
Rarely a Good Call (but in this
case in search of accurate
environmental information to
assist the IVF practice they were
representing) – What
Information/Data Were They
Seeking?

THM: Air Testing Represents a “Snap Shot” in Time

- THM: Air testing represents the conditions at 11:00 on a Tuesday
- THM: The sources of environmental contaminants are exceptionally dynamic.
- THM: The air testing may or may not reflect what is occurring in the IVF environment during the active cycle

Our Objectives Today

- **Discuss the specific role of ambient air quality in successful preimplantation toxicology and embryogenesis.**
- **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 - which airborne pathogens are critical and which are less important to the process.**

Our Objectives Today

- **Discuss 3-3-3-4!**
- **Evaluate current mechanisms of remediation and their effectiveness**
- **Recognize what can you do - what questions can you ask – what solutions exist to assure that your lab design, HVAC system, upstream equipment and protocols are supporting an optimal and consistent laboratory environment?**

3 – 3 – 3 – 4

- 3 categories of airborne pathogens
- 3 sources of air to your laboratory
 - 3 categories of filtration physics
 - 4 THM from a long-term study of ambient air, preimplantation toxicology and mechanisms of remediation

Categories of Air Contamination

- **Volatile Organic Compounds (VOCs)**
 - Ethanol, styrene, toluene, aldehydes
- **Viable particulates**
 - Biological and viral particulates
 - Microbial and fungal pathogens
- **Nonviable particulates**
 - Classification of ISO and Class Rating
 - Non-infectious but serve as “vehicles” for infectious viable 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

Outside Influences Outside of Your Control

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

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



The IVF Laboratory: *Common Constituents of Recirculated Air*

- Tissue cultureware
 - Styrene
 - Toluene
 - Acetone
 - 2-butanone
- Isopropanol, cidex
- Equipment/component off-gassing
 - Trimethylsilanol
 - Hexamethylcyclotrisiloxane

The IVF Laboratory: Common Constituents of Recirculated Air

- HVAC / refrigerants / compressed gasses
 - Chloroethane
 - Dichloro-tetrafluorethane
 - Dichlorodifluoromethane
- CO2 tanks
 - Acetaldehyde
 - Isovaleraldehyde
 - Benzaldehyde
 - Formaldehyde
- Personnel bioburden
 - Particulate
 - Bacteria, Fungal spores, VOCs

Even embryologists shed.....

	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

HVAC System-Specific Organisms

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

Common Embryotoxic Fungal 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

Assuming that air quality was a variable impactful to our IVF process and wanting to remove it as a negative influence.....

Operation within an ISO 5 cleanroom
- *thus removing the variable of air* –

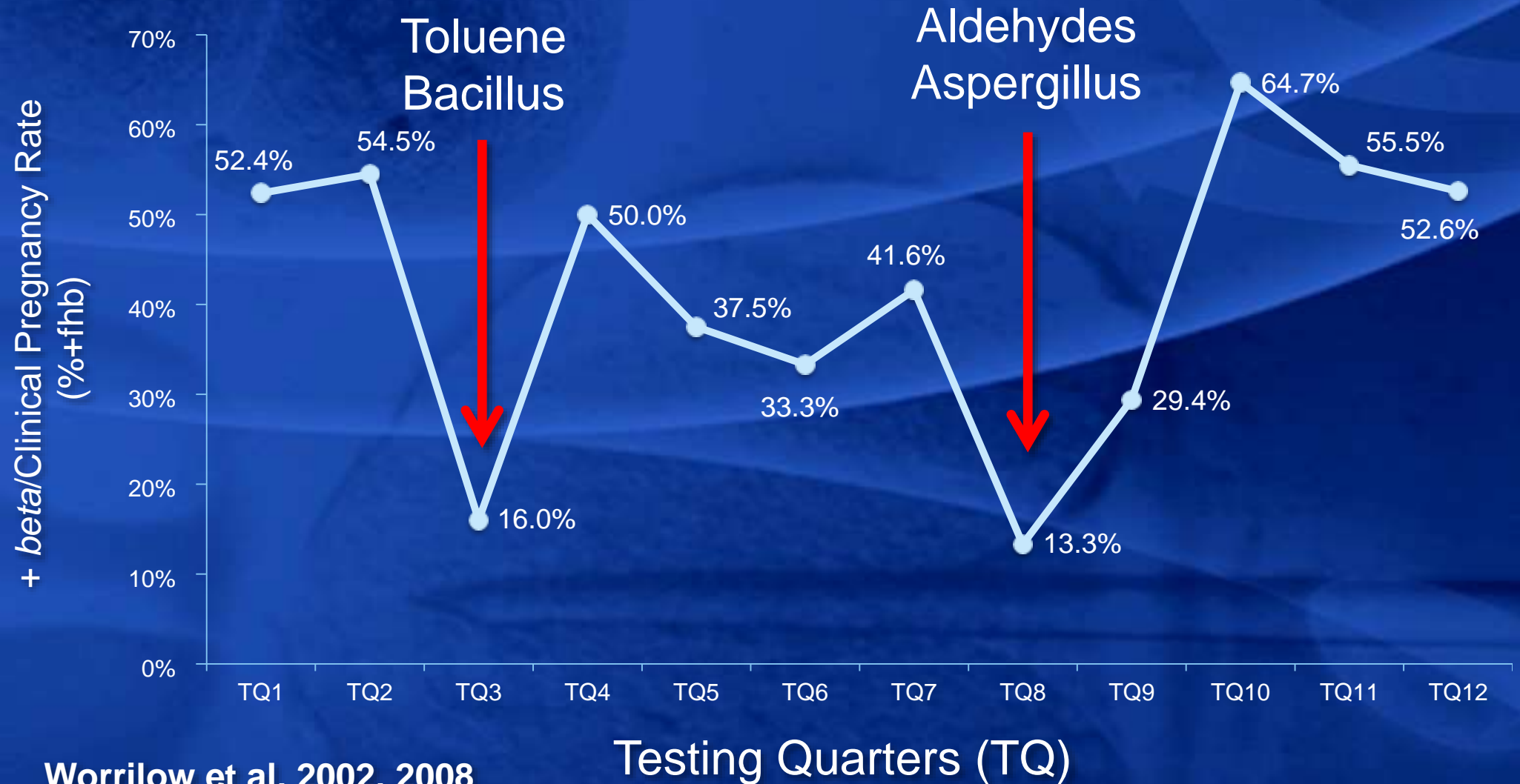
- ISO 5 design incorporating optimal air flow and air dynamics
- Dedicated AHU and sealed plenum
- 30 ACH, + pressure, live monitoring
- Laminar flow

> 300 lbs of carbon, > 150 lbs of KMnO_4 (Cohen, Gilligan and Hall)

- ULPA/UVC final filtration (Boone and Higdon)
- Air flow over critical points of process
 - Cleanroom SOPs followed
 - Quarterly certification

***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, 2008
Worrilow, 2013, 2015, 2017

Testing Quarters (TQ)

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 defined the airborne metrics necessary to consistently support the *in vitro* culture of the human embryo.

THM #1: IVF does not require the traditional cleanroom or ISO 5/6 environment.

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

THM #2: The impact of ppb levels of VOCs on our process and clinical outcomes

**FAQ: Can't we simply
add more carbon and
KMnO₄ to fix the
problem?**

No. Why?

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

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

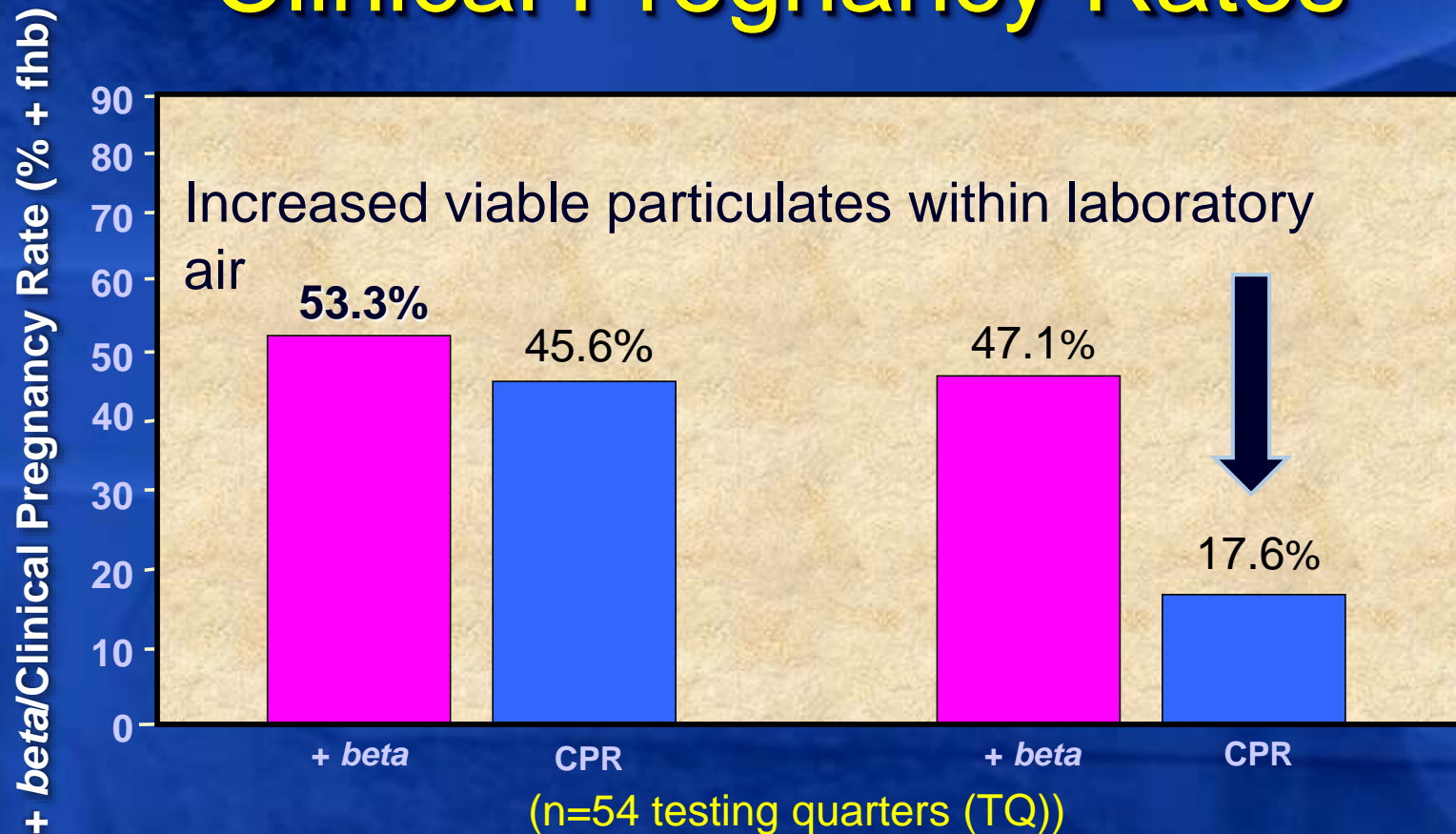
We wish this were true.

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

**THM #3: The impact of
subtle levels of viable
particulates/biologicals on
our process and clinical
outcomes**

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



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

Filtration Physics: Viable Particulates and Fungal VOC sources - 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.
- ❖ **Not all UV are created equal!**

**THM #4: What
HEPA/ULPA filtration and
ISO ratings do and DO
NOT do for our culture
environment**



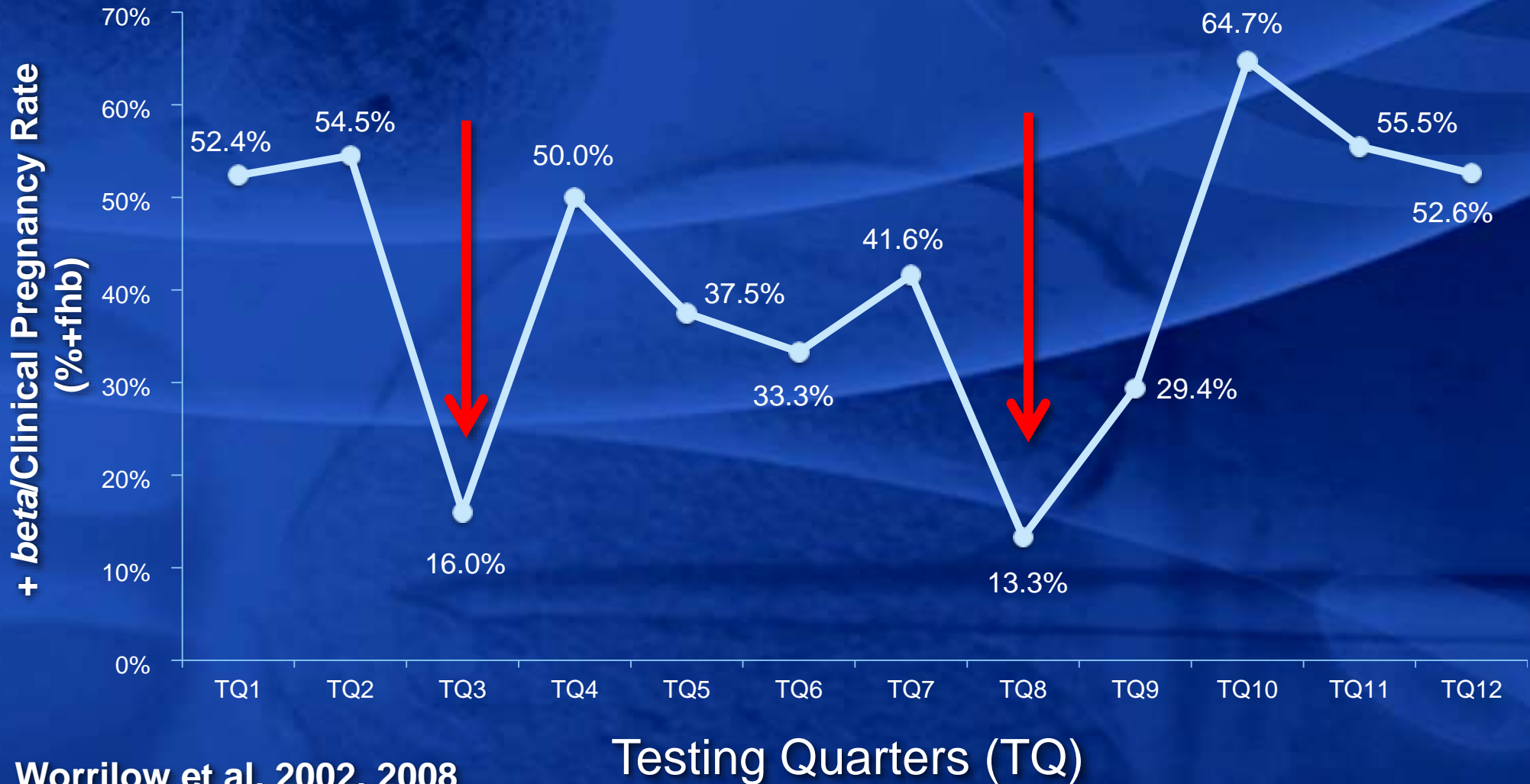
We create the perfect environment for growth.

Filtration Physics: Viable and Nonviable Particulates

HEPA and ULPA Filtration

- HEPA and ULPA filtration are designed to remove or capture particles greater than 0.3 microns in diameter at a 99.97 – 99.99, 99.997 – 99.999% effectiveness rating, respectively.
- HEPA and ULPA filtration remediate by “capture.”

Data Collected Over the 10-Year Study Led to the Identification of the Problem

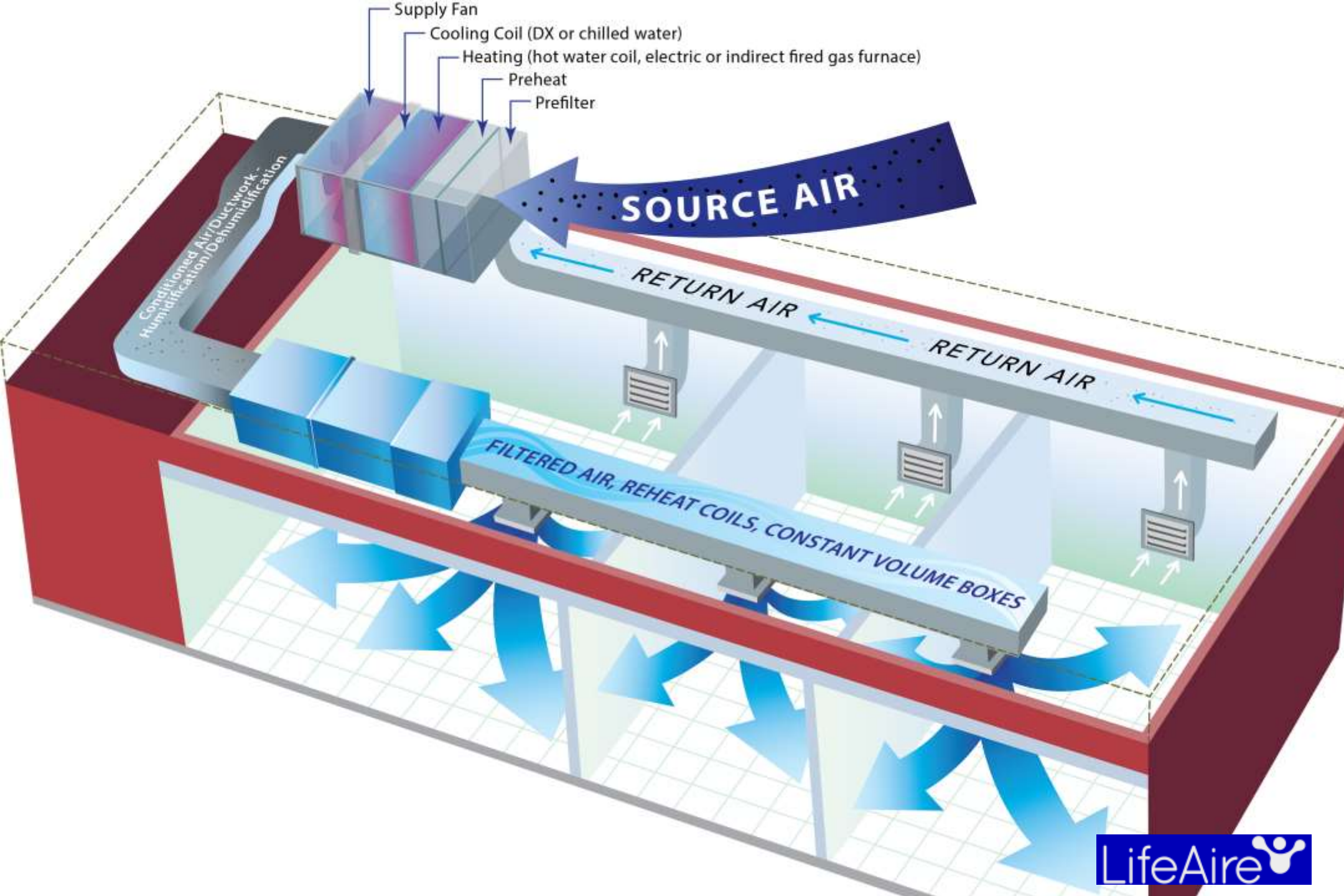


Worrilow et al, 2002, 2008
Worrilow, 2013, 2015, 2016

Testing Quarters (TQ)

Identification of the problem led to a comprehensive understanding and identification of critical airborne metrics: VOC, viable and nonviable particulate levels optimal for consistent culture of the human embryo

And our last objective - know what you have in your HVAC system, pop the ceiling tiles and ask questions....ask lots of questions.....know what you have above those ceiling tiles!
What did you “inherit?”



Critical Components: HVAC System

- Location of practice – what do your neighbors do, ask for PM5 and PM10 environmental data in the area
- Location of air intake relative to outside activity – anticipate change in source air (ie. new construction, shipping docks, parking garages, drycleaners, new traffic patterns)
- Dedicated air handling unit (AHU), proper sizing to deliver positive pressure and adequate air changes/hour – don't let them undersize your needs!

Critical Components: HVAC System

- Water and gas pipelines – cooling effect, dampness, mold growth = microbial growth and fungal VOCs
- Upstream filters – no electrostatic filters
- Dedicated humidity system, DI water only, continual flushing, proper dispersion, moisture = microbial growth
- Proper dehumidification, cooling and heating equipment
- Cooling coils – source of microbials

Critical Components: HVAC System

- Non-shedding ductwork, non-treated (rust inhibitor), externally insulated only (NO formaldehyde-urea insulation)
- Comprehensive air purification system – check for bypass
- Proper return and final diffuser location relative to critical points of process
- Dedicated return air – do not share with other clinical areas if possible (ie. GI lab, ortho joint room, etc.)
- AHU, air filtration/purification system, temperature, humidity, BMS/DDS/BACNET on generator

Air Quality Component and Management

- Consideration of energy efficiency (30 – 25 ACH = \$ savings)
- Proper air flow with no/minimal leakage from adjacent areas; plenums, service ducts, unknown holes in ductwork, leaky switches/outlets, identification of “still” pockets, etc.
- + Delta/positive pressure - 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 SOPs

Air Quality Component and Management

- **Vertical unidirectional air flow with low returns**
- **Proper air changes per hour (ACH)**
- **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 – ability to use recirculated air only during an environmental event**
- **Proper volume of exhaust**

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**
- **LIVE ppb TVOC monitoring optimal**

Additional Environmental Components to Consider

- Walls, paints, flooring and ceiling – no MDPB, no linoleum, low/no VOC materials, wipeable paint/no pigments, heat-welded floors, no adhesives, dovetail edges, no corners, ceiling of plasterboard, gypsum panels with air-tight, silicone gasketing
- 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 (clinical too!)
- Proper “bake-out” of new materials or renovated, expanded or new laboratory or clinical space – off-gassing is a time-dependent reaction and can be accelerated by higher temperatures

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, cidex or other agents 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

**There is always
someone willing to do
it for cheaper!**



When Environmental Solutions Are Proposed: Ask for Data. Proof of Technology. References to Experience in IVF.

- **Do they understand the environment that you are protecting, the sensitivity of the human embryo to its environment?**
- **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?**

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.
- ❖ Subtle levels of airborne VOC and biological contaminants can be impactful to successful human embryogenesis and clinical outcomes – proper mechanisms of filtration physics must be used to comprehensively remediate, remove and control the airborne pathogens.

- ❖ It is critical to control the quality of the ambient air serving your *in vitro* culture environment to optimize successful preimplantation embryogenesis and provide improved levels of patient care.